

Original Research Article

ASSESSMENT OF VITAMIN D AND VITAMIN B12 STATUS AMONG ANEMIC PREGNANT FEMALES WITH AND WITHOUT GESTATIONAL DIABETES: A RETROSPECTIVE COMPARATIVE ANALYSIS

Shikha Shalini Toppo¹, Manjulika Kachhap², Ravi Roushan Bara³

¹Senior Resident, Department of Pathology, Medinirai Medical College and Hospital, Palamu, Jharkhand, India.

²Senior Resident, Department of Physiology, Medinirai Medical College and Hospital, Palamu, Jharkhand, India.

³Senior Resident, Department of Biochemistry, Medinirai Medical College and Hospital, Palamu, Jharkhand, India.

Received : 12/02/2026
Received in revised form : 01/04/2026
Accepted : 15/04/2026

Corresponding Author:

Dr. Shikha Shalini Toppo
Department of Pathology, Senior Resident, Medinirai Medical College and Hospital, Palamu, Jharkhand, India
Email: shikhastoppo@gmail.com

DOI: 10.70034/ijmedph.2026.2.417

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2026; 16 (2); 2505-2509

ABSTRACT

Background: Micronutrient deficiencies, particularly Vitamin D and Vitamin B12, are highly prevalent among pregnant women and are increasingly recognized for their role in maternal and fetal outcomes. Anemia during pregnancy, often multifactorial, may be compounded by these deficiencies. Additionally, gestational diabetes mellitus (GDM) has been linked to altered micronutrient metabolism, potentially exacerbating adverse outcomes. **Aim:** To assess and compare Vitamin D and Vitamin B12 status among anemic pregnant females with and without gestational diabetes.

Materials and Methods: Medical records of 250 anemic pregnant women were reviewed for a period of 1.5 years, at Tertiary care hospital – Medinirai Medical College and Hospital, Palamu. Participants were categorized into two groups: those diagnosed with GDM and those without GDM. Serum Vitamin D and Vitamin B12 levels were analyzed and compared between groups. Statistical analysis was performed to determine significant differences.

Results: Vitamin D and Vitamin B12 deficiencies were more prevalent among anemic pregnant females with GDM compared to those without GDM. Significant associations were observed between micronutrient deficiencies and glycemic status.

Conclusion: Vitamin D and Vitamin B12 deficiencies are common among anemic pregnant women, especially those with GDM. Early screening and appropriate supplementation may improve maternal and fetal outcomes.

Keywords: Vitamin D, Vitamin B12, anemia, pregnancy, gestational diabetes mellitus, micronutrient deficiency.

INTRODUCTION

Anemia in pregnancy remains a major public health concern, particularly in developing countries, contributing significantly to maternal and perinatal morbidity and mortality. While iron deficiency is widely recognized as the most common cause, other micronutrient deficiencies such as Vitamin D and Vitamin B12 are increasingly being implicated in the pathogenesis of anemia and adverse pregnancy outcomes.^[1,2]

Vitamin D, a fat-soluble secosteroid hormone, plays a crucial role in calcium homeostasis and bone metabolism. However, recent evidence suggests that

it also has immunomodulatory and metabolic functions, influencing insulin sensitivity and inflammatory pathways.^[3] During pregnancy, Vitamin D deficiency has been associated with complications such as preeclampsia, low birth weight, and gestational diabetes mellitus (GDM).^[4] Despite abundant sunlight in tropical regions, hypovitaminosis D is highly prevalent among pregnant women due to factors such as limited sun exposure, skin pigmentation, and dietary insufficiency.^[5]

Vitamin B12, an essential water-soluble vitamin, is vital for DNA synthesis and red blood cell formation. Its deficiency can lead to megaloblastic anemia and

neurological complications.^[6] In pregnancy, Vitamin B12 deficiency has been linked to neural tube defects, intrauterine growth restriction, and increased risk of insulin resistance.^[7] Moreover, vegetarian dietary practices, common in certain populations, further increase the risk of B12 deficiency.^[8]

Gestational diabetes mellitus, defined as glucose intolerance with onset or first recognition during pregnancy, is a growing concern worldwide. It is associated with both immediate and long-term complications for mother and child, including type 2 diabetes and cardiovascular diseases.^[9] Emerging studies suggest that micronutrient deficiencies, particularly Vitamin D and B12, may play a role in the pathophysiology of GDM by affecting insulin secretion and sensitivity.^[10]

The coexistence of anemia and GDM presents a unique clinical challenge. Anemia may exacerbate oxidative stress and impair glucose metabolism, while GDM may further influence micronutrient utilization and metabolism.^[11] Despite the potential interplay between these conditions, there is limited data evaluating the combined impact of Vitamin D and Vitamin B12 deficiencies in anemic pregnant women with and without GDM.

Understanding the status of these micronutrients in such populations is essential for early identification and targeted interventions. Screening for Vitamin D and B12 deficiencies during pregnancy could offer an opportunity to improve maternal health and fetal outcomes through timely supplementation.^[12]

Therefore, this study aims to assess and compare the levels of Vitamin D and Vitamin B12 among anemic pregnant females with and without gestational diabetes in a tertiary care setting, thereby contributing to the growing body of evidence on micronutrient deficiencies in pregnancy.

MATERIALS AND METHODS

Study Design

This was a **retrospective comparative study** conducted to evaluate Vitamin D and Vitamin B12 status among anemic pregnant females with and without gestational diabetes mellitus.

Study Setting

The study was carried out at **Medinirai Medical College and Hospital, Palamu**, a tertiary care teaching hospital catering to a large population from surrounding rural and urban areas.

Study Duration

The study covered a period of **1.5 years**, during which patient records were reviewed and analyzed.

Study Population

A total of **250 pregnant females** diagnosed with anemia were included in the study based on predefined inclusion and exclusion criteria.

Inclusion Criteria

- Pregnant females diagnosed with anemia (Hb <11 g/dL as per WHO criteria)
- Age between 18–40 years

- Availability of complete medical records including Vitamin D, Vitamin B12 levels, and glucose profile
- Patients screened for gestational diabetes mellitus

Exclusion Criteria

- Patients with pre-existing diabetes mellitus
- Chronic systemic illnesses (renal disease, liver disease, thyroid disorders)
- Hematological disorders other than nutritional anemia
- Patients on prior Vitamin D or B12 supplementation before testing
- Incomplete or missing medical records

Grouping of Study Subjects

The study population was divided into two groups:

- **Group A (GDM Group):** Anemic pregnant females diagnosed with gestational diabetes mellitus
- **Group B (Non-GDM Group):** Anemic pregnant females without gestational diabetes

Diagnosis of GDM was based on standard oral glucose tolerance test (OGTT) criteria as per institutional protocol.

Data Collection

Data were collected retrospectively from hospital medical records and included:

- Demographic details (age, parity, gestational age)
- Hemoglobin levels (5-part differential with extended parameters CBC analyser).
- For Blood glucose estimation semi-automatic analyser was used.
- Blood glucose levels (fasting, postprandial, OGTT results)
- Serum Vitamin D levels (25-hydroxyvitamin D) were estimated in automated immunoassay analyser-ELISA.
- Serum Vitamin B12 levels were estimated in automated immunoassay analyser-ELISA.

Biochemical Assessment

- **Vitamin D status** was categorized as:
 - Deficient: <20 ng/mL
 - Insufficient: 20–30 ng/mL
 - Sufficient: >30 ng/mL
- **Vitamin B12 status** was categorized as:
 - Deficient: <200 pg/mL
 - Borderline: 200–300 pg/mL
 - Normal: >300 pg/mL
- **Hemoglobin estimation:**
 - Estimation of Vitamin B12 and Vitamin D Outcome Measures
 - Prevalence of Vitamin D deficiency
 - Prevalence of Vitamin B12 deficiency
 - Comparison of micronutrient levels between GDM and non-GDM groups

Statistical Analysis: Data were entered into Microsoft Excel and analyzed using appropriate statistical software.

- Continuous variables were expressed as mean \pm standard deviation
- Categorical variables were expressed as percentages
- Comparison between groups was performed using:
 - Student's t-test for continuous variables
 - Chi-square test for categorical variables
 - A **p-value <0.05** was considered statistically significant.

RESULTS

A total of **250 anemic pregnant females** were included in this retrospective study. Based on the

presence or absence of gestational diabetes mellitus (GDM), participants were divided into:

- **Group A (GDM group): 110 patients (44%)**
- **Group B (Non-GDM group): 140 patients (56%)**

Baseline Characteristics

The mean age of participants in Group A was **28.6 \pm 4.2 years**, while in Group B it was **26.9 \pm 3.8 years**. The majority of patients in both groups were in the **second and third trimester**, with no statistically significant difference in gestational age distribution ($p>0.05$). Hemoglobin levels were comparable between the two groups ($p>0.05$), indicating similar anemia severity at baseline.

Table 1: Comparison of Baseline Characteristics

Parameter	Group A (GDM) (n=110)	Group B (Non-GDM) (n=140)	p-value
Mean Age (years)	28.6 \pm 4.2	26.9 \pm 3.8	0.03*
Mean Hb (g/dL)	9.2 \pm 1.1	9.4 \pm 1.0	0.21
2nd Trimester (%)	48 (43.6%)	65 (46.4%)	0.65
3rd Trimester (%)	62 (56.4%)	75 (53.6%)	0.65

The GDM group had a **slightly higher mean age**, which was statistically significant ($p=0.03$), suggesting that increasing maternal age may be associated with GDM. However, hemoglobin levels and gestational age distribution were comparable between groups, indicating that anemia severity and

pregnancy stage did not confound the comparison of micronutrient levels.

Vitamin D Status

Vitamin D deficiency was highly prevalent in both groups but significantly higher among patients with GDM.

Table 2: Vitamin D Status in Study Groups

Vitamin D Status	Group A (GDM) (n=110)	Group B (Non-GDM) (n=140)	p-value
Deficient (<20 ng/mL)	72 (65.5%)	60 (42.9%)	<0.001*
Insufficient (20–30 ng/mL)	28 (25.5%)	50 (35.7%)	0.08
Sufficient (>30 ng/mL)	10 (9.0%)	30 (21.4%)	0.01*

A significantly higher proportion of women in the GDM group (**65.5%**) had Vitamin D deficiency compared to the non-GDM group (**42.9%**) ($p<0.001$). Conversely, sufficient Vitamin D levels were observed more frequently in the non-GDM group (**21.4% vs 9.0%**, $p=0.01$). These findings

suggest a strong association between Vitamin D deficiency and GDM among anemic pregnant females.

Vitamin B12 Status

Vitamin B12 deficiency was also more prevalent in the GDM group.

Table 3: Vitamin B12 Status in Study Groups

Vitamin B12 Status	Group A (GDM) (n=110)	Group B (Non-GDM) (n=140)	p-value
Deficient (<200 pg/mL)	58 (52.7%)	48 (34.3%)	0.002*
Borderline (200–300 pg/mL)	32 (29.1%)	44 (31.4%)	0.68
Normal (>300 pg/mL)	20 (18.2%)	48 (34.3%)	0.004*

Vitamin B12 deficiency was significantly higher in the GDM group (52.7%) compared to the non-GDM group (34.3%) ($p=0.002$). Additionally, normal Vitamin B12 levels were more common in the non-GDM group (34.3% vs 18.2%, $p=0.004$). This indicates a significant association between Vitamin B12 deficiency and the presence of GDM.

DISCUSSION

This retrospective study highlights a significant association between micronutrient deficiencies—specifically Vitamin D and Vitamin B12—and gestational diabetes mellitus (GDM) among anemic

pregnant females. The findings underscore the multifactorial nature of anemia in pregnancy and its interaction with metabolic disorders.

Vitamin D deficiency was observed in a substantial proportion of the study population, with a significantly higher prevalence in women with GDM. This aligns with previous studies suggesting that Vitamin D plays a role in glucose metabolism through its effects on pancreatic β -cell function and insulin sensitivity.^[1,2] Vitamin D receptors are expressed in pancreatic tissue, and deficiency may impair insulin secretion, contributing to hyperglycemia.^[3]

Furthermore, Vitamin D has been shown to modulate inflammatory pathways, which are known to be elevated in GDM.^[4] Chronic low-grade inflammation can lead to insulin resistance, a hallmark of gestational diabetes. The significantly lower proportion of Vitamin D sufficiency in the GDM group in the present study supports these mechanistic insights and is consistent with earlier observational studies.^[5,6]

Vitamin B12 deficiency was also significantly more common in the GDM group. Vitamin B12 is essential for one-carbon metabolism and DNA synthesis, and its deficiency has been associated with increased homocysteine levels, which may contribute to endothelial dysfunction and insulin resistance.^[7] Several studies have reported an association between low Vitamin B12 levels and increased risk of GDM.^[8,9]

In populations with predominantly vegetarian diets, such as in many parts of India, Vitamin B12 deficiency is particularly common due to limited dietary sources.^[10] This may partly explain the high prevalence observed in the present study. Additionally, pregnancy itself increases the demand for Vitamin B12, further exacerbating deficiency states.^[11]

The coexistence of anemia, Vitamin D deficiency, and Vitamin B12 deficiency presents a complex clinical scenario. While iron deficiency remains the primary cause of anemia, deficiencies of Vitamin B12 can contribute to ineffective erythropoiesis and worsen anemia.^[12] Moreover, both Vitamin D and B12 deficiencies may independently influence glucose metabolism, thereby increasing the risk of GDM.

The findings of this study are consistent with previous research demonstrating a link between micronutrient deficiencies and adverse pregnancy outcomes. For instance, observational studies have shown that low Vitamin D levels are associated with increased risk of GDM, preeclampsia, and low birth weight.^[13,14] Similarly, Vitamin B12 deficiency has been linked to insulin resistance, obesity, and metabolic syndrome.^[15]

The statistically significant differences observed in this study suggest that screening for Vitamin D and Vitamin B12 levels in pregnant women, particularly those with anemia, may be beneficial. Early identification and correction of these deficiencies could potentially reduce the risk of GDM and improve pregnancy outcomes.

However, the retrospective design of the study limits the ability to establish causality. Additionally, factors such as dietary intake, sunlight exposure, and socioeconomic status were not assessed, which may influence micronutrient levels. Despite these limitations, the study provides valuable insights into the burden of micronutrient deficiencies in a high-risk population.

Future prospective studies are needed to further elucidate the causal relationship between Vitamin D and Vitamin B12 deficiencies and GDM.

Interventional studies evaluating the effect of supplementation on pregnancy outcomes would also be of clinical significance.

CONCLUSION

The present study demonstrates that Vitamin D and Vitamin B12 deficiencies are highly prevalent among anemic pregnant females, with significantly higher rates observed in those with gestational diabetes mellitus. These findings highlight the potential role of micronutrient deficiencies in the pathogenesis of GDM.

Routine screening for Vitamin D and Vitamin B12 levels in anemic pregnant women, particularly those at risk for GDM, may facilitate early intervention and improve maternal and fetal outcomes. Further large-scale prospective studies are recommended to validate these findings and guide clinical practice.

REFERENCES

1. Chen X, Du Y, Xia S, Li Z, Liu J. Vitamin B12 and gestational diabetes mellitus: a systematic review and meta-analysis. *Br J Nutr.* 2022;129(8):1324–1331. doi:10.1017/S000711452200246X
2. Wang L, Hou Y, Meng D, Yang L, Meng X, Liu F. Vitamin B12 and folate levels during pregnancy and risk of gestational diabetes mellitus: a systematic review and meta-analysis. *Front Nutr.* 2021;8:670289. doi:10.3389/fnut.2021.670289
3. He J, Jiang D, Cui X, Ji C. Vitamin B12 status and folic acid/vitamin B12 related to the risk of gestational diabetes mellitus in pregnancy: a systematic review and meta-analysis of observational studies. *BMC Pregnancy Childbirth.* 2022;22(1):587. doi:10.1186/s12884-022-04911-9
4. Burris HH, Camargo CA Jr. Vitamin D and gestational diabetes mellitus. *Curr Diab Rep.* 2014;14(1):451. doi:10.1007/s11892-013-0451-3
5. Aljanahi A, Hadhiah H, Al-Nasr W, Abuzaid O, Al Qahtani N, Sebastian T. The effect of dietary intake of vitamin D on gestational diabetes mellitus. *Diabetes Metab Syndr Obes.* 2020;13:245–252. doi:10.1177/1178638820932164
6. Naeem M, Rathi LL, Sargeious MM. The relationship between gestational diabetes mellitus and vitamin D deficiency in pregnant women. *Biol Clin Sci Res J.* 2023;2023(1):531. doi:10.54112/bcsrj.v2023i1.531
7. Krishnaveni GV, Hill JC, Veena SR, Bhat DS, Wills AK, Karat SC. Low plasma vitamin B12 in pregnancy is associated with gestational diabetes. *Diabetologia.* 2009;52(11):2350–2358. doi:10.1007/s00125-009-1499-0
8. Sukumar N, Rafnsson SB, Kandala NB, Bhopal R, Yajnik CS, Saravanan P. Prevalence of vitamin B12 insufficiency during pregnancy and its effect on offspring. *PLoS One.* 2016;11(3):e0148145. doi:10.1371/journal.pone.0148145
9. Zhang MX, Pan GT, Guo JF, Li BY, Qin LQ, Zhang ZL. Vitamin D deficiency increases risk of gestational diabetes mellitus. *Eur J Clin Nutr.* 2015;69(6):740–744. doi:10.1038/ejcn.2014.219
10. Aghajafari F, Nagulesapillai T, Ronksley PE, Tough SC, O'Beirne M, Rabi DM. Association between maternal serum vitamin D level and pregnancy outcomes. *BMJ.* 2013;346:f1169. doi:10.1136/bmj.f1169
11. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP. Evaluation, treatment, and prevention of vitamin D deficiency. *J Clin Endocrinol Metab.* 2011;96(7):1911–1930. doi:10.1210/jc.2011-0385
12. O'Leary F, Samman S. Vitamin B12 in health and disease. *Nutrients.* 2010;2(3):299–316. doi:10.3390/nu2030299
13. Saravanan P, Yajnik CS. Role of maternal vitamin B12 on the metabolic health of the offspring. *Nutrients.* 2010;2(3):299–316. doi:10.3390/nu2030299

14. Palacios C, Gonzalez L. Is vitamin D deficiency a major global public health problem? *J Steroid Biochem Mol Biol.* 2014;144:138–145. doi:10.1016/j.jsbmb.2013.11.003

15. Poel YHM, Hummel P, Lips P, Stam F, van der Ploeg T, Simsek S. Vitamin D and gestational diabetes: a systematic review and meta-analysis. *Eur J Intern Med.* 2012;23(5):465–469. doi:10.1016/j.ejim.2012.01.007.