

Comparison Of Vitamin D, B12 and Folate Levels In Women with and Without Gestational Diabetes Mellitus Reporting to A Tertiary Care Hospital in Lahore

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ABSTRACT

Objective: To compare vitamin D, B12 and Folate levels in women with and without gestational diabetes mellitus (GDM) and to find any association of these markers with GDM.

Study Design: Comparative Cross-sectional study.

Place and Duration of Study: Pathology Department, Combined Military Hospital (CMH), Lahore Pakistan, from Feb 2023 to Aug 2023.

Methodology: Vitamin B12, vitamin D and folate levels were assessed in two groups: those with normal glucose tolerance (Group-A, n=108), and women diagnosed with GDM (Group-B, n=54). The comparison of vitamin levels was conducted using the Mann Whitney U test. The association between these vitamins and fasting blood glucose level was explored using multiple linear regression.

Results: Females with GDM had lower levels of vitamin B12 (156 pmol/L vs 198 pmol/L), vitamin D (48 nmol/L vs 59 nmol/L) and higher levels of serum folate (40 nmol/L vs 29 nmol/L). The difference in serum values of folate ($p=0.003$), vitamin B12 ($p=0.001$) and vitamin D ($p=0.002$) between the GDM and NGT groups was statistically significant. Vitamin B12 deficiency was observed in 23.4% (n=38) of the study population. About 86% females (n=140) either had deficient or insufficient vitamin D levels. Folate excess was recorded in 25% (n=40) of the participants.

Conclusion: Women with Gestational Diabetes Mellitus had comparatively higher serum folate levels and lower levels of vitamins B12 and D as compared to women without GDM, and there is an association of these vitamins with GDM.

Keywords: Folate, Gestational Diabetes Mellitus, Vitamin B12, Vitamin D.

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INTRODUCTION

Gestational diabetes mellitus (GDM) stands as one of the most prevalent endocrine conditions during pregnancy, with a global estimation of 14.0% prevalence. This prevalence differs across income brackets, with rates at 12.7% in low-income, 9.2% in middle-income, and 14.2% in high-income countries.¹ Several systematic reviews have concluded that using supplements like probiotics, inositol, and vitamin D (Vit D) can reduce the risk of GDM effectively or improve its outcomes.² Both Folate or FA and Vit B12 play critical roles in early pregnancy as key nutrients. Consequently, recent attention has shifted towards exploring the effects of additional micronutrient supplements like folic acid (FA) and cobalamin (Vit B12) on GDM.³

Multiple studies have presented conflicting outcomes concerning the relationship between FA,

vitamin B12, and GDM risk.⁴ For instance, the Nurses' Health Study II (NHS II), encompassing 14,533 women, reported that a daily intake of 400 µg of FA before gestation correlated with reduced GDM risk.⁵ Conversely, another cohort study contradicted this, suggesting that daily FA intake in early pregnancy increased insulin resistance and GDM risk by 2.25 times.⁶ These conflicting findings underline the ongoing ambiguity regarding the association of folate with GDM.

Studies examining FA, Vit B12, and Vit D in relation to insulin resistance and GDM risk are ongoing, with very few simultaneously comparing these vitamins in women with and without GDM.^{7,8}

This study aimed to assess the connection between maternal folate, Vit B12, and Vit D with GDM. The findings seek to contribute to early GDM prevention and offer insights into the risks associated with deficiencies or excesses of FA, Vit B12, and Vit D, establishing optimum serum levels during pregnancy without adverse consequences.

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METHODOLOGY

This comparative cross-sectional study was conducted at the Pathology Department, Combined Military Hospital (CMH), Lahore Pakistan, from Feb 2023 to Aug 2023, after obtaining approval from the Institutional Ethical Review Board (letter no. 436/2023).

Inclusion Criteria: Women of any age with a live singleton pregnancy at 24-28 weeks of gestation were included.

Exclusion Criteria: Women with overt diabetes mellitus, history of neural tube defects in previous pregnancies, multiple gestations, severe anemia (haemoglobin <10 g/dl), history of chronic hepatitis or severe liver disease, confirmed deficiencies in B12, Vit D, or folate, and recent B12 or Vit D injections within the last 6 months were excluded.

The sample size, initially calculated to be 90 individuals based on incidence of B12 deficiency with GDM at 51.1% and without GDM at 21.9%.⁹ However to augment statistical power of the study, we included 162 females (108 women with normal glucose tolerance and 54 cases of GDM).

Women with at least one of the below mentioned risk factors were screened for GDM: a BMI of ≥ 30 kg/m², prior GDM, history of previously unexplained stillbirth, history of macrosomia with a birthweight ≥ 4.0 kg, history of diabetes in the family, women with aged ≥ 35 years or a documented history of polycystic ovarian syndrome.

After obtaining informed consent, data on demographics, supplement intake, medical, reproductive, and family history were collected using standardized questionnaires through face-to-face interviews using non-probability convenience sampling. Preconception BMI was computed from self-reported pre-pregnancy weight and height.

All pregnant women underwent a 75-g Oral Glucose Tolerance Test (OGTT) during weeks 24–28 of gestation following an overnight fast of at least 8 hours. Diagnosis of GDM was made according to the criteria recommended by the International Association of Diabetes and Pregnancy Study groups (IADPSG): fasting glucose ≥ 5.1 mmol/L, 1-h glucose ≥ 10.0 mmol/L, or 2-h glucose ≥ 8.5 mmol/L. Those with normal glucose tolerance (NGT) were considered Group-A, while those with gestational diabetes mellitus were allotted to Group-B (Figure-1).

Blood samples were collected by trained staff. Blood Glucose was analysed the same day whereas Vit D, Vit B12, Insulin and Folate were analysed in batches. To cater for the effect of light on folate analysis, lightproof tubes were used during transportation and storage. Analysis of Vit D, Vit B12, insulin and Folate was performed using Electrochemiluminescence immunoassay (ECLIA) on COBAS e601 Analyzer. Plasma glucose was analysed by the electrochemical Hexokinase method using fully automated Chemistry analyzer COBAS c501. All measurements were conducted in the Pathology department of CMH Lahore. Three standard solutions of quality controls (QC1, QC2, and QC3) were used as daily QC. B12 deficiency was defined as: <203.3 pg/ml or <150 pmol/l. Folate levels <10 nmol/l and >45 nmol/l were defined as folate deficiency and folate excess respectively (16). Serum 25OHD concentrations ≥ 75 , 25-75 and <25 nmol/L, were defined as Vitamin D sufficiency, insufficiency, and deficiency, respectively.¹⁰

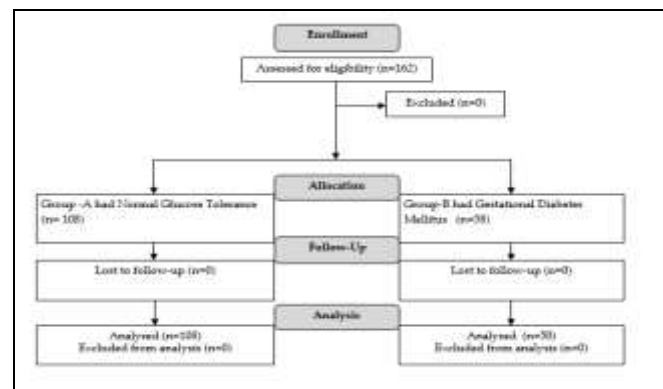


Figure-1: Patient Flow Diagram (n = 162)

Statistical Package for Social Sciences (SPSS) version 24 was used for data analyses. To assess the distribution of data, Shapiro-wilk test and Kolmogorov-Smirnov test were used. Based on these tests none of the measured and calculated parameters in this study had a normal distribution. As a result, nonparametric tests were used for statistical analysis. Qualitative variables were presented as frequencies and percentages. Quantitative data was presented in the form of medians and interquartile ranges. Comparisons between groups for qualitative variables was performed using Chi Square test. Comparisons between females with GDM and normal glucose tolerance (NGT), for quantitative variables like Vit B12, Vit D, Fol, Insulin and fasting glucose were performed by Mann-Whitney U test. Homeostasis

model assessment for insulin resistance (HOMA-IR) served as a measure of insulin resistance. To explore the relationship between various vitamins and fasting blood glucose levels, multiple linear regression was utilized, adjusting for gestational age and gravidity. A Receiver Operating Characteristic (ROC) curve was employed to determine appropriate cutoff points for serum levels of Vitamins B12, D, folate, insulin, and fasting glucose, indicating a potential association with GDM. Statistical significance was set at a p -value ≤ 0.05 .

RESULTS

Among the 162 females, 108 had normal glucose tolerance (NGT, Group-A), while those with gestational diabetes mellitus (GDM, Group-B) comprised of 54 women. The median age was 28(24-31) years. The age in the study sample ranged from 20-40 years. Median BMI was 26 (23.5-29.7) kg/m², while the range was from 17-36 kg/m². Comparison of Vit B12, Vit D, Fol and other quantitative variables among women with GDM and NGT are presented in Table-I.

Within the studied population, 39% were categorized as overweight, while 25% fell into the obese category. The median (interquartile range) concentrations of serum Folate, Vit B12 and Vit D were 32(22-44) nmol/L, 183(152-219) pmol/L and 55(46-65) respectively. Females with GDM had lower levels of Vit B12 (156 pmol/L vs 198 pmol/L), Vit D (48 nmol/L vs 59 nmol/L) and higher levels of serum Folate (40 nmol/L vs 29 nmol/L). The difference in serum values of Folate ($p=0.003$), Vit B12 ($p=0.001$) and Vit D ($p=0.002$) between the two groups was statistically significant. About 86% females ($n=140$) either had deficient or insufficient Vit D levels (<75 nmol/L). Vit B12 deficiency <150 pmol/L was observed in 23.4% (38/162) of the study population. Folate excess >45 nmol/L was recorded in 25% (40/162) of the participants. Females with GDM also had high fasting glucose (5.3 mmol/L vs 4.5 mmol/L) and higher insulin levels compared to women with NGT (12.7 μ U/ml vs 6.0 μ U/ml). Median HOMA-IR value in females with GDM were also higher, 2.90 (1.3-5.5) as compared to females with NGT, 1.30 (0.9-2.1).

In comparison to women without GDM, those diagnosed with GDM tended to be older ($p=0.001$), had a pre-pregnancy weight within the overweight or obese range ($p=0.001$), had previous occurrences of GDM ($p=0.001$), and had a greater prevalence of first-degree family history of diabetes ($p=0.001$).

Comparison of qualitative variables between women with NGT and GDM is presented in Table-II.

Multiple linear regression showed there was moderate association of fasting glucose with vit D, vit B12 and Fol ($r^2=0.50$, $p<0.001$).

A Receiver Operating Characteristic (ROC) curve was constructed to determine optimal cutoff points for serum levels of Vitamins B12, D, folate, insulin, and fasting glucose as potential indicators of GDM. For Fol area under curve (AUC) was 0.748 (Figure-2). A level of 28.5 nmol/L had sensitivity of 85% and specificity of 50 % for predicting GDM. AUC for Vit B12 and Vit D were 0.262 and 0.290 respectively, so levels were not calculated. For Insulin AUC was 0.835. A level of 7.9 μ U/mL had sensitivity of 80% and specificity of 70 % for predicting GDM. For Fasting Glucose, AUC was 0.885. A level of 4.75 mmol/L had sensitivity of 87% and specificity of 70 % for predicting GDM.

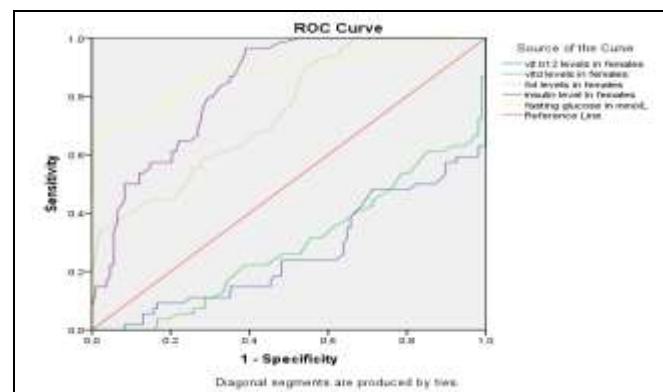


Figure-2: ROC curves for Folate, Vit B12, Vit D, Glucose (F) and Insulin (F)

DISCUSSION

In our study, we conducted a comparative analysis of Vitamin D, Vitamin B12, and Folate levels between women with and without GDM, exploring their association with fasting glucose levels. Our findings indicated a higher incidence of GDM among individuals exhibiting elevated folate levels and decreased levels of Vitamin B12 and Vitamin D.

In our study population, 38 women (23.4%) experienced Vitamin B12 deficiency i.e levels <150 pmol/L. The results are slightly lower according to another study, in which 32.9% of women had Vit B12 insufficiency.¹¹ The relationship between serum Vitamin B12 levels and GDM has been examined in small prospective studies, showing a correlation between lower Vitamin B12 levels, higher HOMA IR values, and GDM at 28-30 weeks of gestation.^{12,13} One

Table-I: Comparison of Basic Characteristics, Vitamin B12, Vitamin D, Folate levels across Groups (n=162)

Variables	All patients Median (IQR)	Group-A (n=108) Median (IQR)	Group-B (n=58) Median (IQR)	p-value
Age (years)	28.0(24.0-31)	27.0(23.0-31.0)	30.0(25.0-36.0)	0.005
Gravida	2.9(2.0-4.0)	2.9(1.4-3.9)	3.1(1.5-4.7)	0.377
BMI (kg/m ²)	26.0(23.5-29.7)	25.0(21.0-29.0)	29.0(24.0-33.0)	0.001
Glucose (F) (mmol/L)	4.7(4.5-5.1)	4.5(4.3-4.7)	5.3(5.0-5.6)	0.001
Insulin (F) (μU/ml)	8.4(5.6-12.0)	6.0(4.0-11.0)	12.7(7.0-21.0)	0.001
HOMA IR	1.8(1.0-2.7)	1.3(0.9-2.1)	2.9(1.3-5.5)	0.001
Vit B12 (pmol/L)	183.0(152.0-219.0)	198.0(165.0-260.0)	156.0(114.0-202.0)	0.001
Folate (nmol/L)	32.0(22.0-44.0)	30.0(18.0-39.0)	40.0(28.0-52.0)	0.003
Vit D (nmol/L)	55.0(46.0-65.0)	58.0(51.0-70.0)	48.0(32-59)	0.002

Table-II: Comparison of Characteristics across Groups (n=162)

Variables	Group-A (n=108) n (%)	Group-B (n=58) n (%)	p-value
Previous history of GDM	14 (42.4%)	19 (57.6%)	0.001
Family history of Diabetes	37 (51.4%)	35 (48.6%)	0.001
BMI >30kg/m ²	18 (43.9%)	23 (56.1%)	0.001
History of Htn and proteinuria	12 (48.0%)	13 (52.0%)	0.012
Age >35 years	11 (52.4%)	10 (47.6%)	0.001
History of Macrosomia	5 (71.4%)	2 (28.6%)	0.81

study reported a twofold increase in GDM risk with Vitamin B12 levels <203.3 pg/ml.¹⁴ A UK based study by Soukumar et al. has demonstrated that vitamin B12 deficiency increases the risk of GDM by 2.59 times.¹⁵ Our study also demonstrated that females with GDM had lower Vit B12 levels (158 pmol/L vs 225 pmol/L). Multiple studies have reported associations between increased Vitamin B12 levels and reduced GDM risk.^{16,17} Various mechanisms have been proposed to explain Vitamin B12's protective effects against diabetes, such as its impact on homocysteine metabolism and its potential association with insulin resistance.¹⁸

In our study folate excess >45 nmol/L was seen in 25% (40/162) of the study participants. Females with GDM were having higher levels of serum Folate (40 nmol/L vs 28 nmol/L). Studies examining periconceptional Folic Acid Supplementation (FAS) and subsequent GDM risk have presented conflicting results. While one study reported a lowered GDM risk with FAS before conception, contradictory findings were observed for FAS during early pregnancy in another study.^{16,17} A case-control study highlighted a higher risk of GDM with a higher serum folate/vitamin B12 ratio at 24–28 weeks of gestation.¹⁹ Sobczynska-Malefora et al. reported an 11% increased risk of GDM among women with higher serum FA concentrations, even after adjusting for various factors.²⁰

In our study vitamin D insufficiency/deficiency was very high. About 86% females either had deficient or insufficient vit D levels (<75 nmol/L). Numerous prior studies have highlighted the prevalence of vitamin D insufficiency in pregnant women, ranging from 33% in Australia to 70.6% in Iran. Ethnicity-based data suggests lower 25OHD levels during pregnancy in Asian women compared to European and American counterparts.²⁰⁻²²

In our investigation, we observed diminished 25OHD levels in the GDM group compared to the NGT group, indicating an association between low vitamin D levels and GDM. Additionally, we noted an inverse relationship between serum 25OHD and insulin levels among GDM patients, aligning with findings in other ethnic populations. Studies by Maghbooli *et al.*, and Zhang *et al.* reported a higher prevalence of low vitamin D levels in GDM patients during the second trimester (at week 16; 25OHD <50 nmol/L: 33% vs. 14%) and third trimester (between weeks 24-28; 25OHD <12.5 nmol/L: 44.2% vs. 23.5%).^{12,13} Conversely, an Indian study suggested that while vitamin D deficiency (VDD) was prevalent in Indian mothers, it wasn't directly linked to GDM.¹⁴

LIMITATIONS OF STUDY

Our study had certain limitations. There was a lack of preconceptional data on Folate, Vitamin D, and Vitamin B12 levels, along with detailed dietary habits. Additionally, our cohort primarily comprised women from low-middle to

middle socioeconomic backgrounds, likely having poor nutritional statuses before pregnancy. Furthermore, a single measurement of serum Folate, B12, and 25OHD levels in the third trimester may not wholly represent their status throughout pregnancy.

CONCLUSION

Women with Gestational Diabetes Mellitus had comparatively higher serum folate levels and lower levels of vitamins B12 and D as compared to women without GDM, and there is an association of these vitamins with GDM.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

NUK & AS: Data acquisition, data analysis, critical review, approval of the final version to be published.

MS & MZR: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

HA: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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