Original Article

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Biochemistry Section

Status of Vitamin B12 Deficiency among Lactating Mothers at Six Weeks Postpartum at a Tertiary Care Hospital, West Bengal, India

RITUPARNA MAJI[1], ANUBHAV MUKHERJEE[2], MOUSUMI DUTTA[3], SURAJIT LAHIRI[4], SWATI BHATTACHARYYA[5]

ABSTRACT

Introduction: Vitamin B12 (vit B12) deficiency is a preventable disorder and has dire consequences on the neurological development of the infant. The only source of vit B12 for exclusively breastfed newborns is their mother's milk. So, determining the status of maternal vit B12 levels during early lactation is vital. In India, very few studies have documented vit B12 deficiency among lactating mothers, especially eastern region remains unexplored.

Aim: To estimate the prevalence of vit B12 deficiency among healthy lactating mothers attending the postpartum clinic at six weeks at a Tertiary Care Hospital of West Bengal, India and also to compare the haematological values between vit B12 deficient and sufficient groups among the lactating mothers.

Materials and Methods: An observational cross-sectional study was conducted in the Department of Biochemistry in collaboration with postpartum clinic of Calcutta National Medical College, West Bengal, India. The study was carried out over a period of two months, from July 2022 to August 2022. Serum vit B12, serum ferritin and haematological parameters {Haemoglobin (Hb), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC)} were measured in 121 apparently healthy lactating women attending the postpartum clinic for a regular check-up at six weeks. Detailed socioclinical history and dietary history was taken from each participant and these variables were compared between vit B12 deficient and sufficient groups. Pearson's Chi-square test, Fisher's-exact test, Mann-Whitney U test were applied to analyse the data.

Results: The total study population comprised of 121 mothers with an average age of 25.65±3.71 years and 75% of them consumed non vegetarian diet. A prevalence of 19 (15.7%) deficiency of vit B12 was found among the lactating mothers at six weeks postpartum. Mothers who were vegetarian, multigravida and with complaints of paraesthesia were more associated to cobalamin deficiency. The vit B12 deficient mothers had significantly higher erythrocyte indices than the sufficient group but none of the values were in the megaloblastic range.

Conclusion: The vit B12 deficiency was prevalent during early lactation in 19 (15.7%) lactating mothers. The first postnatal visit at six weeks might be the most feasible period to screen and supplement these women to prevent long term sequelae in both mother and child. Haematological parameters were of limited use in identifying vit B12 deficiency during lactation.

Keywords: Cobalamin, Infant, Lactation, Postnatal, Pregnancy

INTRODUCTION

The vit B12 plays an important role in the maintenance of normal erythropoiesis, cell reproduction, nucleoprotein and myelin synthesis. Vitamin B12 insufficiency during lactation may cause anaemia and neurological damage in both the mother and the breastfed child [1]. The vit B12 status of the mother influences the vit B12 status of the infant. A cobalamin-deficient mother is likely to produce breast milk with low levels of the vitamin [2]. These infants may present with failure to thrive, irritability, apathy, anorexia, refusal to solid feeds, delay and regression of neurological development, hypotonia, seizure and pancytopenia [3]. The effects are more pronounced in exclusively breastfed infants where animal-based complimentary feeding is not yet introduced. Also, a recent study from India has shown that low vit B12 may contribute to depressive symptoms among mothers in the vulnerable postpartum period [4]. Hence, determining the cobalamin status of lactating women is vital for both the mother and the infant.

The vit B12 level of <200 pg/mL (150 pmol/L) is universally considered to be threshold of deficiency [5]. Current estimate of prevalence of vit B12 deficiency of overall Indian population is said to be at 47.19% while during pregnancy ranges from 43% to 74% [6]. This depleted cobalamin reserve is expected to persist and even aggravate in the postpartum period. But data documenting vit B12 deficiency among lactating mothers is very limited, not only in India but worldwide. Moreover, these studies estimated vit B12 at different periods of lactation, ranging from 48 hours to six months postdelivery [7-10].

The data should be interpreted keeping in mind that plasma vit B12 levels in maternal blood have considerable physiological variations due to haemodynamic effects of pregnancy. Studies have shown that vit B12 levels decrease significantly during pregnancy and recover to normal values within 6-8 week postpartum [11,12]. Also, cobalamin status depends on the dietary preference (vegetarian/ non vegetarian) and socioeconomic status of the mother and is supposed to vary for different population [13]. With this background, the primary objective of present study was to estimate the prevalence of serum vit B12 deficiency in lactating women at six weeks postpartum. The period of six weeks postpartum was chosen because the haemodynamic effects of pregnancy were expected to be stabilised by then [11,12]. Also, mothers undergoing hospital delivery are routinely advised to visit the postpartum clinic at six weeks. So, maximum number of mothers could be approached at this time. The classical clinical manifestation of vit B12 deficiency is megaloblastic anaemia (MCV>100 fL) [13]. Hence, haematological values can also be of use in identifying vit B12 deficiency during lactation.

The present study aimed to estimate the prevalence of serum vit B12 deficiency in lactating women at six weeks postpartum in a tertiary care hospital of West Bengal, India, and also to compare the haematological values between vit B12 deficient and sufficient groups among the lactating mothers, so as ascertain whether vit B12 deficient mothers present with megaloblastic anaemia or not.

MATERIALS AND METHODS

An observational cross-sectional study was conducted as a short-term project in the Department of Biochemistry in collaboration with postpartum clinic of Calcutta National Medical College, West Bengal, India. The study was carried out over a period of two months, from July 2022 to August 2022. The authors followed the guidelines of the Helsinki Declaration (1975, revised in 2013). Ethical approval was obtained from the Institutional Ethical Committee (IEC) (EC-CNMC/2022/19), and written informed consent was obtained from all participants in a language they understood.

Inclusion criteria: The study included apparently healthy lactating mothers who attended the postpartum clinic for a regular check-up at six weeks postpartum. Participants had to have a single term pregnancy with an uncomplicated course of gestation, be cooperative, and willing to donate a venous sample for analysis.

Exclusion criteria: Women with a history of multiple pregnancies, regular intake of any multivitamin supplements during and after delivery, acute illnesses such as fever, serious medical disorders like gestational diabetes, hypothyroidism, pregnancy-induced hypertension, hepatitis, renal insufficiency, coronary artery disease, any complications during pregnancy, or clinical evidence of blood-borne diseases like Human Immunodeficiency Virus (HIV), hepatitis B or C, syphilis were excluded from the study.

Sample size calculation: The required sample size (n) was calculated using the formula:

$n = z^2pq/d^2$

$n = (1.96)^2 \times 0.46 \times 0.54/(0.09)^2$

$n = 118$

where n is the sample size, d is the relative error (which is 20% of p), p is 0.46 [12], q is 1-p (0.54), and z is 1.96. This calculation was done considering a 95% confidence interval, two-tailed test. A final sample size of 121 participants was included in the study who fulfilled the selection criteria.

Study Procedure

After obtaining written informed consent, participants were interviewed using a predesigned and pretested schedule. In addition to age and gravida, a detailed history regarding the type of diet consumed (vegetarian/non vegetarian) was recorded, as a previous study suggests that vitamin B12 deficiency is more common in vegetarians [14]. The history of common symptoms of vitamin B12 deficiency, such as headache, fatigue, tingling, and numbness (paresthaesia), was also recorded [13].

Laboratory investigations: An 8 mL venous blood sample was collected from each participant following universal precautions for venipuncture. Of this, 5 mL was collected in a vacuum evacuated tube containing a clot activator (red topped), allowed to stand for 30 minutes, centrifuged at 3000 rpm for 10 minutes, and the serum was separated for biochemical analysis of serum total vitamin B12 and serum ferritin. The remaining 3 mL blood was collected in an Ethylenediaminetetraacetic Acid (EDTA) tube (lavender topped) for haematological estimations. All samples were analysed within 8 hours on the day of collection or stored at -20°C.

Parameters assessed: Serum total vit B12 was analysed using the Siemens ADVIA Centaur Immunoassay system by competitive immunoassay using direct chemiluminescent technology [15]. The reference intervals for serum total vitamin B12 were 200 to 700 pg/mL, and vitamin B12 deficiency was defined as serum vitamin B12 <200 pg/mL [5]. Serum ferritin was analysed using the Siemens ADVIA Centaur Immunoassay system two-site sandwich immunoassay with direct chemiluminometric technology [15]. The reference intervals for serum ferritin were 12-95 ng/mL [8].

Haematological parameters: It included Hb (reference interval: 12-16 g/dL), MCV (reference interval: 80-100 fL), MCH (reference interval: 27-32 pg), and MCHC, reference interval: 32-36 g/dL) [15], were measured using an automated cell counter. All laboratory investigations were performed using pre-validated standardised methods with a co-efficient of variation within 10%.

STATISTICAL ANALYSIS

The data were entered into a Microsoft Excel sheet and checked for any erroneous entries. The data were analysed using principles of descriptive and inferential statistics with Microsoft Excel and Statistical Package for Social Sciences (SPSS) version 20.0. The results were expressed in terms of percentages (%), means, Standard Deviations (SD), medians, and interquartile ranges. The data were presented in tabular form. The normality of the data was tested using the Kolmogorov-Smirnov and Shapiro-Wilk tests. For non parametric data, the Mann-Whitney test was applied and the bivariate association between dependent and independent variables was tested using Pearson's Chi-square test and Fisher's-exact test.

RESULTS

The prevalence of vit B12 deficiency (<200 pg/mL) among the 121 lactating mothers was calculated to be 19 (15.7%). Vitamin B12 deficiency was significantly higher among the multigravida mothers 15 (33.3%) out of 75, compared to primigravida mothers 4 (5.3%) out of 76. Additionally, 16 (53.3%) out of 30 vegetarian mothers were found to be vitamin B12 deficient, compared to only 3 (3.3%) out of 91 in the non vegetarian group (p <0.001). A significant association was found between the presence of tingling and numbness sensation and vitamin B12 deficiency (p-value= 0.005). None of the mothers complaining of fatigue were found to be vit B12 deficient. No significant association was found between the history of headaches among the two groups [Table/Fig-1]. Laboratory parameters of study subjects has been presented in [Table/Fig-2].

Factors	n (%)	Vit B12 deficient mothers, n (%)	p-value
Age (in years), Mean±SD	25.65±3.71		
Gravida			
1	76 (62.8)	4 (5.3)	<0.001*
≥2	45 (37.2)	15 (33.3)	
Dietary habits			
Non vegetarian	91 (75.2)	3 (3.3)	<0.001* (By Fisher's-exact test)
Vegetarian	30 (24.8)	16 (53.3)	
Headache			
Absent	55 (45.5)	8 (14.5)	0.806
Present	66 (54.4)	11 (16.7)	
'Pins and Needles' sensation			
Absent	63 (52.1)	4 (6.3)	0.005*
Present	58 (47.9)	15 (25.9)	
Fatigue			
Absent	95 (78.5)	19 (20)	0.012*
Present	26 (21.4%)	0	

[Table/Fig-1]: Distribution of study subjects according to their vitamin B12 status and different socioclinical characteristics.
(Pearson's Chi-square test) (N=121)

Parameters	Median (IQR)	Range	Reference intervals [8,15]
Vitamin B12 (pg/mL)	382 (324-553.50)	139-829	200-700
Hb (g/dL)	12 (11.5-13.1)	10.6-14.9	12-16
MCV (fL)	78.2 (75-81.1)	64.3-96.2	80-100
MCH (pg)	25.5 (23.4-27.7)	19.5-32.1	27-32
MCHC, g/dL	32 (30.6-32.7)	29.1-34.4	32-36
Ferritin, ng/mL	20.60 (14.95-30.7)	3.6-54.9	12-95

[Table/Fig-2]: Laboratory parameters of study subjects.
Hb: Haemoglobin; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration

In the present study, the vitamin B12 deficient mothers had significantly higher MCV, MCH, and MCHC values compared to the sufficient group (p-values 0.005, 0.001, and 0.001, respectively). However, there was no significant difference in Hb and ferritin levels between the two groups (p-values were 0.054 and 0.371, respectively) [Table/Fig-3].

Haematological parameter	Vit B12 status	Mean±mean value±SD	Mean rank	Mann-Whitney U	p-value
Hb, (g/dL)	Sufficient (n=102) Deficient (n=19)	12.15±1.08 12.76±1.25	58.36 75.18	699	0.054
MCV (fL)	Sufficient (n=102) Deficient (n=19)	77.49±6.26 83.34±6.88	57.11 81.89	572	0.005*
MCH (pg)	Sufficient (n=102) Deficient (n=19)	25.44±3.30 27.81±2.18	56.23 86.63	482	0.001*
MCHC (g/dL)	Sufficient (n=102) Deficient (n=19)	31.61±1.39 32.73±0.45	55.76 89.11	435	0.001*
Ferritin (ng/mL)	Sufficient (n=102) Deficient (n=19)	23.49±12.85 23.88±10.54	59.77 67.61	843	0.371

[Table/Fig-3]: Distribution of study subjects according to their vitamin B12 status and different haematological parameters.
Mann-Whitney U test; Hb: Haemoglobin; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration

DISCUSSION

The present study found the prevalence of vitamin B12 deficiency to be 15.7% among lactating mothers at six weeks postpartum. If borderline deficiency is considered, i.e., vit B12 values between 200 to 300 pg/mL, then around 21.5% of mothers would potentially be deficient [6]. The prevalence of vit B12 deficiency observed in the present study was comparatively lower than previously reported in the Indian population, which has ranged from 16% to 77% among adult Indians and has been linked to a vegetarian diet [6]. Similar studies documenting the prevalence of vit B12 deficiency during lactation have been tabulated in [Table/Fig-4] [4,8-10].

Authors of the study	Place/Year of study	Time of study (postpartum)	Sample size	Non vegetarian (%)	Prevalence of vit B12 deficiency (%)
Mittal M et al., [8]	Delhi, India/ 2017	1 to 6 months	100	54	46
Reischl-Hajiabadi AT et al., [9]	Germany/ 2022	0 to 20 weeks	121	66	41.1
Casterline JE et al., [10]	Guatemala/ 1997	3 months	113	--	33.3
Dhiman P et al., [4]	Puducherry, India/ 2021	6 weeks	434	95	3.5
Present study	Kolkata, India/ 2023	6 weeks	121	75	15.7

[Table/Fig-4]: Summary of studies documenting vitamin B12 deficiency status in lactating mothers [4,8-10].

In a study by Mittal M et al., out of 100 mothers with infants aged one to six months, 46% were found to be deficient in vitamin B12. Only 35.2% of non vegetarian mothers were deficient compared to 72.4% of vegetarian mothers [8]. A study from Germany detected 41.1% cobalamin deficiency in 121 mothers through newborn screening at 0-20 weeks postpartum [9]. Casterline JE et al., in a similar study evaluating the status of vitamin B12 in 113 lactating women and their infants at three months of lactation in Guatemala, reported a prevalence of 33.3% [10].

The comparatively lower prevalence of vit B12 deficiency in the present study group might be attributed to the predominance of a non vegetarian diet consumed by the mothers. Diet has consistently been a major factor in determining the prevalence of vitamin B12 status. The present study results were similar to a study from South India where the prevalence of vit B12 deficiency at six weeks postpartum in 434 mothers was only 3.5%. Around 95% of their study population consumed a mixed diet [4]. Similarly, 75% of the present study's population was on a non vegetarian diet, and vitamin B12 deficiency was significantly lower in this group than in the vegetarians. There was a lack of reference intervals for serum vitamin B12 during pregnancy and lactation. Using non pregnant cut-offs could have also led to an overestimation of deficiency status in these studies, as serum vitamin B12 values tend to decrease steadily during the course of pregnancy [7].

In the present study, authors observed that vit B12 deficiency was more common among multigravida mothers than primigravida mothers. The Estimated Average Requirement (EAR) for adults is set at 2.2 µg/day, based on the amount estimated to maintain normal serum vitamin B12 concentrations and normal haematological status in half of the adult population. The Recommended Dietary Allowance (RDA) is increased to 2.6 µg/day in pregnancy to support daily transfer to the foetus. The RDA for lactating women is 2.8 µg/day to replace secretion of the vitamin in breast milk [5]. If an adult woman is not properly supplied with the required vitamin B12 intake during the course of pregnancy and lactation, the mother may develop an underlying deficiency that could be worsened by another cycle of childbirth. It was also seen that 15 out of the 19 vitamin B12 deficient mothers complained of paresthesia. However, the literature suggests that neurological features develop in the early course of vit B12 deficiency compared to haematological findings [13].

Approximately 47% of the population was anaemic, with Hb concentrations less than 12 gm%. The median values of other haematological parameters such as MCV, MCH, MCHC, and ferritin were on the lower side, indicating that iron deficiency anaemia was rampant among the study subjects. None of the deficient mothers had megaloblastic features (MCV>100 fL). Surprisingly, the MCV and MCHC values were significantly higher in the cobalamin deficient group compared to the sufficient group, though the individual values were within the normal range. This may be because the haematologic changes caused by vit B12 deficiency may be masked by concomitant iron deficiency [11]. Iron deficiency anaemia can obscure the presence of macrocytosis. In women with anaemia, the co-existence of iron, folate, and vit B12 deficiency has been reported in previous studies [13]. This also implies that the vit B12 status of the mother needs to be determined even in the presence of iron deficiency anaemia.

The vit B12 is neither routinely estimated nor supplemented during pregnancy or lactation. However, the present study has documented that almost one-sixth of the lactating mothers were cobalamin deficient. These mothers had not been identified and replenished during early gestation, and because of the further increased demand, the vit B12 deficiency might have aggravated over the course of pregnancy and manifested during lactation. Researchers have found lower cord blood vit B12 and higher urinary methylmalonic acid levels in infants born to deficient mothers [8]. A vit B12 deficient mother also has a lower vit B12 concentration in breast milk [9]. Moreover, an infant during the first six months depends only on exclusive breastfeeding and fails to get adequate vit B12 from other dietary sources. In fact, a study by Taneja S et al., from Delhi, India showed that the median level of cobalamin was much lower in breastfed children compared to non breastfed children [16]. Not only in children, but vit B12 deficiency has also been associated with maternal postpartum depression [4].

Vitamin B12 deficiency might have a catastrophic effect on the child's neurological and haematological development and result in long-term sequelae. Many children with symptomatic vitamin B12 deficiency show improvement in clinical symptoms after supplementation with the vitamin. However, long-term neurological and intellectual outcomes following severe deficiency are poor, resulting in permanent deficits [13]. So, the earlier the intervention,

the better the outcome for the child. But early detection during pregnancy is complicated because there is no consensus on the cut-off or reference intervals of serum vit B12 or the functional biochemical markers to correctly diagnose B12 deficiency during pregnancy [7].

As the serum vit B12 levels return to pre-pregnancy levels within a few weeks postpartum, these deficient women can be identified during their first visit to the postpartum clinic at six weeks. Since there are no guidelines for routine estimation of serum vit B12 in lactating mothers, efforts may be targeted to screen them, with special emphasis given to those who were vegetarian, multigravida, or complaining of paresthesia, as per the present study. The haematological parameters were of limited use in diagnosing cobalamin deficiency among mothers, possibly because of the concomitant presence of iron, folate, and vit B12 deficiency, and also because vitamin B12-dependent anaemia does not develop until late in the course of deficiency [13].

Limitation(s)

The present study has some limitations. Firstly, the functional markers of vitamin B12 deficiency, such as holotranscobalamin and Methylmalonic Acid (MMA), were not measured. Secondly, correlating maternal serum vitamin B12 levels with maternal breast milk vitamin B12 levels and urinary MMA in infants could have strengthened the study. Thirdly, the study sample was not representative of the whole population.

CONCLUSION(S)

The present study found that 15.7% of lactating mothers who came to the postpartum clinic for their first visit (six weeks postpartum) were vitamin B12 deficient. Factors such as vegetarianism, multiple pregnancies, and complaints of paresthesia were associated with cobalamin deficiency, and these factors could be considered during screening of mothers. Haematological parameters were not helpful in identifying vit B12 deficiency in these mothers. Further research is needed to determine the cut-offs for diagnosing vitamin B12 deficiency during pregnancy and lactation, to correlate functional markers of vit B12 deficiency with serum vitamin B12 levels in mothers, and to compare maternal vitamin B12 levels with infant vit B12 deficiency.

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PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Biochemistry, Calcutta National Medical College, Kolkata, West Bengal, India.
2. Undergraduate Student, Department of Biochemistry, Calcutta National Medical College, Kolkata, West Bengal, India.
3. Associate Professor, Department of Obstetrics and Gynaecology, Calcutta National Medical College, Kolkata, West Bengal, India.
4. Assistant Professor, Department of Community Medicine, Bankura Sammilani Medical College, Bankura, West Bengal, India.
5. Professor, Department of Biochemistry, Calcutta National Medical College, Kolkata, West Bengal, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Surajit Lahiri,
23/2/1, D.P.P Road, Kolkata-700047, West Bengal, India.
E-mail: drlahiri83@gmail.com

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