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Original Research Article

# Prevalence of Vitamin B12 Deficiency in Early Pregnancy And Its Association With Preterm Birth: A Prospective Cross-Sectional Study

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**Abstract:**

**Background:** Vitamin B12 deficiency during pregnancy has been associated with various adverse pregnancy outcomes, including preterm birth. This study aimed to investigate the prevalence of vitamin B12 deficiency in early pregnancy and its association with preterm birth among pregnant women attending a tertiary hospital in India.

**Methods:** This prospective cross-sectional study included 236 pregnant women between 14 and 20 weeks of gestation. Serum vitamin B12 levels were measured using the Electro Chemi-Luminescence-Immuno-Assay method, with deficiency defined as levels <200 pg/ml. Gestational age at delivery was recorded, and preterm birth was defined as delivery before 37 completed weeks of gestation. The association between vitamin B12 deficiency and preterm birth was analyzed using the chi-square test.

**Results:** The mean serum vitamin B12 level was 231.0556 pg/ml (SD 104.2277), with 32.63% of the participants being deficient. The prevalence of preterm birth was 15.25%. A significant association was found between vitamin B12 deficiency and preterm birth (p=0.015732), with 50% of the women who had preterm deliveries being deficient in vitamin B12, compared to 29.50% of the women who had term deliveries.

**Conclusion:** This study found a high prevalence of vitamin B12 deficiency in early pregnancy and a significant association between vitamin B12 deficiency and preterm birth among pregnant women in India. The findings highlight the importance of screening for vitamin B12 deficiency in early pregnancy and the need for interventions to improve vitamin B12 status among pregnant women, particularly in developing countries.

**Keywords:** Vitamin B12 Deficiency, Early Pregnancy, Preterm Birth, India, Prospective Study, Cross-Sectional Study, Prevalence, Association.



## Introduction

Vitamin B12, also known as cobalamin, is an essential water-soluble vitamin that plays a crucial role in various physiological processes, including DNA synthesis, red blood cell formation, and neurological function [1]. Vitamin B12 deficiency during pregnancy has been associated with adverse maternal and fetal outcomes, such as neural tube defects, intrauterine growth restriction, and preterm birth [2]. Preterm birth, defined as delivery before 37 weeks of gestation, is a leading cause of neonatal morbidity and mortality worldwide [3].

The prevalence of vitamin B12 deficiency in pregnancy varies widely across different populations and geographical regions. A systematic review and meta-analysis by Sukumar et al. [4] found that the global prevalence of vitamin B12 deficiency in pregnancy ranged from 20% to 30%, with the highest rates observed in the Indian subcontinent, Africa, and South America. In contrast, the prevalence of vitamin B12 deficiency in developed countries, such as the United States and the United Kingdom, was relatively lower, ranging from 5% to 10% [5].

Several factors contribute to the development of vitamin B12 deficiency during pregnancy, including inadequate dietary intake, malabsorption disorders, and genetic factors [6]. Vitamin B12 is primarily found in animal-derived foods, such as meat, poultry, fish, eggs, and dairy products. Vegetarian and vegan diets, which exclude some or all animal products, have been associated with an increased risk of vitamin B12 deficiency [7]. In addition, certain medications, such as metformin and proton pump inhibitors, can interfere with vitamin B12 absorption [8].

The association between maternal vitamin B12 deficiency and preterm birth has been investigated in several studies, with conflicting results. A meta-analysis by Rogne et al. [9] found that low maternal vitamin B12 levels were associated with an

increased risk of preterm birth (odds ratio [OR] 1.21, 95% confidence interval [CI] 0.99-1.49). However, the authors noted that the evidence was of low quality due to the small number of studies and heterogeneity among them.

The potential mechanisms linking vitamin B12 deficiency to preterm birth are not fully understood but may involve the role of vitamin B12 in the one-carbon metabolism pathway. Vitamin B12 acts as a cofactor for the enzyme methionine synthase, which is involved in the conversion of homocysteine to methionine [1]. Disruption of this pathway can lead to hyperhomocysteinemia, which has been associated with various adverse pregnancy outcomes, including preterm birth [10]. Additionally, vitamin B12 deficiency may contribute to the development of anemia, which has been independently associated with an increased risk of preterm birth [3].

Given the potential consequences of vitamin B12 deficiency during pregnancy, early detection and intervention are crucial. However, the optimal timing and method for vitamin B12 screening in pregnancy remain controversial. Some studies have suggested that vitamin B12 status should be assessed in early pregnancy, as this is a critical period for fetal development and the establishment of maternal vitamin B12 stores [6]. Others have argued that screening should be targeted towards high-risk populations, such as vegetarians, vegans, and women with a history of bariatric surgery or malabsorption disorders [7].

The methods used for assessing vitamin B12 status in pregnancy include serum vitamin B12 measurement, methylmalonic acid (MMA) assay, and holotranscobalamin (HoloTC) assay [1]. Serum vitamin B12 is the most commonly used marker, but its sensitivity and specificity for detecting true vitamin B12 deficiency have been questioned [5]. MMA and HoloTC are considered more reliable indicators of vitamin B12 status but are not widely available in clinical practice [6].

In this prospective cross-sectional study, we aimed to investigate the prevalence of vitamin B12 deficiency in early pregnancy and its association with preterm birth in a cohort of pregnant women attending antenatal care at a tertiary hospital in India. We hypothesized that vitamin B12 deficiency in early pregnancy would be associated with an increased risk of preterm birth. The findings of this study could inform the development of screening and intervention strategies for vitamin B12 deficiency in pregnancy, with the ultimate goal of reducing the burden of preterm birth and improving maternal and neonatal outcomes.

## Aims and Objectives

The primary aim of this prospective cross-sectional study was to investigate the prevalence of vitamin B12 deficiency in early pregnancy among pregnant women attending antenatal care at a tertiary hospital in India. The secondary objective was to examine the association between vitamin B12 deficiency in early pregnancy and the risk of preterm birth.

## Materials and Methods

**Study Design and Setting:** This prospective cross-sectional study was conducted in the Department of Obstetrics and Gynecology at Tata Motors Hospital, Jamshedpur, Jharkhand, India.

**Study Duration:** The study was carried out from September 2017 to March 2019. The enrollment period was from September 2017 to September 2018, and all participants were followed up until delivery, which concluded in March 2019.

**Study Population:** The study population consisted of pregnant women who met the inclusion criteria and visited the Department of Obstetrics and Gynecology at Tata Motors Hospital for antenatal care during the study period.

**Sample Size:** The sample size was calculated using the formula: $n = Z2 \times P(1-P) / d2$, where n is the sample size, Z is the standard normal variate (1.96 for a 95% confidence interval), P is the expected prevalence of vitamin B12 deficiency in the population based on previous studies, and d is the absolute error or precision (margin of error). Considering a prevalence of 19% based on the study by Sukumar et al., a confidence interval of 95%, and a margin of error of 0.05, the calculated sample size was 236.

**Inclusion and Exclusion Criteria:** Pregnant women aged between 18 and 35 years, who were at 14 to 20 weeks of gestation and registered for antenatal care at the hospital, were included in the study after obtaining informed consent. Women were excluded if they were on medication for chronic illnesses such as diabetes mellitus, hypertension, heart disease, or thyroid disorders; had diagnosed hemoglobinopathies like thalassemia or sickle cell anemia; tested positive for hepatitis B surface antigen, human immunodeficiency virus, or syphilis; had multiple gestations; or were lost to follow-up.

**Data Collection and Laboratory Analysis:** After obtaining approval from the Institutional Ethics Committee, all eligible pregnant women were enrolled in the study during their first antenatal visit. A structured questionnaire was administered to collect sociodemographic data, medical history, and dietary habits. Venous blood samples were collected from the participants between 14 and 20

weeks of gestation after an overnight fast of 8 hours. Serum vitamin B12 levels were measured using the Electro Chemi-Luminescence-Immuno-Assay (ECLIA) method on a COBAS e 411 analyzer. Vitamin B12 deficiency was defined as serum levels below 200 pg/mL.

**Follow-up and Outcome Assessment:** The participants were followed up throughout their pregnancy, and the gestational age at delivery was recorded. Preterm birth was defined as delivery before 37 completed weeks of gestation. The association between vitamin B12 deficiency in early pregnancy and preterm birth was analyzed.

**Statistical Analysis:** Data were analyzed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, N.Y., USA). Categorical variables were expressed as frequencies and percentages, while continuous variables were presented as mean ± standard deviation. The association between vitamin B12 deficiency and preterm birth was assessed using the chi-square test or Fisher's exact test, as appropriate. A p-value of less than 0.05 was considered statistically significant.

**Ethical Considerations:** The study protocol was approved by the Institutional Ethics Committee of Tata Motors Hospital, Jamshedpur, India. Written informed consent was obtained from all participants before enrollment, and the study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

## Results

A total of 250 pregnant women were enrolled in the study, of which 14 were excluded due to various reasons, including lost to follow-up (5), twin gestation (3), hepatitis B surface antigen positivity (2), human immunodeficiency virus positivity (1), and overt diabetes (2). The remaining 236 pregnant women were included in the final analysis (Table 1).

The mean serum vitamin B12 level of all study participants was 231.0556 pg/ml (SD 104.2277). Among the 236 participants, 159 (67.37%) had normal vitamin B12 levels (>200 pg/ml), while 77 (32.63%) were found to be deficient (<200 pg/ml) (Table 1).

The mean gestational age at delivery among all study participants was 266.6652 days (SD 11.2) (Table 1). The distribution of gestational age at delivery revealed that 200 (84.75%) women had term deliveries (>37 weeks), while 36 (15.25%) experienced preterm births (<37 weeks) (Table 2).

The association between gestational age at delivery and maternal serum vitamin B12 levels was found to be statistically significant (p=0.015732) (Table 2). Among the women who had preterm deliveries, 18 (50.00%) had normal vitamin B12 levels, while the other 18 (50.00%) were deficient. In contrast, among the women who had term deliveries, 141 (70.50%) had normal vitamin B12 levels, and 59 (29.50%) were deficient.

The age distribution of the study participants showed that the majority (61.02%) were in the age group of 20-30 years, followed by 38.56% in the 30-40 years age group, and only 0.42% in the 18-20 years age group (Table 3). The mean age of the participants was 28.1 years (SD 4.1269). There was no statistically significant association found between maternal age and serum vitamin B12 levels (p=0.78).

Regarding the parity distribution, 100 (42.37%) participants were primigravida, while 136 (57.63%) were multigravida (Table 4). Among the primigravida women, 65 (65.00%) had normal vitamin B12 levels, and 35 (35.00%) were deficient. The distribution of normal and deficient vitamin B12 levels among multigravida women was similar, with no statistically significant association between parity and serum vitamin B12 levels (p=0.86).

**Table 1: Vitamin B12 levels and mean gestational age of the study participants**

| Vitamin B12 Levels | No. of pregnant women (%) | Mean Vitamin B12 (SD) | Mean gestational age in days (SD) |
|---|---|---|---|
| Normal (>200pg/ml) | 159 (67.37%) | 231.0556 (104.2277) | 266.6652 (11.2) |
| Deficient (<200pg/ml) | 77 (32.63%) | | |
| Total | 236 (100.00%) | | |

**Table 2: Distribution of gestational age at delivery and its association with maternal serum Vitamin B12 levels**

| Gestational age at delivery | No. of pregnant women (%) | No. of pregnant women with Normal B12 (%) | No. of pregnant women with deficient B12 (%) | P value |
|---|---|---|---|---|
| Preterm (<37weeks) | 36 (15.25%) | 18 (50.00%) | 18 (50.00%) | 0.015732 |
| Term (>37weeks) | 200 (84.75%) | 141 (70.50%) | 59 (29.50%) | |
| Total | 236 (100.00%) | 159 (67.37%) | 77 (32.63%) | |

**Table 3: Age distribution, mean age, and association of maternal age with serum vitamin B12 levels**

| Age (in Years) | Frequency (%) | Mean age (SD) | Normal Vit B12 (%) | Deficient Vit B12 (%) | P value |
|---|---|---|---|---|---|
| 18 - <20 | 1 (0.42%) | 28.1 (4.1269) | 1 (100.00%) | 0 (0.00%) | 0.78 |
| 20 - <30 | 144 (61.02%) | - | 97 (67.36%) | 47 (32.64%) | |
| 30 - <40 | 91 (38.56%) | - | 61 (67.03%) | 30 (32.97%) | |
| Total | 236 (100.00%) | - | 159 (67.37%) | 77 (32.63%) | |

**Table 4: Parity distribution and association between parity and maternal serum Vitamin B12 levels**

| Gravida | Frequency (%) | Normal Vit B12 (%) | Deficient Vit B12 (%) | P value |
|---|---|---|---|---|
| 1 | 100 (42.37%) | 65 (65.00%) | 35 (35.00%) | 0.86 |
| 2 | 64 (27.12%) | 42 (65.63%) | 22 (34.37%) | |
| 3 | 45 (19.07%) | 33 (73.33%) | 12 (26.67%) | |
| 4 | 19 (8.05%) | 13 (68.42%) | 6 (31.58%) | |
| 5 | 8 (3.39%) | 6 (75.00%) | 2 (25.00%) | |
| Total | 236 (100.00%) | 159 (67.37%) | 77 (32.63%) | |

## Discussion

The present study investigated the prevalence of vitamin B12 deficiency in early pregnancy and its association with preterm birth among pregnant women attending a tertiary hospital in India. The findings revealed that 32.63% of the participants had vitamin B12 deficiency (<200 pg/ml) in early pregnancy, and there was a significant association between vitamin B12 deficiency and preterm birth (p=0.015732).

The prevalence of vitamin B12 deficiency in this study is consistent with the findings of a systematic review and meta-analysis by Sukumar et al., which reported a global prevalence of vitamin B12 deficiency in pregnancy ranging from 20% to 30% [4]. However, the prevalence in the current study is higher than that reported in developed countries such as the United States and the United Kingdom, where the prevalence ranges from 5% to 10% [5].

A study by Krishnaveni et al. conducted in South India found a similar prevalence of vitamin B12 deficiency (43%) among pregnant women [11]. The higher prevalence of vitamin B12 deficiency in developing countries like India can be attributed to various factors, including dietary habits, socioeconomic status, and lack of food fortification programs [12].

The significant association between vitamin B12 deficiency and preterm birth in this study is consistent with the findings of a meta-analysis by Rogne et al., which reported an increased risk of preterm birth (odds ratio [OR] 1.21, 95% confidence interval [CI] 0.99-1.49) among women with low vitamin B12 levels [9]. However, the evidence from the meta-analysis was of low quality due to the small number of studies and heterogeneity among them.

A prospective cohort study by Dwarkanath et al. in India found that women with vitamin B12 deficiency (<150 pmol/L) had a significantly higher risk of preterm birth (adjusted OR 3.16, 95% CI 1.12-8.93) compared to women with normal vitamin B12 levels [13]. The study also found a significant association between vitamin B12 deficiency and intrauterine growth restriction (adjusted OR 2.55, 95% CI 1.08-6.02).

In contrast, a study by Woude et al. in the Netherlands found no significant association between vitamin B12 status and preterm birth (OR 1.05, 95% CI 0.63-1.76) [14]. The discrepancy in findings could be due to differences in study populations, sample sizes, and the definition of vitamin B12 deficiency used in the studies.

The mechanism underlying the association between vitamin B12 deficiency and preterm birth is not fully understood. However, it is hypothesized that vitamin B12 deficiency may lead to the accumulation of homocysteine, which can cause oxidative stress, inflammation, and endothelial dysfunction, thereby increasing the risk of adverse pregnancy outcomes, including preterm birth [15].

The current study did not find a significant association between maternal age, parity, and vitamin B12 status, which is consistent with the findings of previous studies [16,17]. However, some studies have reported an association between advanced maternal age and vitamin B12 deficiency [18].

The strengths of this study include its prospective design, the use of a standardized method for measuring vitamin B12 levels, and the inclusion of a representative sample of pregnant women attending a tertiary hospital. However, the study has some limitations. First, the study was conducted in a single center, which may limit the generalizability of the findings. Second, the study did not assess other factors that may influence vitamin B12 status, such as dietary intake and genetic polymorphisms.

In conclusion, this study found a high prevalence of vitamin B12 deficiency in early pregnancy and a significant association between vitamin B12

deficiency and preterm birth among pregnant women in India. The findings highlight the importance of screening for vitamin B12 deficiency in early pregnancy and the need for interventions to improve vitamin B12 status among pregnant women, particularly in developing countries. Future studies should investigate the effectiveness of vitamin B12 supplementation in reducing the risk of preterm birth and other adverse pregnancy outcomes.

## Conclusion

This prospective cross-sectional study investigated the prevalence of vitamin B12 deficiency in early pregnancy and its association with preterm birth among pregnant women attending a tertiary hospital in India. The study found a high prevalence of vitamin B12 deficiency (32.63%) in early pregnancy, which is consistent with the findings of previous studies conducted in developing countries. The study also found a significant association between vitamin B12 deficiency and preterm birth (p=0.015732), with 50% of the women who had preterm deliveries being deficient in vitamin B12, compared to 29.50% of the women who had term deliveries.

The findings of this study highlight the importance of screening for vitamin B12 deficiency in early pregnancy, particularly in developing countries where the prevalence of deficiency is high. Early detection and intervention can help reduce the risk of adverse pregnancy outcomes, including preterm birth. The study also underscores the need for public health interventions to improve vitamin B12 status among pregnant women, such as dietary diversification, food fortification, and supplementation programs.

Future research should focus on investigating the effectiveness of vitamin B12 supplementation in reducing the risk of preterm birth and other adverse pregnancy outcomes, as well as exploring the underlying mechanisms of the association between vitamin B12 deficiency and preterm birth. Additionally, studies should be conducted in diverse populations to assess the generalizability of the findings and to identify population-specific risk factors for vitamin B12 deficiency.

In conclusion, this study provides valuable insights into the prevalence of vitamin B12 deficiency in early pregnancy and its association with preterm birth in a developing country setting. The findings emphasize the need for routine screening and intervention strategies to improve vitamin B12 status among pregnant women and to reduce the burden of adverse pregnancy outcomes, particularly in resource-limited settings.

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