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Low Serum Cobalamin is a Risk Factor for Gestational Diabetes

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AB and UM conceived the idea. KW, AB and UM designed the study. AK, KW, AB and UM critically evaluated the manuscript. KW, AK, SE and SF processed patients. TAS analyzed and compiled the results. SF and TAS wrote the manuscript. SE and FAR reviewed the manuscript. TAS supervised the research.

Key words
B12, Cobalamin, Biomarker, Gestational diabetes, Fasting blood sugar level.

ABSTRACT

Research shows that serum Vitamin B12 levels are reduced in diabetes including gestational diabetes. We sought to compare serum cobalamin between pregnant females diagnosed with gestational diabetes mellitus (GDM) and healthy controls and to propose a cut off value for serum B12 levels as a predictive biomarker for GDM. Study design was Quantitative cross-sectional study. This study was carried out at Lady Atchison Hospital/Mayo Hospital Lahore, Pakistan from January to December 2016. Cobalamin levels were measured in women diagnosed with GDM (n=59) and controls (n=41). GDM was diagnosed when fasting blood sugar levels were 95 mg/dL or above. The levels were compared using Mann Whitney U test. Multiple linear regression was used to investigate the association of cobalamin with fasting blood sugar level after adjusting for gestational age and gravidity. ROC curve analysis was conducted to assess the suitability cobalamin as a predictive biomarker for GDM. Median cobalamin were significantly reduced in cases (108.95±92 pg/mL) as compared to controls (173±90 pg/mL) at $p<0.001$. Low vitamin B12 levels ($\beta = -0.29$, $p=0.004$) significantly predicted high fasting blood sugar levels in pregnancy even after adjusting for gestational age and gravidity. ROC curve analysis revealed the optimal cutoff point of serum cobalamin for predicting gestational diabetes to be 113 pg/mL at which sensitivity and specificity were was 56.9% (95%CI 44.1-68.8%) and 80.5 % (95%CI 65.6-89.9%) at $P<0.0001$. It was concluded that low serum cobalamin levels may be a risk factor and may predict the development of GDM.

INTRODUCTION

Gestational diabetes mellitus is defined as presence of hyperglycemia in pregnant women who did not suffer from diabetes mellitus prior to their pregnancy (American Diabetes Association, 2014). In our country conflicting results have been reported. Previous studies from our population have reported frequencies of GDM ranging from 4.2% to 26% (Bibi *et al*., 2015; Iqbal *et al*., 2007; Rahman *et al*., 2007) However a latest high powered study shows that 17.2% of pregnant local females in their second trimester suffer from gestational diabetes (Fatima *et al*., 2017).

GDM is a serious disorder with health related implications for not only the mother but also the unborn child. For the mother, it is associated with increased long term risks of diabetes, metabolic syndrome and cardiovascular disorders. Pregnant woman are also at risk of polyhydramnios, pregnancy toxaemia, urinary tract infection, candidiasis, higher incidence of premature childbirth and cesarean delivery. The most common fetal complications are macrodome, spontaneous abortion, congenital malformation, intrauterine death (Ornoy, 2011). Such individuals are at 7 fold increase in risk of developing T2DM subsequently (Bellamy *et al*., 2009).

Various risk factors have been identified for GDM. Previous studies have shown that older maternal age (≥ 25 years), high parity (DeSisto, 2014), pre-pregnancy body mass index (BMI) more than thirty (30) kg/m^2 , GDM during previous pregnancies, a previous macrosomic baby (>4.0 kg), family history of diabetes, and certain Asian and African ethnicities put women at a higher risk of developing GDM (American Diabetes Association, 2014).

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Vitamin B12 is an essential water soluble vitamin that plays a vital role in the physiological dynamics of human body ranging from production of erythrocytes on one hand to optimal nervous system functioning on the other. Whenever there is depletion of vitamin B12 because of poor dietary source or increased cell turn over, the deficiency can manifest as wide range of symptoms. If not detected and treated timely, these medical symptoms can present within a short period of time. It has been suggested that vitamin B12 deficiency may be treated by parental or high dose oral cobalamin therapy (Stabler, 2013).

Pregnant females are at an increased risk of B12 deficiencies as they are in a state of high cellular turnover and increased overall dietary requirements especially vitamin B12. The rapidly growing fetus consumes vitamin B12 from the mother's body, thus posing a threat of deficiency if dietary requirements are not met. In the mother classical cobalamin deficiency features may be produced including macrocytic red blood cells with or without anaemia, ovalocytosis, hyper segmented white blood cells, pancytopenia, atrophic glossitis, stomatitis, malabsorption due to villi atrophy and mucositis (Rush *et al*., 2014). The fetus will also bear the consequences of low cobalamin levels. An uncorrected deficiency will not only lead to impaired fetal growth in utero but may also make the fetus susceptible to a multitude to chronic diseases including diabetes mellitus, fatty liver disease, cardiovascular diseases, depression and even cancer in future (Rush *et al*., 2014).

As various international studies have recently hinted towards an association of vitamin B12 deficiency with GDM, we sought to explore this relationship in a cohort of local women in their second and third trimester. We also constructed a receiver operative curve (ROC curve) to investigate the suitability of serum cobalamin as a predictive biomarker for GDM.

MATERIALS AND METHODS

The study was conducted from January 2016 to December 2016. Sample size was calculated to be 90 (45 in each group) estimated by using 5% level of significance and 90% power of test with expected percentage of B12 deficiency with GDM as 51.1% and without GDM as 21.9% (Sukumar *et al*., 2016). However to increase the power, 100 patients were sampled (41 controls and 59 cases). The subjects were booked in antenatal clinics of Lady Aitchison Hospital, Lady Willington Hospital and Mayo Hospital, Lahore. Cases were women in their second and third trimesters who were diagnosed with gestational diabetes mellitus according to criteria defines by American Diabetes Association (Behan, 2017) on the basis of 75g OGTT. Gravidity was defined as total number of recalled pregnancies regardless of the outcome. Gestational age was determined from the first day of last menstrual period. Controls were women in their second and third trimesters with normal blood sugar profiles. Informed consent was obtained from all study participants. The fasting blood samples were collected in laboratory and submitted there for lab analysis (Roche E-170 Vitamin B12 "ECLIA").

Results were represented as mean±SD and or Median±IQR as some data were normally distributed (age) and others were skewed (gestational age, FBS, RBS and Vitamin B12 levels). Mann Whitney U test was used to compare Vitamin B12 levels. Women with fasting blood sugar 95 mg/dL or above were diagnosed with gestational diabetes. Women with B12 levels of 150 pg/mL or less were considered deficient. Odds ratio with 95% confidence intervals were calculated to see if cobalamin deficient women were more likely to develop GDM. A multiple linear regression model was created to investigate the association between fasting blood sugar levels and vitamin B12 levels after adjusting for the effect of gestational age and gravidity. The variables, vitamin B2 levels, fasting blood sugar levels and gestational age were all log transformed before entering them in the regression model as they were skewed in their distribution. A receiver operating characteristic curve (ROC) was plotted to identify the cut-off point for serum vitamin B12 as suggestive of gestational diabetes. A two-sided $P<0.05$ was considered statistically significant.

Table I.- Characteristics of all subjects (n=100).

	Mean	SE	SD
Age (years)	26.95	0.46	4.57
Gestational age (weeks)	37.53	0.32	3.05
Fasting blood sugar levels (mg/dL)	117.77	4.62	46.22
Random blood sugar levels (mg/dL)	155.73	7.37	73.70
Vitamin B12 levels (pg/ml)	149.44	8.87	88.21

RESULTS

The subjects were pregnant women aged 26.95±4.57 (mean±SD) years old. Their average gestational age was 37.53±3.05 weeks. Amongst the 58 women with GMD, 39 (67%) were B12 deficient whereas in case of women without GDM (n=41) only 16 (39%) were deficient in vitamin B12. Vitamin deficient females were 3.21 (95% CI=1.39-7.38) times more likely to develop GDM than those with normal serum B12 levels

The mean characteristics of the subjects are shown in Tables I and II.

Table II.- Comparison of subjects with gestational diabetes and controls.

	Control (n=41)		Gestational diabetes (n=59)		'p' value
	Mean±SD	Median±IQR	Mean±SD	Median±IQR	
Age (years)	26.24±4.25	26±7	26.37±4.33	26±7	0.19*
Gestational age (weeks)	38.64±2.18	39±3	38.64±2.18	30±3	0.001**
Gravida (total pregnancies)	2.05±1.86	2±3	-	3±2	<0.001**
Fasting blood sugar levels (mg/dL)	80.00±9.54	82±16	144.05±43.68	135±58	<0.001**
Random blood sugar levels (mg/dL)	98.24±17.33	97±26	194.98±71.88	187.5±118	<0.001**
Vitamin B12 levels (pg/ml)***	179.81±91.05	173±90	127.97±80.18	108.95±92	<0.001**

*, Independent samples t test was used to compare means since age is normally distributed; **, Mann Whitney t test was used because the distribution of these variables was significantly; ***, B12 levels were not available for 1 subject.

Table III.- Factors affecting fasting blood sugar levels in a linear regression model.

Risk factors log for Fasting blood sugar (mg/dL) levels	Unstandardized coefficients		Standardized coefficients	Sig.	95% CI for B	
	B	Std. Error	Beta		Lower bound	Upper bound
Constant	7.56	1.46		<0.001	4.659	10.452
Log of vitamin B12 (mg/dL)	-0.20	0.07	-0.29	0.004	-0.328	-0.064
Log gestational age (weeks)	-0.57	0.39	-0.14	0.15	-1.354	0.210
Log gravidity	0.40	0.14	0.28	0.01	0.119	0.677

Adjusted R^2=0.22 ANOVA for model p<0.001.

The two groups were matched for age (p=0.19). There were statistically significant differences between random and fasting blood sugar levels. Vitamin B12 levels were overall low in all subjects (Mean±SD =149.44±88.21 pg/mL). However, median B12 levels were significantly reduced in women with gestational diabetes (108.95±92 pg/mL) as compared to controls (173±90 pg/mL) at p<0.001 (Fig. 1).

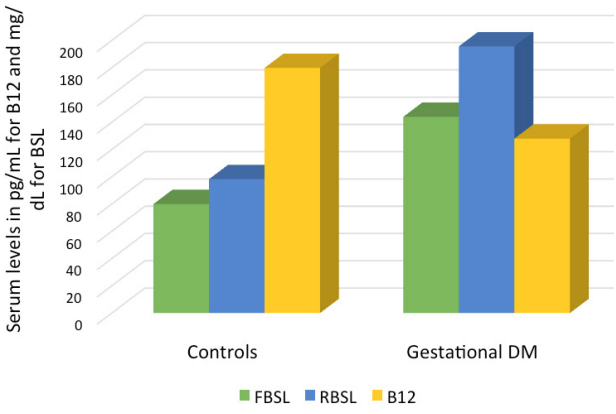

Fig. 1. Vitamin B12, fasting and random blood sugar levels in patients with gestational diabetes and controls

As a sensitivity analysis we adjusted for the confounding effects gestational age and gravidity. Multiple linear regression analysis was conducted to test if vitamin B12 levels, gestational age and gravidity significantly predicted fasting blood sugar levels. The results of the regression indicated the two predictors explained 22 % of the variance (R^2 =0.22; p<0.001). It was found that low vitamin B12 levels (β = -0.29, p=0.004) and gravidity (β =-0.28, p=0.01) significantly predicted high fasting blood sugar levels in pregnancy. ROC curve analysis revealed the optimal cutoff point of serum cobalamin for indicating gestational diabetes to be 113 pg/mL at which sensitivity was 56.9% (95%CI 44.1-68.8%), specificity was 80.5 % (95%CI 65.6-89.9%), PPV was 80.5% and NPV was 56.9%. The area under curve (AUC) was 71% (95% CI, 60.9-81.2%) at *P*<0.0001 (Fig. 2 and 3).

DISCUSSION

The most noteworthy finding of our study is the association of low serum Vitamin B12 levels (β= -0.29, p=0.004) with high fasting blood sugar levels and gestational diabetes in pregnant females even after adjusting for gravidity (β = -0.28, p=0.01) and gestational age (β = -0.57, p=0.21).

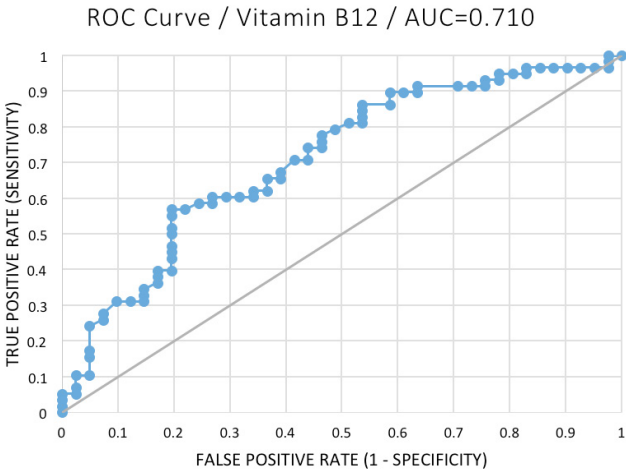

Fig 2: Receiver Operature Curve for Vit B12 as diagnostic marker for gestational diabetes showing area under curve to be 71%

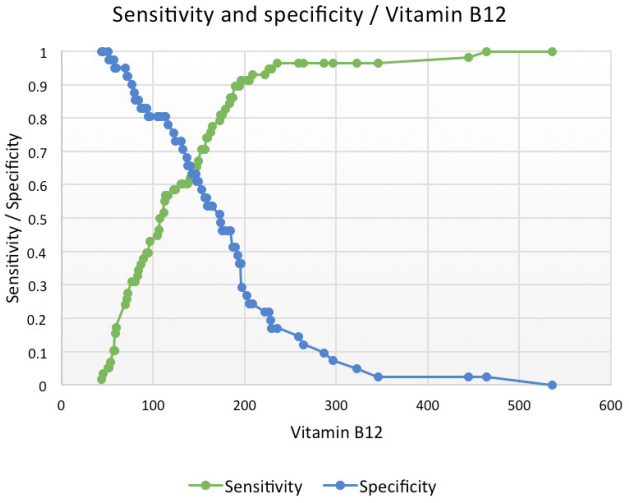

Fig 3: Receiver Operature Curve for Vit B12 showing 113 pg/mL as the optimum cut-off point for diagnosis of gestational diabetes with maximum sensitivity and specificity.

There is substantial literature available associating Vitamin B12 with insulin resistance. However many of these study attribute the finding of low B12 in diabetes to use of metformin (Chapman *et al*., 2016; Karimi and Omrani, 2016; Valdés-Ramos *et al*., 2015). In our cohort none of the women were previously diabetic or taking metformin but this association was still noted. The association we discovered between low B12 levels and GDM is consistent with several international studies from different ethnicities and nationalities.

In 2009, an Indian study by Krishnaveni *et al*. (2009) first reported a link between vitamin B12 deficiency and risk of adiposity, insulin resistance and GDM. They first brought to notice that GDM was two times more frequent among patients with vitamin B12 deficiency than among non-deficient pregnant females. Similar to Krishnaveni *et al*. (2009), we too discovered that B12 deficient pregnant females were 3.2 times more likely to develop GDM. A few years later Knight *et al*. (2015) replicated the findings of the 2009 Indian study on a cohort of white British pregnant females. They checked this association in a cohort with different ethnicity, nutritional status, dietary habits and standards of living compared to the Indian study. However, they too reported the same trend of B12 showing inverse correlation with fasting sugar level in pregnant females(r= -0.09; p=0.006) (Knight *et al*., 2015). Yet another recent UK based retrospective study by Sukumar *et al*. (2016) validated this link further by demonstrating that B12 deficient women were at 2.59 times higher odds of developing gestational diabetes (Sukumar *et al*., 2016). These results also support our findings.

Although B12 deficiency is shown to be associated with GDM, it must be noted that cobalamin deficiency was in 39% of our healthy controls as well. A longitudinal study by Milman *et al*. (2006) showed low cobalamin levels existed in normal pregnancies especially during later stage. This result is consistent with our study as low cobalamin levels were found in both normal pregnancies and those with GDM but deficiency was more frequent in GDM group (67%). Thus it is reasonable to believe that GDM has a multifactorial etiology and B12 deficiency may be one of these factors.

Serum B12 levels may also be used as predictive biomarker for gestational diabetes in pregnant females and such females may be monitored closely to prevent maternal and neonatal complications. GDM is diagnosed after the onset of the second trimester which means that the fetus has been potentially exposed to high sugar levels and associated metabolic derangement of GDM throughout the first trimester. This can lead to fetal metabolic programming possibly through epigenetic modifications (Mansell and Saffery, 2017) which can make the fetus susceptible to a variety of chronic diseases as an adult (Hanson and Gluckman, 2014; Plagemann, 2011). Timely detection and management of GDM prevent these adverse outcomes for the mother and child. Predictive biomarkers need to be developed to fulfill this need (Horvath *et al*., 2010). A number of predictive biomarkers have recently been proposed including adipokines and various inflammatory mediators (Brink *et al*., 2016).

Several clinical risk prediction models have also been proposed to predict GDM. These models include variables like ethnicity, family history, history of GDM, body mass index. These model have shown impressive AUCs of

77% (Van Leeuwen *et al*., 2010) and 82% (Thériault *et al*., 2014). Adding an objective serum based biomarker to this clinical risk prediction model is likely to improve predication rates. We propose B12 levels as such a marker. Our results show that even when considered independently it has promising ROC characteristics. It gives an AUC of 71% (95% CI, 60.9-81.2%) at $P<0.0001$. Adding it to a clinical risk prediction model will most likely improve the predictive value of such a model. This can be explored in future studies.

There are several limitations to our study. There is lack of pre-pregnancy anthropometric data, pre-conception vitamin B12 levels and details of dietary habits. However cohort comprised of women belonging to low socioeconomic class who were availing the services of a government hospital and are all likely to have poor nutritional status even prior to their pregnancies. Furthermore the association between low B12 levels and GDM risk has been established previously in an Indian cohort with vegetarian dietary habit and low BMIs (Krishnaveni *et al*., 2009) as well as a British cohort with diet rich in animal protein and higher BMI (Sukumar *et al*., 2016). We thus feel confidence in our findings.

Secondly, our sample size was modest, however we sampled women from the same socioeconomic status, ethnicity and there was no statistical difference in their age. Thus the effects of these confounders were minimized and we feel that the current sample size provided enough power investigate the association in this particular cohort and the results may be extrapolated to a similar local population. Finally our study is cross sectional study which by nature cannot establish causation. In future we intend to conduct an interventional study to investigate the risk reduction in GDM with B12 treatment.

CONCLUSION

Serum Vitamin B12 levels may be a risk factor for development of gestational diabetes.

Statement of conflict of interest

We have no conflicts of interest to declare

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