National Board of Examinations - Journal of Medical Sciences
Volume 4, Issue 7, Pages 1044–1057, July 2026
DOI 10.61770/NBEJMS.2026.v04.i07.005

ORIGINAL ARTICLE

Association of Maternal Cobalamin Status in Gestational Diabetes Mellitus: A Prospective Cross-Sectional Study

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Accepted: 19-June-2026 / Published Online: 2-July-2026

Abstract

Background: Gestational Diabetes mellitus is the most common complications affecting pregnancy which impacts the maternal as well as foetal outcome. Maternal obesity is one among the various risk factors for GDM which can be contributed by Cobalamin (Vitamin B12) deficiency. So, we aimed to measure Serum Cobalamin levels in GDM mothers and compared with Non GDM mothers. **Materials and Methods:** We conducted a Prospective cross - sectional study including 50 antenatal mothers with GDM and 50 healthy non GDM antenatal controls. We measured Serum cobalamin levels, OGCT, FBS, BMI in both the groups and compared using independent t test and chi square tests. The correlation between serum cobalamin and other parameters were done by Pearson correlation and ROC curve analysis was done to measure the cut off of Cobalamin in predicting GDM. **Results:** Maternal serum cobalamin was significantly lower in GDM mothers than controls (246.78 ± 60.52 pg/ml vs 410.89 ± 145.9 pg/ml p<0.001). OGCT, FBS and BMI were significantly increased in GDM mothers than controls (p value <0.001). OGCT and FBS showed significant negative correlation with Cobalamin (p value <0.001). ROC curve analysis of serum cobalamin showed cut-off threshold value as 301.7 pg/ml with AUC of 0.954 (95% C.I = 0.892-0.986) with a statistically significant p value (p < 0.001). **Conclusion:** Low Maternal serum Cobalamin levels are significantly associated with GDM and it can be utilised as a biomarker to indicate gestational diabetes in pregnant women. Vit B12 supplements can be added to high-risk antenatal mothers.

Keywords: Gestational Diabetes mellitus, Serum Cobalamin, Oral Glucose Challenge Test, Maternal age, Body Mass index

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Graphical Abstract

Association of Maternal Cobalamin status in gestational diabetes mellitus: A Prospective cross-sectional study

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Background
Gestational diabetes mellitus (GDM) is a common pregnancy complication associated with adverse maternal and fetal outcomes. Emerging evidence suggests that maternal vitamin B12 deficiency may contribute to obesity, insulin resistance, and GDM. This study aimed to evaluate the association between maternal vitamin B12 levels and GDM by comparing affected women with healthy antenatal mothers.

Objectives To study the association of maternal Vitamin B12 levels with GDM and to compare with healthy antenatal mothers.

Methods
We conducted a Prospective cross - sectional study including 50 antenatal mothers with GDM and 50 healthy non GDM antenatal controls. We measured Serum cobalamin levels, OGCT, FBS, BMI in both the groups and compared using independent t test and chi square tests. The correlation between serum cobalamin and other parameters were done by Pearson correlation and ROC curve analysis was done to measure the cut off of Cobalamin in predicting GDM.

Results

Serum cobalamin (in pg/mL)	Group 1- GDM group	Group 2 Control group	Total	Fischer exact test value	P value
< 200	14 (28%)	0	14 (14%)		
200-299	26 (52%)	3 (6%)	29 (29%)	61.836	<0.001*
≥ 300	10 (20%)	47 (94%)	57 (57%)		

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Conclusions Low Maternal serum Cobalamin levels are significantly associated with GDM and it can be used as a biomarker to indicate gestational diabetes in pregnant women. Vit B12 supplements can be added to high-risk antenatal mothers.

Introduction

Gestational Diabetes mellitus is the most common complications affecting pregnancy. It is categorized by varying degrees of carbohydrate intolerance and hyperglycemia that is observed only during pregnancy [1]. The prevalence of GDM was estimated to be approximately 14% globally. Its prevalence is higher in Asian countries accounting for about 20.8% [2]. Both the mother and the fetus are impacted by the effects of GDM. In Fetus, Macrosomia, preterm birth, stillbirth, shoulder dystocia, and unexplained fetal deaths are just a few of the pregnancy complications that can be caused by gestational diabetes mellitus. Poor glycemic control during pregnancy, vulnerability to infections, cephalo-pelvic disproportion, and type II diabetes in later life are all maternal consequences [3–5]. It has been observed that the adverse effects for the fetus might have happened as early as around 20th week which is well prior to diagnose GDM [6]. It is very crucial to identify the risk factors and prevent GDM to decrease adverse fetal and maternal outcomes.

Maternal obesity is one of the common modifiable risk factors. Maternal Vitamin B12 levels are inversely associated with maternal BMI [7]. It has been hypothesized that low vitamin B12, at a cellular level can cause adipocyte dysfunction and obesity-related complications [8]. Vitamin cobalamin (vitamin B12) along with folic acid is an important micronutrient needed for the synthesis of protein, DNA and lipids, in a series of cellular reactions which is collectively called as one-carbon metabolism [9,10]. The cobalamin vitamin is important for the development of red blood cells, neural growth, and brain myelination [1,11,12]. Homocysteine is converted into the methyl donor, methionine as part of one carbon metabolism, which also requires cofactors vitamin B12 and folate. Furthermore, the alteration of methyl malonyl-CoA to succinyl-CoA in mitochondria requires the coenzyme B12, and in its absenteeism, an accumulation of the previous substance inhibits fatty acid oxidation and encourages lipogenesis [13,14]. According to a recent systematic review, vitamin B12 insufficiency in pregnant women around the world was more common in all trimesters (20%–30%) [15]. Low B12 levels during pregnancy have an impact on the health of the mother and fetus, including maternal obesity, maternal and fetal insulin resistance, and adverse lipid profile in the newborns [16–18].

In a prospective, longitudinal study in early pregnancy, vitamin B12 deficiency independently helped in predicting gestational diabetes mellitus (GDM) and type 2 diabetes (T2D) at 5-years after post-delivery. The fact that this association was mediated by obesity highlights the possible contribution of low vitamin B12 levels to adipocyte dysfunction [16]. Currently, the issue of vitamin B12 deficiency and gestational diabetes mellitus (GDM) has been hardly hinted in medical literature, hence there is a need to study the association of maternal cobalamin levels and GDM. In this study, we aimed to study the association of maternal Vitamin B12 levels with GDM and to compare with health antenatal mothers.

Materials and Methods

Study Participants and Methods

We conducted hospital-based prospective cross-sectional study in Department of Biochemistry after obtaining approval from Institutional Ethics Committee (Madras Medical College) vide letter no: 11052021 dated on 05-05-2021. The study participants were recruited from Obstetrics OPD during the period of March 2021– March 2022. The written informed consent was obtained from the Study Participants after explaining about the study protocol.

Study Population

In this study 100 Antenatal mothers were selected from antenatal OPD based on the inclusion and exclusion criteria and are categorized into 2 groups.

Group I – Cases involving 50 antenatal mothers with Gestational Diabetes Mellitus

Group II – Controls involving 50 apparently healthy antenatal mother

Inclusion criteria

Antenatal mother of age group 20-40 years in > 20 weeks of gestation with OGCT values > 140 mg/dL were included and the diagnosis of GDM was made with 3 sample OGTT using the recommendations given by the International Association of the Diabetes and Pregnancy Study Groups Consensus Panel [19].

Exclusion criteria

Antenatal mother with Pre gestational diabetes mellitus, Multiple pregnancy, Previous history of large babies, chronic hypertension and those received B12 injection in last six months or those on B12 supplements and women undergoing concurrent Metformin pharmacological therapy were excluded from the study.

Study Procedure

Detailed demographic and clinical information, including age, height, weight, and relevant medical history, were recorded for all participants. Following a comprehensive clinical examination, 5 mL of venous blood was collected from the antecubital vein under strict aseptic conditions. The blood sample collected in a serum tube was allowed to clot, and serum was separated by centrifugation at 3000 rpm for 15 minutes. The separated serum was used for the estimation of overnight (atleast 8 hrs) fasting blood glucose, urea, and creatinine levels. Approximately 2 mL of serum was aliquoted and stored at −20°C until further analysis of serum cobalamin levels.

Study Parameters

Serum cobalamin levels were estimated using the Electrochemiluminescence Immunoassay (ECLIA) technique on the Cobas e 411, a fully automated immunoassay analyzer. Fasting blood Sugar (FBS) was analyzed by the hexokinase endpoint method. Blood urea estimation was performed using the kinetic urease–glutamate dehydrogenase method, while serum creatinine was measured by Jaffe's kinetic method with an IDMS-traceable calibrator. All biochemical analyses were carried out in accordance with the laboratory's standard operating procedures, and internal quality control measures were employed throughout the study period. Body mass index (BMI) was calculated for each participant using the recorded height and weight measurements.

Statistical analysis

Continuous variables were summarized as mean ± standard deviation (SD) or median with interquartile range (IQR), as appropriate. The normality of data distribution was assessed using the Kolmogorov–Smirnov test. Comparative analyses were performed using the independent t-test for continuous variables and the chi-square test for categorical variables. The relationship between serum vitamin B12 levels and other study parameters was evaluated using Pearson's correlation analysis, while Receiver Operating Characteristic (ROC) curve analysis was carried out to assess the diagnostic performance of the studied variables. Statistical analyses were conducted using SPSS software version 21.0, and a p-value less than 0.05 was considered statistically significant.

Results

In this study the GDM group, 68% were primi, 16% were 2nd gravida 6% were 3rd gravida, 8% were 4th gravida and 2% were 5th gravida. In the controls, 64% were primi, 24% were 2nd gravida and 12% were 3rd gravida. There was no statistically significant difference between the two groups by Fischer exact test. In the GDM group, 8% were less than 28 weeks, 78% were from 29 to 34 weeks and 14% were more than 34 weeks. In the controls, 6% were less than 28 weeks, 66% were from 29 to 34 weeks and 28% were more than 34 weeks. There was no statistically significant difference between the two groups by Fischer exact test (Table 1).

Table 1. Clinical Parameters of the study participants in both the groups (n=100)

Variables		Group I - GDM	Group II - Control	Total	Fischer exact test value	P value
Gravida	Primi	34 (68%)	32 (64%)	66 (66%)	6.461	0.131
	2	8(16%)	12 (24%)	20 (20%)		
	3	3 (6%)	6 (12%)	9 (9%)		
	4	4 (8%)	0	4(4%)		
	5	1 (2%)	0	1 (1%)		
Gestational age	≤ 28 weeks	4 (8%)	3 (6%)	7 (7%)	2.980	0.259
	29 to 34 weeks	39 (78%)	33 (66%)	72 (72%)		
	>34 weeks	7 (14%)	14 (28%)	21 (21%)		
Family history of DM	Yes	28 (56%)	8 (16%)	36 (36%)	17.361	**<0.001***
	No	22 (44%)	42 (84%)	64 (64%)		

In GDM group, 56% had a family history of Diabetes and 44% did not have a family history of Diabetes. In controls group, 16% had a family history of Diabetes and 84% did not have a family history of Diabetes. The difference between the two groups with respect to family history of Diabetes mellitus was statistically significant by Chi square test.

The mean age of the study participants in Gestational diabetes mellitus group was 25.06 ± 4.24 years and the mean age of study participants in control group was 23.84 ± 3.67 years. There was no statistically significant difference between the two groups by independent t test. The two groups are comparable with respect to age of the study participants. The mean weight and BMI of the participants in GDM group was significantly higher than in controls (p Value < 0.001). Systolic BP was significantly higher in GDM mothers than control mothers and no statistically significant difference is seen in diastolic BP. OGCT values were significantly higher in GDM mothers than Non GDM mothers (p Value < 0.001). Fasting blood sugar was significantly higher in GDM mothers than control mothers (p Value < 0.001). Serum Cobalamin levels were significantly lower in GDM mothers than Non GDM mothers (246.78 ± 60.52 Vs 410.89 ± 145.9, p Value < 0.001) (Table 2).

Table 2. Study parameters in in both the groups (n=100)

Parameters	Group I – GDM	Group II -Control	T test value	P value
Age (in Yrs)	25.06 ± 4.24	23.84 ± 3.67	1.536	0.128
Weight (kg)	59.20 ± 5.35	55.08 ± 3.57	4.524	**<0.001***
BMI (kg/m^2)	23.87 ± 1.54	22.68 ± 1.07	4.445	**<0.001***
Systolic BP (mm Hg)	112.92 ± 5.99	108.64 ± 7.85	30.64	**0.003***
Diastolic BP (mm Hg)	73.16 ± 5.17	71.28 ± 4.97	0.118	0.906
OGCT (mg/dL)	160.74 ± 15.37	113.90 ± 14.27	0.852	**<0.001***
FBS (mg/dl)	137.44 ± 26.71	88.90 ± 12.11	11.703	**<0.001***

Serum cobalamin (pg/mL)	246.78 ± 60.52	410.89 ± 145.9	7.344	**<0.001***
Blood urea(mg/dl)	26.30 ± 3.91	23.98 ± 4.59	2.718	**0.008***
Serum creatinine (mg/dL)	0.828 ± 0.185	0.840 ± 0.199	0.312	0.756

*p values < 0.05 are considered statistically significant.

In renal function parameters, Blood Urea levels were significantly higher in GDM mother with p value of 0.008 and serum creatinine levels showed no statistical difference between GDM mothers and control. We have compared both GDM and Control group based on the serum cobalamin levels and the difference between two groups with respect to serum cobalamin were statistically significant by Fischer Exact test (p value < 0.001) (Table 3 and Figure 1).

Table 3. Serum cobalamin of study participants

Serum cobalamin (in pg/mL)	**Group 1- GDM group**	**Group 2 Control group**	**Total**	**Fischer exact test value**	**P value**
< 200	14 (28%)	0	14 (14%)	61.836	**<0.001***
200-299	26 (52%)	3 (6%)	29 (29%)		
≥ 300	10 (20%)	47 (94%)	57 (57%)		

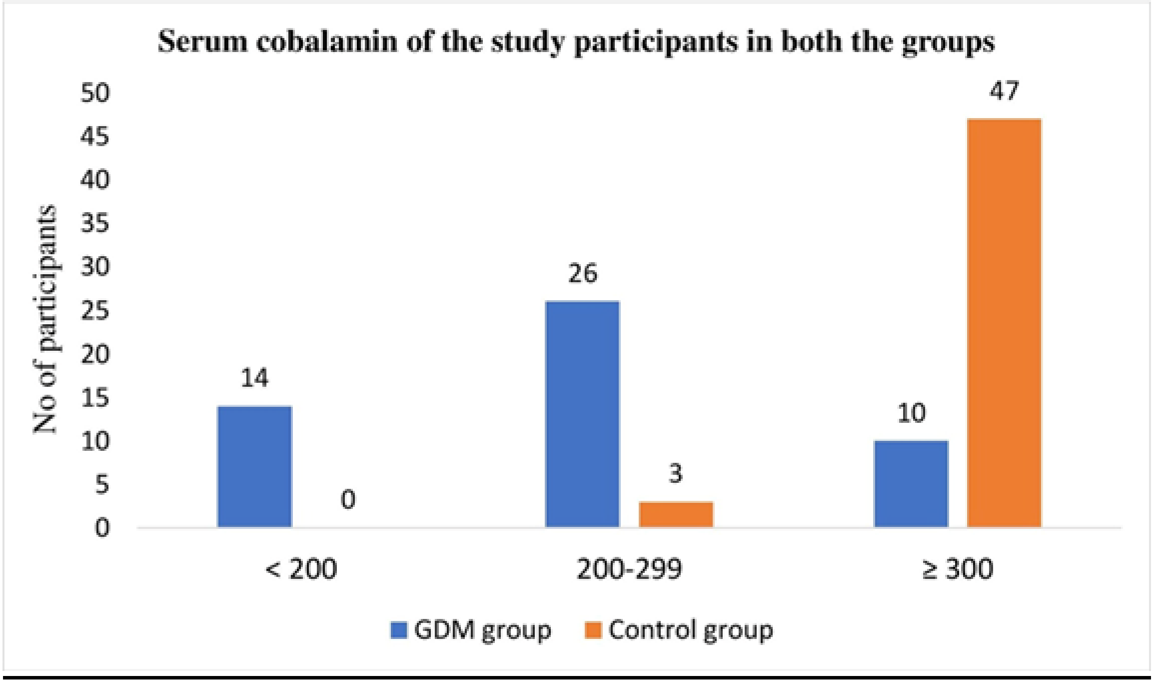

Figure 1. Serum cobalamin of the study participants in both the groups

We have correlated the study parameters with Serum cobalamin levels. OGCT and FBS showed statistically significant strong negative correlation (r= -0.764, P < 0.001*) and (r= -0.728, P < 0.001*), respectively (Figures 2 and 3). There was no correlation between Serum cobalamin with age, gestational age, BMI, Blood urea and serum creatinine (Table 4).

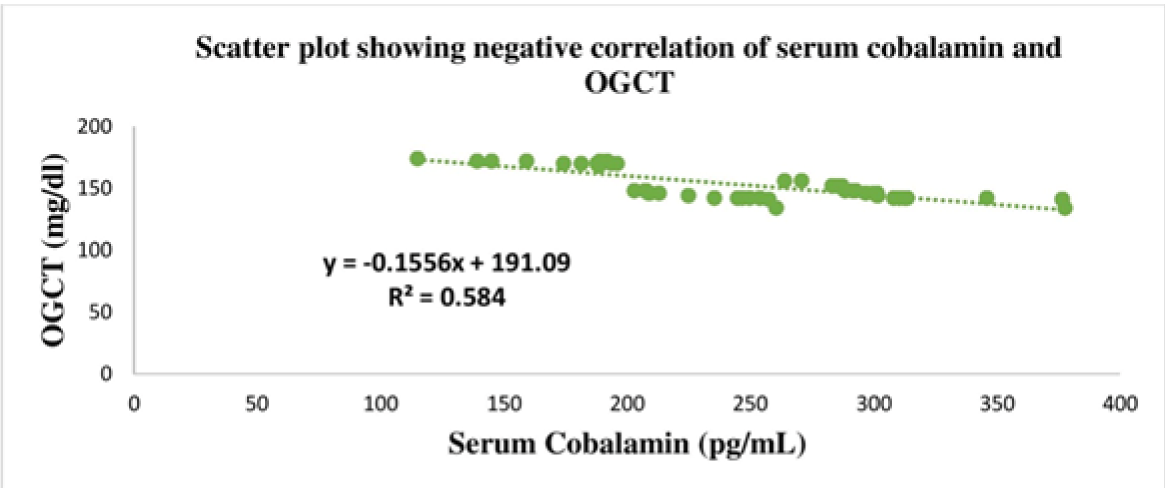

Figure 2. Scatter plot showing negative correlation of serum cobalamin and OGCT

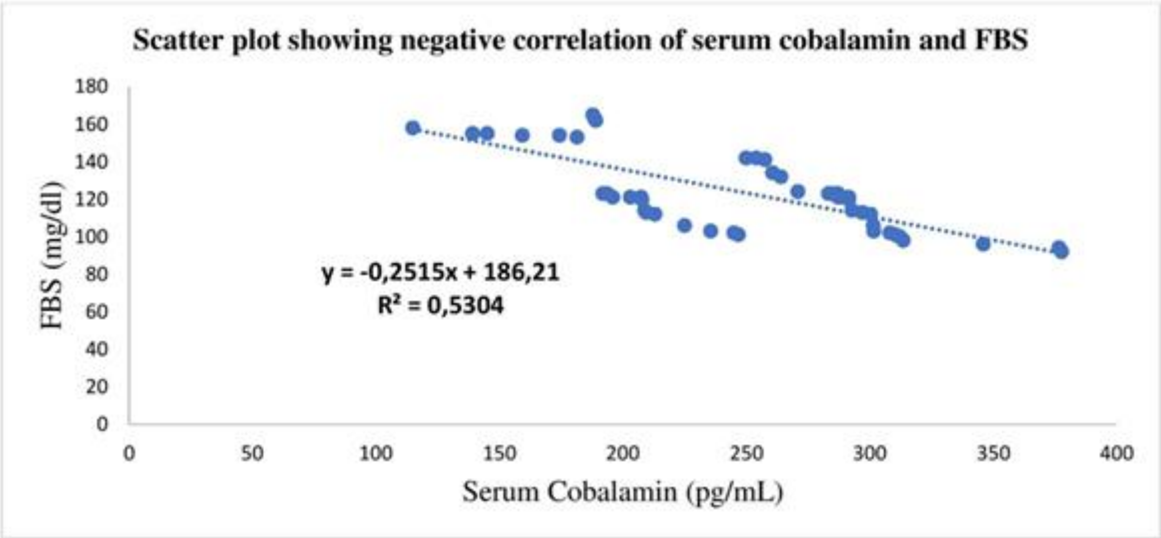

Figure 3. Scatter plot showing negative correlation of serum cobalamin and FBS

Table 4. Correlation of serum cobalamin in GDM group

Parameter	**Pearson correlation coefficient**	**P value**
Age	-0.064	0.660
Gestational age	-0.225	0.116
BMI	-0.135	0.350
OGCT	-0.764	**<0.001***
FBS	-0.728	**<0.001***
Blood urea	0.056	0.699
S. Creatinine	0.064	0.661

The Receiver Operating Characteristic (ROC) curve analysis of serum cobalamin showed cut-off threshold value as 301.7 (95% C.I = 287.2 to 313.6), with a sensitivity of 86%, and a specificity of 92%. The area under the curve (AUC) was 0.954 (95% C.I = 0.892-0.986) with a statistically significant p value ($p < 0.001$) (Table 4).

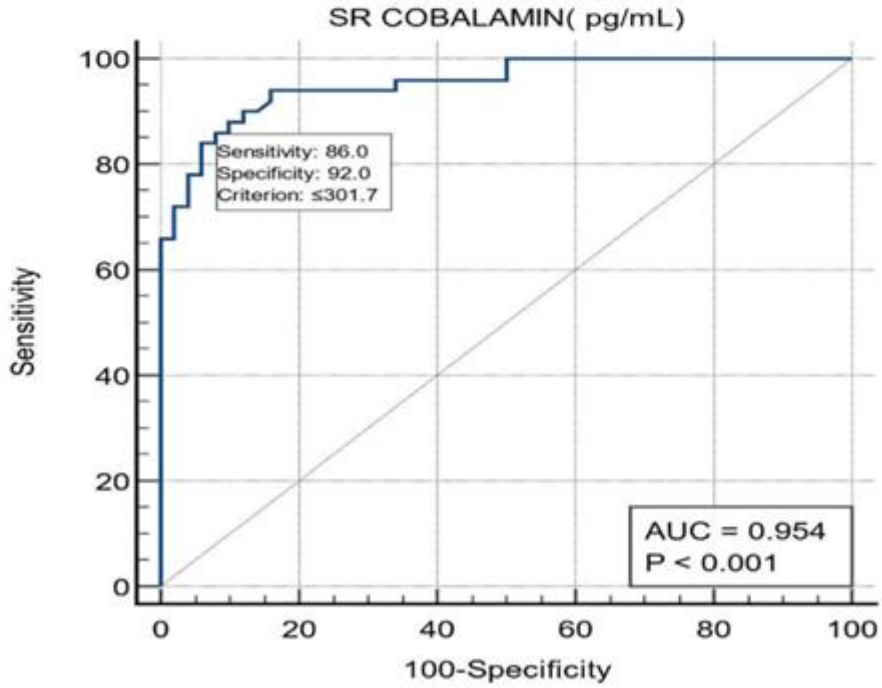

Figure 4. ROC curve for Serum Cobalamin levels

Discussion

We have conducted a hospital based cross sectional study to find the serum cobalamin levels in GDM and non GDM Antenatal mothers. We have found that maternal serum cobalamin levels were low in GDM mothers than non GDM mothers and a negative correlation was seen with cobalamin and OGCT as well FBS levels which was similar to previous studies conducted by Saher Fatima1 et al. [20] and Aesha Sadaf Rizwan et al. [21]. With a cut off of 300 pg/ml, our study has found 40 (80%) of 50 GDM mother had low cobalamin and only 3(6%) out of 50 non GDM mothers. The correlation we found in our study was in line with several international studies from various ethnicities and countries. Since metformin therapy was an exclusion criterion in the current study, the data hint to a more general vitamin B_{12} insufficiency. Prior studies had shown that hypovitaminosis B_{12} was common in GDM as a result of metformin therapy. Maternal obesity being a modifiable risk factor for GDM as well as Insulin resistance, we have calculated BMI in both groups and it showed significant difference between both groups. Krishnaveni et al. [22] found a link between insulin resistance, obesity, serum cobalamin insufficiency and GDM.

The Receiver Operating Characteristic (ROC) curve analysis of serum cobalamin showed cut-off threshold value as 301.7 pg/ml with a significant AUC which was higher than optimal cutoff point for indicating gestational diabetes noted by Ambreen Butt et al. [23] (301.7 Vs 113 pg/mL). The exact mechanism by which low maternal B12 contributing to the development of GDM is not well known. According to Adaikalakoteswari et al. [24], low cobalamin levels during pregnancy can increase the levels of adipose-derived circulating microRNAs, which may contribute to the development of insulin resistance. Low B12 levels will lead to hyperhomocysteinemia which in turn can have harmful effects on pancreatic β-cell metabolism and insulin secretion [25]. To support these facts, a trial conducted by Setola et al. [26] showed an improvement in insulin resistance after supplementation with Cobalamin. Therefore, it is considered that there are multiple contributing reasons to GDM, with B_{12} deficiency being one of the major ones.

Limitations

The sample size of the present study was relatively small. However, the participants were recruited from a similar socioeconomic background and ethnic group, and no significant difference in age was observed among them, thereby minimizing potential confounding effects related to these factors. Dietary habits and preconception vitamin B12 status of the participants were not assessed, which may have influenced the study findings. Additionally, biochemical markers such as homocysteine and methylmalonic acid, which are considered more sensitive and specific indicators of vitamin B12 deficiency, were not measured. The inclusion of these parameters could have provided a more comprehensive assessment of vitamin B12 status.

Conclusions

Maternal serum cobalamin levels may serve as a useful biomarker for identifying the risk of gestational diabetes mellitus (GDM) during pregnancy. Pregnant women with low serum cobalamin concentrations can be monitored more closely, enabling early intervention and reducing the risk of adverse outcomes for both the mother and the fetus. Since GDM is often diagnosed only after the first trimester, the fetus may already have been exposed to elevated maternal glucose levels and associated metabolic disturbances during a critical period of development. Such exposure may contribute to fetal metabolic programming through epigenetic modifications, thereby increasing susceptibility to various chronic metabolic disorders later in life.

Early screening, timely diagnosis, and appropriate management of GDM can help prevent these unfavorable maternal and fetal outcomes. Furthermore, vitamin B12 supplementation should be considered for populations at risk of deficiency, particularly in developing countries where malnutrition remains prevalent. Adequate vitamin B12 status is important for optimal neurological development and may influence epigenetic mechanisms that affect long-term health. Therefore, serum vitamin B12 levels should be taken into account when developing predictive and prognostic models for GDM, especially among women with established risk factors for the condition.

Authors' Contributions

SP has contributed to the conceptualization and definition of the intellectual content of the manuscript, design of the study and Manuscript preparation. MAV contributed to the literature search, manuscript editing, and manuscript review. TU contributed towards data acquisition Statistical analysis, Manuscript review and editing. SP will act as the corresponding author of the manuscript

Data availability statement

The datasets generated and analysed in this study are available from the corresponding author on reasonable request. They are not publicly shared because they contain sensitive information that could indirectly identify participants.

Ethical approval

This study has been approved by the Institution Ethics Committee, Madras Medical College, Chennai. Ref.No:11052021 dated: 05-05-2021.

Informed Consent

Written informed consent was obtained from all participants after explaining the study procedures, potential risks and benefits. Consent covered both participation and publication of anonymised findings, with assurance of confidentiality and data privacy.

Conflicts of interest

The authors declare that they do not have conflict of interest.

Funding

No funding was received for conducting this study.

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