Prevalence of Vitamin B12 Deficiency and Its Associated Risk Factors among Pregnant Women of Rural South India: A Community-based Cross-sectional Study

Anitha Mohanraj Barney1,2, Vinod Joseph Abraham1, Sumita Danda2, Anne George Cherian1,3, S. Vanitha4
Departments of 1Community Health, 2Clinical Genetics, 3Obstetrics and Gynecology and 4Clinical Biochemistry, Christian Medical College, Vellore, Tamil Nadu, India

Abstract

Introduction: Vitamin B12 is essential for the normal functioning of the nervous system and for the formation of red blood cells. Vegetarian diet, low socioeconomic status, and social and religious reasons are known risk factors of its deficiency. Pregnant women, children, and the elderly are vulnerable groups. Indians have the highest prevalence, but the data among pregnant women in the rural setting is lacking. Objectives: The objective of this study is to assess the prevalence of Vitamin B12 deficiency and its associated factors among pregnant women of rural South India. Materials and Methods: A cross-sectional study was conducted to recruit consecutive 120 multigravida women with ≤20 weeks of gestation, attending the mobile doctor run clinic of Kanjyambadi block, Vellore. A structured questionnaire was administered, and blood samples were collected. Results: The prevalence of Vitamin B12 deficiency (<200 pg/mL) and anemia (Hb ≤10.5 g/dL) was 55% and 17.5%, respectively. Only 11.7% were B12 deficient and anemic. Past history of abortion (odds ratio [OR] = 0.5), fatigue (OR = 0.4), and low B12 intake (OR = 2) was associated only in the bivariate analysis. First trimester (OR = 3.9) and obesity (OR = 9.6) were found to be independent risk factors of Vitamin B12 deficiency. Conclusion: Our study showed a high prevalence of Vitamin B12 deficiency in pregnancy in rural India. Some risk factors were identified. However, studies with a higher sample size will be beneficial to study the associated risk factors better.

Keywords: Anemia, B12 deficiency, nutrition, pregnancy

INTRODUCTION

Vitamin B12 is a water-soluble micronutrient, which helps in the formation of red blood cells and is also essential for the normal functioning of the nervous system and brain. Along with folic acid, Vitamin B12 is needed for fatty and amino acid metabolisms and DNA synthesis and also plays a significant role in the conversion of homocysteine to methionine, which is required for the synthesis of neurotransmitters and phospholipids. It is naturally produced by microbial synthesis, and the main dietary sources are of animal origin. Other important sources are fermented foods and uncooked plant-based food contaminated with B12 producing bacteria or algae. Vitamin B12 deficiency can result from malabsorption, intestinal disorders, and low levels of binding proteins and the use of medications such as proton-pump inhibitors and metformin. People consuming vegetarian diet are at a higher risk. Other common causes are low socioeconomic status and social and religious reasons for nonconsumption of meat. Several studies highlight South Asian ethnicity as a risk factor. India has the highest prevalence of Vitamin B12 deficiency, ranging from 47% to 71% in adults.

During pregnancy, mother needs more nutrients, especially micronutrients. The Indian Council of Medical Research recommends a dietary intake of 1 μg/day of Vitamin B12 for adults and 1.5 and 1.2 μg/day for pregnancy and lactation, respectively. Fast and processed food intake and decreased consumption of fruits, vegetables, and meat lead to a high prevalence of Vitamin B12 deficiency in pregnancy in rural India. Some risk factors were identified. However, studies with a higher sample size will be beneficial to study the associated risk factors better.
micronutrient deficiencies. In pregnancy, it leads to adverse outcomes. We decided to conduct this study since there is a lack of studies to assess the prevalence of Vitamin B12 deficiency and its risk factors in pregnancy in rural South India.

Methods

This study was approved by the Institutional Review Board. This is a cross-sectional study done from August 2017 to May 2018. The calculated sample size was 100, based on a prevalence of 51% with a relative precision of 20%. In order to account for a dropout rate of 20%, 120 samples were included. Multigravida <20 weeks of gestational age who were the residents of a rural block of Vellore district were included in the study. Known Vitamin B12 deficiency, those on Vitamin B12 supplements and known cases of malabsorption, gastrectomy, and irritable bowel syndrome or any other gastric disorders which is known to cause Vitamin B12 deficiency were excluded from the study.

Consecutive pregnant women from the mobile clinic in Kaniyambadi block, which covers a population of approximately 120,000 were recruited. An informed consent was obtained. A structured questionnaire which included a 24-h dietary recall was administered. Calibrated vessels and spoons were used to quantify the intake of solids (rice) and liquids (milk or ghee, etc.). A blood test was obtained to check for serum Vitamin B12 levels and hemoglobin. Estimation of Vitamin B12 and hemoglobin was done in a NABL accredited laboratory by Roche e602 and Sysmax KX 21, respectively, the instruments are calibrated daily. Vitamin B12 <200 pg/ml was taken as deficient and hemoglobin ≤10.5 g/dL was diagnosed as anemia. Although the WHO recommends 11 g/dL as the cutoff for anemia all throughout pregnancy, the CDC recommends a trimester based cutoff. The latter recommends 10.5 g/dL in the second trimester. Since 60% of the pregnant women in our study were in the second trimester and since 10.5 g/dL was used as the cutoff to treat B12 deficiency with anemia in pregnancy, we took 10.5 g/dL as the cutoff. In view of recent literature showing a possible increased risk of autism to the yet-to-be born child due to high maternal Vitamin B12 levels, only those with B12 deficiency and anemia were treated with 500 μg of Vitamin B12 orally, once a day till delivery. If there was a deficiency in Vitamin B12 but the absence of anemia, the hemoglobin was repeated after 1–2 months. If there was a significant drop in hemoglobin of >1 g/dL (more than what is explainable by the physiological effects of pregnancy), then the treatment was commenced with Vitamin B12 at similar doses.

The data were entered using epidata V 3.1 and analyzed using IBM SPSS 25.0 (SPSS South Asia Pvt Ltd., Kacharkanhalli, Bangalore, Karnataka, India). Descriptive statistics for the continuous variables were calculated as mean and standard deviation. Frequencies and percentages were calculated for the categorical variables. Continuous variables were dichotomized for bivariate analysis. The Chi-square test was performed to assess the statistical significance of the risk factors, and odds ratio was calculated to assess the strength of association. Multiple logistic regression analysis was performed to find out the independent risk factors.

Results

A total of 120 pregnant mothers were included. By B G Prasad Scale, 15% of the mothers were in the lower class of socioeconomic status (SES), 35% of the mothers were in the lower middle-class SES, and 22.5% of them were in the middle class. Eighty-three percent of the mothers had completed at least high school. Eighty-eight percent of the mothers were homemakers, despite many being qualified. Ten percent of the mothers were obese, 19.2% were preobese, and an additional 18.3% were overweight. Sixty percent of the mothers were in their second trimester. In all half of them had not consumed folic acid at the time of recruitment into the study. The mean days of folic acid consumption were only twenty. The most common clinical manifestations were fatigue (25%), pallor (24.8%), breathlessness (20.8%), and fainting (19.2%). Among the known risk factors of B12 deficiency, consanguinity was the most prevalent (25%) followed by the usage of RO water (13.3%). The most prevalent previous maternal and neonatal risk factors/outcomes were previous one or two abortions (32.5%), low birth weight (15.8%), short stature (11.67%), and previous LSCS (9%).

The prevalence of B12 deficiency and anemia was 55% and 17.5%, respectively. Only 11.67% had B12 deficiency with anemia and were treated with Vitamin B12 supplements. As shown in the consort diagram in Figure 1, 52 women underwent repeat hemoglobin in 1 or 2 months. Thirteen of them had a drop in hemoglobin of more than 1 g% and were also treated. The rest 39 with low B12 levels were not treated for reasons mentioned previously.

In the bivariate analysis [Table 1], those with obesity (BMI >30 kg/m²) had higher odds ratio (OR = 4.6) of being B12 deficient. Low PCV (OR = 2.3) and the presence of any comorbidity (OR = 3.4) also had a positive association for

![Figure 1: Consort diagram](image-url)
Table 1: Association between variables and Vitamin B12 deficiency status

| Variables                        | Groups                  | Vitamin B12 deficient (<200 pg/ml) (n=66), n (%) | Vitamin B12 normal (200-950 pg/ml) (n=54), n (%) | P    | OR   | 95% CI  |
|----------------------------------|-------------------------|-----------------------------------------------|-----------------------------------------------|-------|-------|---------|
| **Sociodemographic variables**   |                         |                                               |                                               |       |       |         |
| Age (years)                      | ≥26 (n=57)              | 57.9                                          | 42.1                                          | 0.59  | 1.3   | 0.6-2.5 |
|                                  | <26 (n=59)              | 52.4                                          | 47.6                                          |       |       |         |
| Education                        | ≤High school (n=57)     | 49.1                                          | 50.9                                          | 0.22  | 0.6   | 0.3-1.3 |
|                                  | ≥Higher secondary (n=63)| 60.3                                          | 39.7                                          |       |       |         |
| Occupation                       | Homemaker (n=102)       | 55.2                                          | 44.2                                          | 1.0   | 1.1   | 0.4-3.2 |
|                                  | Working (n=18)          | 53.3                                          | 46.7                                          |       |       |         |
| Per capita monthly income (INR)  | ≤2500 (n=82)            | 54.9                                          | 45.1                                          | 1.0   | 1.0   | 0.4-2.1 |
|                                  | >2500 (n=38)            | 55.3                                          | 44.7                                          |       |       |         |
| BMI (kg/m²)                      | ≥30 kg/m² (n=12)        | 83.3                                          | 16.7                                          | 0.03  | 4.6   | 1.0-22.1|
|                                  | <30 kg/m² (n=108)       | 51.9                                          | 48.1                                          |       |       |         |
| Obstetric history                |                         |                                               |                                               |       |       |         |
| Gestational age                  | First trimester (n=48)  | 43.8                                          | 56.2                                          | 0.04  | 0.5   | 0.2-0.9 |
|                                  | Second trimester (n=72) | 62.5                                          | 37.5                                          |       |       |         |
| Folic acid consumption           | No (n=56)               | 53.6                                          | 46.4                                          | 0.86  | 0.9   | 0.4-1.9 |
|                                  | Yes (n=64)              | 56.2                                          | 43.8                                          |       |       |         |
| Number of abortions              | ≥1 (n=42)               | 42.9                                          | 57.1                                          | 0.05  | 0.5   | 0.2-1.0 |
|                                  | No abortion (n=78)      | 61.5                                          | 38.5                                          |       |       |         |
| High-risk pregnancy              | Yes (n=30)              | 60                                            | 40                                            | 0.67  | 1.3   | 0.6-3.0 |
|                                  | No (n=90)               | 53.3                                          | 46.7                                          |       |       |         |
| Dietary intake (24 h recall)     |                         |                                               |                                               |       |       |         |
| Calories (Kcal)                  | ≤1600 (n=57)            | 63.2                                          | 36.8                                          | 0.06  | 1.9   | 0.9-3.9 |
|                                  | >1600 (n=63)            | 47.6                                          | 52.4                                          |       |       |         |
| Iron (g)                         | ≤22 (n=78)              | 55.1                                          | 44.9                                          | 1.0   | 1.0   | 0.5-2.1 |
|                                  | >22 (n=42)              | 54.8                                          | 45.2                                          |       |       |         |
| Vitamin B12 (µg)                 | ≤1.5 (n=79)             | 60.8                                          | 39.2                                          | 0.05  | 2.0   | 1.0-4.2 |
|                                  | >1.5 (n=41)             | 43.9                                          | 56.1                                          |       |       |         |
| Calcium                          | ≤800 (n=77)             | 59.7                                          | 40.3                                          | 0.11  | 1.7   | 0.8-3.6 |
|                                  | >800 (n=43)             | 46.5                                          | 53.5                                          |       |       |         |
| Known risk factors               |                         |                                               |                                               |       |       |         |
| Consanguinity                    | Yes (n=29)              | 58.6                                          | 41.4                                          | 0.4   | 1.2   | 0.5-2.8 |
|                                  | No (n=91)               | 53.8                                          | 46.2                                          |       |       |         |
| Consumption of RO water          | Yes (n=22)              | 54.5                                          | 45.5                                          | 1.0   | 0.9   | 0.3-2.4 |
|                                  | No (n=98)               | 55.1                                          | 44.9                                          |       |       |         |
| Family history of B12 deficiency | Yes (n=2)               | 50.0                                          | 50.0                                          | 1.0   | 0.8   | 0.1-13.3|
|                                  | No (n=118)              | 55.1                                          | 44.9                                          |       |       |         |
| Clinical manifestations          |                         |                                               |                                               |       |       |         |
| Nocturnal cramping               | Yes (n=25)              | 48.0                                          | 52.0                                          | 0.5   | 0.7   | 0.3-1.7 |
|                                  | No (n=95)               | 56.8                                          | 43.2                                          |       |       |         |
| Fatigue                          | Yes (n=30)              | 40.0                                          | 60.0                                          | 0.04  | 0.4   | 0.2-1.0 |
|                                  | No (n=90)               | 60.0                                          | 40.0                                          |       |       |         |
| Pallor                           | Yes (n=29)              | 41.4                                          | 58.6                                          | 0.07  | 0.5   | 0.2-1.1 |
|                                  | No (n=91)               | 59.3                                          | 40.7                                          |       |       |         |
| Angular stomatitis               | Yes (n=6)               | 33.3                                          | 66.7                                          | 0.4   | 0.4   | 0.1-2.2 |
|                                  | No (n=114)              | 56.1                                          | 43.9                                          |       |       |         |
| Premature greying                | Yes (n=6)               | 83.3                                          | 16.7                                          | 0.16  | 4.3   | 0.5-38.3|
|                                  | No (n=114)              | 53.5                                          | 46.5                                          |       |       |         |
| Blood cell parameters            |                         |                                               |                                               |       |       |         |
| PCV (%)                          | ≤35 (n=58)              | 65.5                                          | 34.5                                          | 0.02  | 2.3   | 1.1-4.8 |
|                                  | >35 (n=62)              | 45.2                                          | 54.8                                          |       |       |         |

Contd...
Table 1: Contd...

| Variables | Groups | Vitamin B12 deficient (<200 pg/ml) (n=66), n (%) | Vitamin B12 normal (200-950 pg/ml) (n=54), n (%) | P     | OR          | 95% CI |
|-----------|--------|---------------------------------|---------------------------------|-------|-------------|--------|
| Hemoglobin (g/dL) | ≤10.5 (n=21) | 66.7                           | 33.3                           | 0.17  | 1.8         | 0.7-4.9 |
|           | >10.5 (n=99) | 52.5                          | 47.5                          |       | 1           |        |
| MCV (fL)  | ≤82 (n=55)   | 58.2                          | 41.8                          | 0.32  | 1.3         | 0.6-2.6 |
|           | >82 (n=65)   | 52.3                          | 47.7                          |       | 1           |        |
| MCH (pg)  | ≤28 (n=55)   | 61.8                          | 38.2                          | 0.11  | 1.7         | 0.8-3.5 |
|           | >28 (n=65)   | 492                           | 50.8                          |       | 1           |        |

MCV: Mean corpuscular volume, PCV: Packed-cell volume, MCH: Mean corpuscular hemoglobin, OR: Odds ratio, CI: Confidence interval

being B12 deficient with statistical significance. Gestational age in the first trimester (OR = 0.5), past history of abortion (OR = 0.5), and fatigue (OR = 0.4) had a protective effect. In the multiple logistic regression [Table 2], after adjusting for confounding factors, first trimester (OR = 3.9; 95% CI 1.4–10.6) and obesity (OR = 9.6; 95% CI 1.3–63.1) were found to be independent risk factors of Vitamin B12 deficiency.

**Discussion**

High prevalence of Vitamin B12 deficiency (55%) among pregnant mothers observed by us is similar to studies previously published from a semiurban area in Bangalore,[13] with a prevalence of 51% and in Pune[14] among rural and urban pregnant women, with 80% and 65% prevalence of Vitamin B12 deficiency, respectively.

The prevalence of anemia was 17.5%. However, only 11.7% had B12 deficiency and anemia. Our study results match a previous study done by Remacha et al.,[17] where only 18% of the participants had both anemia and B12 deficiency. In our study, the mean MCV was 82.07 (normal range 63.5–97.9) and hence normal. Literature agrees that macrocytosis[15] is not always present in cobalamin deficiency. MCV is normal or low also in mixed anemias. However, iron studies were not done in our study to demonstrate this.

A total of 27 mothers (22.5%) were treated with oral Vitamin B12 supplements 500 μg once daily till delivery. Others who were Vitamin B12 deficient but not anemic were not treated because of the evolving evidence of the risk of autism to the unborn child, in those with higher maternal serum B12 levels at delivery. In a cohort study from the United States,[15] the risk of autism in the child increased by 17 times with higher B12 levels (>600 pmol/L) in the mother. In our study, Vitamin B12 was supplements orally because the gastrointestinal absorption in pregnancy is higher,[19] and it is economical, practical, and convenient.

In the bivariate analysis, the mothers with obesity (BMI >30 kg/m²) had higher odds (OR = 4.6) of being B12 deficient with statistical significance. This finding persisted in the multiple logistic regression with increasing odds (OR = 9.6; 95% CI 1.3–63.1) and statistical significance (P = 0.023).

A retrospective study conducted by Prameela et al.[20] in Mysore, India, found that obese women have increased risk of eclampsia, instrumental delivery, LSCS, IUGR, and PPH. Another prospective study by Koduri et al.[21] among 262 pregnant women with BMI >30 also found an increased risk of complications among obese patients. Hence, it could be postulated that B12 deficiency could be one of the pathways for adverse maternal outcomes in the obese group. However, this remains to be studied further.

Among the parameters of the obstetric history, the gestational age and the past history of abortion were associated with B12 deficiency with statistical significance. In the multivariate analysis, mothers in the first trimester had nearly four times higher odds of being B12 deficient when compared with those in the second trimester (OR = 3.9; 95% CI = 1.4–10.6). Forty percent of our mothers were in the first trimester at the time of recruitment. A study[22] which was done to examine the changes in dietary intake from the first to the second trimester of pregnancy found that intake of many micronutrient intakes increased substantially from the first to the second trimester. The increased requirement of Vitamin B12 coupled with decreased intake in the first trimester due to reasons such as morning sickness could have resulted in the increased odds of Vitamin B12 deficiency in the first trimester.

Those who had a history of at least one abortion in the past had a lower prevalence of B12 deficiency with odds of 0.5 (95% CI = 0.2–1.0) when compared to the mothers who had no previous abortion. However, after adjusting for confounders, this association did not persist (OR = 2.0; 95% CI = 0.8–4.7).

A well-conducted French case–control study[23] looked at Vitamin B12 as a risk factor for early recurrent abortion. They also conducted an analysis of five similar studies including theirs and concluded that there was a significant relationship between Vitamin B12 deficiency and early recurrent abortion. A genetic study by Puri et al.[24] from North India found that methylenetetrahydrofolate reductase C677T (MTHFR C677T) genetic polymorphism was associated with higher homocysteine levels which leads to recurrent abortions. Since Vitamin B12 deficiency also causes the elevation of homocysteine levels, this could be the reason for recurrent pregnancy loss in these women.
positive association though not statistically significant. In the multivariate regression analysis, none of these showed statistically significant association. Nonvegetarian diet is the most important dietary source of Vitamin B12. However, in a systematic review and metaanalysis, Sukumar et al. found that the prevalence of Vitamin B12 deficiency was high in nonvegetarian populations as well.

Among the clinical manifestations, those who had the symptom of fatigue had a statistically significant lower odds (OR = 0.4; 95% CI = 0.2–1.0) of being B12 deficient compared with those who did not have fatigue, but this association did not persist on the multivariate analysis. Those who had premature greying had higher odds (OR = 2.5; 95% CI = 0.2–24.9) of being B12 deficient, though not statistically significant. This did not persist in the multivariate analysis. Case–control studies done on premature greying showed that Vitamin B12 deficiency is a significant risk factor for the same.

The strength of our study is that the samples were representative of the entire Kaniyambadi block, having recruited consecutive 120 mothers spread over all the 89 villages. The limitation of the study is that the sample size was not powered to assess all the associated factors. Although obesity and first trimester were identified as the independent risk factors of Vitamin B12 deficiency, other factors such as premature greying, history of early neonatal death, and abortion, which might have had independent association could not be proved due to the limited sample size. Another limitation is that concomitant iron deficiency anemia was not looked into. Furthermore, the maternal and neonatal outcomes in the current pregnancy were not looked into, which could have assessed the impact of Vitamin B12 deficiency on these.

**Conclusion**

The prevalence of Vitamin B12 deficiency among pregnant women in rural South India is considerably high despite most of them being nonvegetarians. First trimester and obesity were found to be independent risk factors of Vitamin B12 deficiency. However, studies with a higher sample size are needed to study associated risk factors better and to consider the need for screening and supplementation of B12 in pregnancy. The genetic basis behind the low Vitamin B12 levels needs to be explored in future studies.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

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