



SERUM COBALAMIN STATUS OF PREGNANT WOMEN SUFFERING FROM GESTATIONAL DIABETES MELLITUS.

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ABSTRACT... Objectives: Determining serum cobalamin levels in Pregnant Women suffering from Gestational Diabetes mellitus (GDM) presenting at our tertiary care hospital. **Study Design:** Case control study. **Setting:** Department of Gynecology and Medicine, SMBB Medical College Layari General Hospital Karachi. **Period:** January 2016 to April 2017. **Material & Methods:** Sample of 100 pregnant women in 2nd and 3rd trimester was selected into; 50 controls and 50 GDM cases through convenient sampling. GDM was defined as pregnant women with fasting blood sugar ≥ 100 mg/dL. 5 ml blood was collected; 3 ml put into EDTA tubes for complete blood counts and 2 ml for sera. Blood glucose was estimated by hexokinase method, HbA1c by colorimetric method and cobalamin by ECLIA assay method. SPSS software 21.0 (IBM, Inc USA) was used for data analysis using Student t-test and Chi-square test ($P \leq 0.05$). **Results:** Age of control was 30.43 ± 1.49 years and 29.95 ± 1.27 years in cases. Gestational age was 33.67 ± 2.69 weeks in controls and 34.75 ± 2.53 weeks in cases. Control and cases shows serum cobalamin levels of 316.34 ± 113.77 pg/ml and 253.5 ± 121.32 pg/ml respectively ($P=0.009$). Serum cobalamin deficiency was noted in 68% of cases and 40% of controls ($P < 0.05$). Glycemic control was bad in majority of cases. Serum cobalamin shows inverse correlation with random blood glucose, fasting blood glucose and Glycated HbA1. **Conclusion:** We found low serum cobalamin levels in pregnant women suffering from gestational diabetes mellitus that showed inverse correlation with random and fasting blood glucose and glycemic control.

Key words: Gestational Diabetes Mellitus, Cobalamin, Glycemic Control.

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INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance and hyperglycemia first time observed during pregnancy.¹ GDM complicates approximately 7% of pregnancies accounting for >0.2 million cases per annum.¹⁻³

Prevalence of GDM ranges from 1-14% of pregnancies. The variation depends on the population studied and diagnostic methodology of glucose testing. Previous studies²⁻⁴ from South East Asia reported GDM prevalence range of 4.2% to 26%. However, a recent study showed GDM prevalence of 17.2% in pregnant female during second trimester.⁵

GDM is a serious metabolic disorder for mother

and fetus. In mothers, the long term risk of DM is increased. Also the risk of metabolic syndrome and cardiovascular disease is increased in GDM. Pregnant GDM female are at increased risk of urinary tract infections, sepsis, pregnancy toxemia, and polyhydramnios. Incidence of premature child birth and cesarean section is increased too. Many of the fetal complications have been reported such as macrosomia, intrauterine death, congenital malformations, and spontaneous abortion.⁶

Risk of developing type 2 DM is 7 times increased in subsequent pregnancies.⁷ Many risk factors have been implicated for developing GDM, such as multiparity, maternal age ≥ 25 years and BMI >30 Kg/m² before pregnancy.⁸

Female suffering from GDM is prone to developing frank DM in next pregnancies, and post partum period. Female with history of macrosomia (>4.0 kg) of fetus, positive family history of DM, Asian and African ethnicities are at high risk of developing GDM in subsequent pregnancies.¹⁻⁷

Vitamin cobalamin (vitamin B₁₂) is an essential nutrient for the cell growth, maturation and cell division along with folic acid. Vitamin cobalamin plays role in the red blood cell production, neuronal growth and myelination of brain.^{1,5,8} Vitamin cobalamin deficiency is prevalent in the country.⁹

¹⁰ Dietary vitamin cobalamin deficiency or due to increased demands as during pregnancy, it manifests as grave medical symptoms and complications in the mother and fetus. Delayed cobalamin replacement presents with rapid appearance of symptoms within short time period. Vitamin cobalamin deficiency may be treated by high dose oral supplements or parenteral route.¹¹ Pregnant women are at high risk of vitamin cobalamin deficiency because of poor nutritional status and high cell turnover in the body. Rapidly growing fetus consumes more vitamin cobalamin from the maternal blood thus putting them on increased risk of this vitamin that may be deficient in the maternal diet. If dietary requirements are not met, vitamin cobalamin deficiency ensues.¹²

Vitamin cobalamin deficiency manifests as glossitis, stomatitis, red buffy tongue, and malabsorption due to villi atrophy. Mucositis is also noted. Peripheral blood smear shows macrocytic erythrocytes with or without anemia. Poikilocytosis, anisocytosis, ovalocytosis, hypersegmented neutrophils and pancytopenia may occur.^{12,13}

Fetus may develop intrauterine growth retardation, fatty liver disease and poor mental development due to defective myelination in the central nervous system. Such babies are prone to developing a multitude to chronic diseases in future such diabetes mellitus (DM), depression, cardiovascular disorders and even cancer.¹² Currently, the issue of vitamin cobalamin

deficiency and GDM has been hotly hinted in medical literature^{12,13}, hence there is a need to explore this association of Vitamin cobalamin and GDM in our indigenous population. The present study was planned to explore the Vitamin cobalamin status in Pregnant Women with Gestational Diabetes mellitus presenting at our tertiary care hospital.

MATERIAL & METHODS

The present case control study was conducted at the Department Gynecology and Obstetrics, Shaheed Muhtrama Benazir Bhutto Medical College Layari General Hospital Karachi, Sindh, Pakistan from January 2016 to April 2017. Ethical approval of research protocol was applied for the ERC (ethical review committee). Sample size was calculated by 'sampling for proportions'. Sample size was calculated to be 95 by using 5% α -level of significance and power of test of 90% at an expected % of vitamin cobalamin deficiency in GDM subjects as 51% and without GDM as 21.9%.⁷ But in order to increase the power, 100 subjects were divided into 2 groups; Group 1– cases of gestational diabetes mellitus and Group 2 – controls. Cases were selected by convenient sampling. GDM cases and control were selected according to the inclusion and exclusion criteria at the booked antenatal clinics of Layari General Hospital. GDM was defined according to the American Diabetes Association (ADA) criteria of 75g OGTT.¹⁴ GDM was defined as pregnant women with fasting blood sugar ≥ 100 mg/dL.¹⁴

Gestational age (GA) was calculated by recalling the first day of LMP (last menstrual period). Gravida was defined as total number of pregnancies regardless of the conception outcome. GDM cases were diagnosed as pregnant women with fasting blood sugar ≥ 100 mg/dL as cited.¹⁴ A sample of 50 GDM patients were tagged cases. Similar number of age and gender matched pregnant female without diabetes mellitus were diagnosed as controls (n=50). Inclusion criteria for controls were pregnant female in 2nd and 3rd trimester of pregnancy with normal blood fasting sugar levels. Inclusion criteria for cases were defined as; 2nd and 3rd trimester of pregnancy of age 25 - 40 years who remembered the exact

LMP date. And GDM patients being treated with metformin are strictly excluded. Pregnant female who suffered from abdominal tuberculosis, pancreatic disease, Gastroesophageal reflux disorder, strict vegetarians, renal disease, and chronic liver disease were excluded. Pregnant female using multivitamin pills since beginning of first trimester were excluded. Volunteers were informed about the purpose of study and willing volunteers were asked to sign the consent form. Findings of research interest were noted in a proforma of study protocol designed by the researcher. Patient history, gravidity, LMP, GA Clinical problem, and blood findings were noted. Confidentiality of patient's records was ensured. Patients were informed that the results and biodata will never be publicized and shall remain confidential. Volunteers were asked for blood sampling. Willing participants were informed that the blood sampling will cause no harm and blood will be used only for the testing of glucose and vitamin levels. Participants were intimated that the laboratory expense will be paid by the researcher not by the patients.

Height, weight and body mass index were calculated. Systemic blood pressure was measured by mercury sphygmomanometer. Willing volunteers were asked to lie on examination couch. A tourniquet was tightly applied above the cubital fossa to make the vein prominent. Skin over prominent vein was sterilized with alcohol swab. Disposable syringe (BD, USA) was used for venesection to draw 5 ml blood. 3 ml of blood sample was put into EDTA tubes for complete blood counts. Fasting blood sugar was estimated by glucose hexokinase method and hemoglobin A1 glycosylated (HbA1c) by Colorimetric method. Serum cobalamin levels were estimated by using the ECLIA (Electro-chemiluminescence immunoassay) method.

Serum cobalamin levels were defined as deficiency <200 pg/mL; marginal deficiency 200 - 299 pg/mL and normal cobalamin at ≥ 300 pg/mL.¹⁵ Data variables were typed on Excel sheet and copied to SPSS software 21.0 (IBM, Inc USA). Age, gestational age, gravidity, fasting blood sugar, HbA1c and serum cobalamin were

analyzed by Student's t-test. Serum cobalamin categories of deficiency, marginal deficiency and normal were cross tabulated using Chi-square test. Pearson's correlation was used for the correlation of serum cobalamin with RBG, FBG, HbA1c and gestational age. All data variables were analyzed at 95% confidence interval ($P \leq 0.05$).

RESULTS

Age of control was 30.43 ± 1.49 years and 29.95 ± 1.27 years of cases. Gestational age and gravida in control and cases were matched as shown in Table-I. Gestational age was 33.67 ± 2.69 weeks in controls and 34.75 ± 2.53 weeks in GDM cases.

All of the control (2.44 ± 0.50) and GDM cases (2.42 ± 0.49) were gravida 2 or 3. Hemoglobin (g/dl), hematocrit (%), RBC counts (million/ μ L) and Platelets (million/ μ L) are shown in Table-I. Random blood glucose (RBG) was noted as 136.58 ± 9.43 mg/dl in controls and 220.16 ± 82.69 mg/dl in cases. Fasting blood glucose (FBG) in control and cases was noted as 88.56 ± 7.87 and 155.67 ± 48.73 mg/dl respectively ($P=0.0001$). Glycated HbA_{1c} in cases was 7.66 ± 1.33 % compared to 5.43 ± 0.58 % in controls. Serum cobalamin (mean \pm SD) in control and cases were noted as 316.34 ± 113.77 (range 113- 513.1) pg/ml and 253.5 ± 121.32 (range 110 - 513.5) pg/ml ($P=0.009$) (Table-II). Sufficient serum cobalamin (≥ 300 pg/mL) was found in 30 (60%) controls compared to 16 (32%) GDM cases.

Marginal deficiency (cobalamin 200- 299 pg/mL) was noted in 11 (22%) of cases compared to 15 (30%) of controls. 23 (46%) of cases revealed deficient serum cobalamin (<200 pg/mL) compared to 5 (10%) in controls. Total 68% cases and 40% controls revealed serum cobalamin deficiency (Table-III). ($P < 0.05$). Serum cobalamin shows significant inverse correlation with RBG ($r = -0.196$, $P=0.008$), FBG ($r = -0.226$, $P=0.001$) and HbA1c ($r = -0.361$, $P=0.0001$) as shown in Table-IV. Figures-1-3 show the graphical distribution of negative correlation of serum cobalamin.

	Control	Cases	P-Value
Age (years)	30.43±1.49	29.95±1.27	0.086
Gestational age	33.67±2.69	34.75±2.53	0.091
Gravida	2.44±0.50	2.42±0.49	0.889
Hematocrit (Hct.) (%)	38.01±3.31	38.12±3.11	0.279
Hemoglobin (g/dl)	10.90±0.57	10.76±0.71	0.739
RBC counts (million/ μ L)	3.23±0.43	3.20±0.46	0.192
Platelet (million/ μ L)	3.72±0.40	3.62±0.41	0.0001
Random Glucose (mg/dl)	136.58±9.43	220.16±82.69	0.0001
Fasting Glucose (mg/dl)	88.56±7.87	155.67±48.73	0.0001
Glycemic control (HbA1c %)	5.43±0.58	7.66±1.33	0.009

Table-I. Demographic features and biochemical blood findings

Serum cobalamin (pg/ml)	Control	Cases
Mean (pg/ml)	316.34	253.50
Standard Deviation	113.77	121.32
Standard Error Mean	16.09	17.15
95% Confidence Interval		
- Lower	16.16	16.15
- Upper	109.51	109.52
Range (pg/ml)	113- 513.1	110 – 513.5
t-value	2.67	
P-value	0.009	

Table-II. Serum cobalamin in control and cases

Serum Cobalamin	Control	Cases	X ² -Value	P-Value
≥300 pg/mL	30 (60%)	16 (32%)	16.4	0.0001
200 - 299 pg/mL	15 (30%)	11 (22%)		
<200 pg/mL	5 (10%)	23 (46%)		
Total	50	50		

Table-III. Serum cobalamin in control and cases

	r-value	P-Value
Age	- 0.099	0.17
Gestational Age	- 0.064	0.089
RBS	- 0.196	0.008
FBS	- 0.226	0.001
HbA1c	- 0.361	0.0001

Table-IV. Pearson `s correlation of serum cobalamin in cases

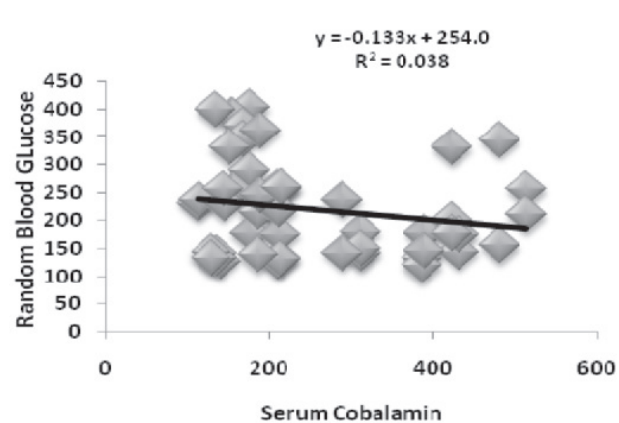


Figure-1. Scatter plot showing negative correlation of serum cobalamin and random blood glucose

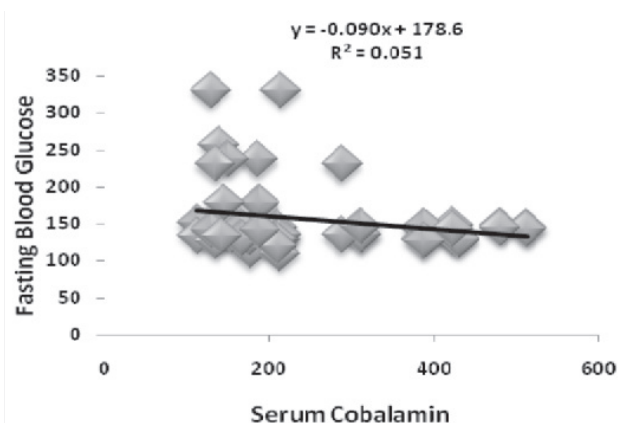


Figure-2. Scatter plot showing negative correlation of serum cobalamin and Fasting blood glucose

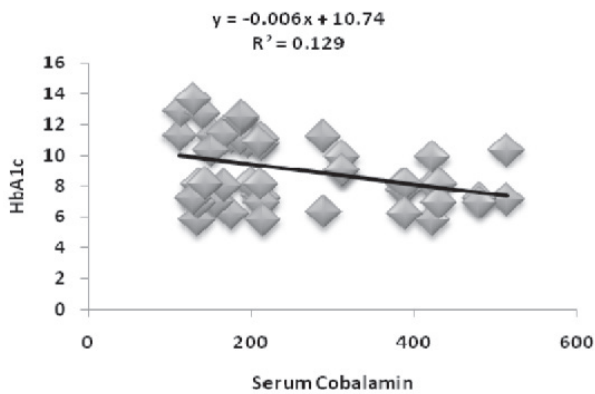


Figure-3. Scatter plot showing negative correlation of serum cobalamin and Glycemic control (HbA1c)

DISCUSSION

The present hospital based study reports serum cobalamin deficiency in 68% of gestational diabetes mellitus (GDM) cases and 40% of controls. Other noteworthy finding of present study is negative correlation of serum cobalamin with random ($r=-0.196$, $p=0.008$) and fasting blood glucose ($r=-0.226$, $p=0.001$) and glycated hemoglobin ($r=-0.361$, $p=0.0001$). The findings of low serum cobalamin are in agreement with previous studies.^{7,13,16-18}

The Milman *et al*¹⁶ conducted a longitudinal study on the cobalamin levels among pregnant women and proved low cobalamin in normal pregnancies. The finding of low serum cobalamin in gestational diabetes mellitus (GDM) was reported by a previous study from India.¹⁷

They reported positive link of serum cobalamin deficiency with insulin resistance, adiposity and gestational diabetes mellitus. Krishnaveni *et al*¹⁷ further added that the risk of GDM is twice more frequent pregnant women with cobalamin deficiency than cobalamin non-deficient pregnant women. The findings of above study support the serum cobalamin deficiency in GDM of present study. Few years later, the Knight *et al.* (2015)¹⁸ examined the British cohort of pregnant women for serum cobalamin. They¹⁸ studied a cohort of white British pregnant women of different ethnicity with different dietary habits, nutritional status and livings standards of society.

They¹⁸ reported serum cobalamin was low in pregnant women and revealed inverse correlation of serum cobalamin with fasting blood glucose ($r= -0.09$; $p=0.006$) similar to present study. The findings of Knight *et al*¹⁸ are in full agreement with present study as negative correlation of serum cobalamin with random ($r=-0.196$, $p=0.008$) and fasting blood glucose ($r=-0.226$, $p=0.001$) and Glycated hemoglobin ($r= -0.361$, $p=0.0001$) was noted. A year later, a UK based retrospective study by Sukumar *et al*¹³ proved the link of low serum cobalamin in pregnant women suffering from GDM and regression analysis model revealed a 2.59 times higher odds of developing GDM in serum cobalamin deficient pregnant women.¹³

The findings of Sukumar *et al*¹³ are in keeping with the observations of present study. A recent study by Ambreen *et al* (2017)⁷ analyzed the serum cobalamin levels in pregnant and reported 67% deficiency of serum cobalamin in GDM and 39% of controls. The findings of Ambreen *et al* (2017)⁷ are validated by the present study that observes serum cobalamin deficiency in 68% of gestational diabetes mellitus (GDM) cases and 40% of controls. The Ambreen *et al* (2017)⁷ further noted negative correlation of serum cobalamin fasting blood glucose ($\beta= -0.29$, $p=0.004$), gravidity ($\beta = -0.28$, $p=0.01$) and gestational age ($\beta = -0.57$, $p=0.21$). The above findings are comparable to the negative correlation of serum cobalamin with random ($r=-0.196$, $p=0.008$) and fasting blood glucose ($r=-0.226$, $p=0.001$) and Glycated hemoglobin ($r= -0.361$, $p=0.0001$) of present study. Hence the findings of present study are worth reporting for future studies. Serum cobalamin deficiency is prevalent^{9,10} in the country due to nutritional deficiency added by economical constraints putting the pregnant ladies and children at highest risk.

Hence the finding of 40% cobalamin deficiency of present study is justified in the present scenario of nutritional deficiencies particularly in the developing countries. Many of previous studies¹⁹⁻²¹ have attributed the serum cobalamin deficiency in diabetics to the metformin therapy but metformin intake was an exclusion criterion in the present study. Negative correlation of

serum cobalamin with RBG, FBG, and HbA1c is important finding that show the glycemic control will be deviated more to abnormal side with concomitant cobalamin deficiency in pregnant women suffering from GDM. Serum cobalamin levels should be closely monitored for prevention of materno-fetal complications. Intrauterine life of fetal metabolic programming may adversely affect through epigenetic modifications making the fetus prone to chronic disease in adult life.^{22,23} Timely correction of cobalamin deficiency and diabetic management of GDM may prevent the adverse fetomaternal outcomes. As the bad glycemic control is also a risk factor for the materno-fetal outcome hence this needs to be addressed by the treating obstetricians and physicians. The findings of present study in the light of national and international literature are worth to report. The limitations of present study include – first; small sample size, second; anthropometric data of pre-pregnancy serum cobalamin, nutritional status and dietary habits are not clear, third; cross sectional study design, hence findings cannot be generalized. However, the strength of study lays in its prospective study design, and exclusion and inclusion criteria.

CONCLUSION

We found low serum cobalamin levels in pregnant women suffering from gestational diabetes mellitus and showed inverse correlation with random and fasting blood glucose and glycemic control. The present hospital based study reports 68% deficiency of serum cobalamin in gestational diabetes mellitus. Cobalamin supplements should be recommended to this particular population at risk of improving maternal, neonatal and child outcome, giving emphasis on neurological processes and epigenetic modification.




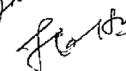
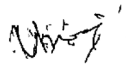
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2	Sadia Saeed	Concept, Materials handling, Interpretation lab investigations, Manuscript write up, Proof reading.	
3	Syeda Fariha Hasnny	Literature review, Materials handling, compilation of results, statistical analysis, Manuscript write up, Proof reading,	
4	Nathumal Maheshwari	Correspondence.	
5	Urooj Tabassum	Concept, Materials handling, Collection of materials, compilation of results, Statistical analysis, manuscript write.	
6	Arshad Ali	Concept, Materials handling, Interpretation lab investigations, Manuscript write up, Proof reading.	