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Original Article

Prospective Evaluation of Hematological Biomarkers and Maternal–Infant Vitamin B12 Status in Exclusively Breastfed Infants

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ABSTRACT

Introduction: Vitamin B12 deficiency during early infancy is an important yet frequently under-recognized nutritional problem, particularly among exclusively breastfed infants whose micronutrient status depends largely on maternal under-recognized nutritional problem, particularly among exclusively breastfed infants whose micronutrient status depends largely on maternal vitamin B12 stores, dietary practices, antenatal supplementation and lactational adequacy. The nonspecific clinical presentation of deficiency may delay diagnosis, especially in resource-limited pediatric settings. Routine hematological biomarkers may provide useful screening support, but their interpretation must be integrated with maternal–infant biochemical assessment.

Aim: To prospectively evaluate routine hematological biomarkers and maternal–infant vitamin B12 status for early detection and monitoring of vitamin B12 deficiency in exclusively breastfed infants.

Methods: This prospective descriptive hospital-based study included 150 exclusively breastfed infant–mother dyads attending pediatric outpatient, immunization and inpatient services of a tertiary care hospital during the approved study period after institutional ethics approval. Infants aged 1–6 months who were exclusively breastfed were enrolled after written maternal consent. Clinical history, maternal dietary pattern, infant feeding details, anthropometry, examination findings, infant complete blood count and serum vitamin B12 levels of both mother and infant were recorded. Infant vitamin B12 status was categorized as deficient, borderline or sufficient. Hematological biomarkers were compared across vitamin B12 categories, and maternal–infant concordance was assessed.

Results: Among 150 exclusively breastfed infants, 68 (45.3%) were vitamin B12 deficient, 36 (24.0%) had borderline levels and 46 (30.7%) were sufficient. Maternal vitamin B12 deficiency was observed in 74 mothers (49.3%). Among infants of vitamin B12-deficient mothers, 52 (70.3%) were also deficient, showing significant maternal–infant concordance (χ^2=51.86, $p<0.001$). Pallor was observed in 42 (28.0%) infants, poor weight gain in 31 (20.7%), irritability or lethargy in 27 (18.0%) and developmental concern in 24 (16.0%). Anemia was present in 62 (41.3%), macrocytosis in 43 (28.7%) and raised red cell distribution width in 57 (38.0%). Deficient infants had lower hemoglobin and higher mean corpuscular volume and red cell distribution width than sufficient infants.

Conclusion: Vitamin B12 deficiency was common among exclusively breastfed infants and was strongly linked with maternal deficiency. Maternal–infant dyad assessment, supported by routine hematological biomarkers, may improve early recognition and monitoring.

Keywords: *Vitamin B12 deficiency; Exclusively breastfed infants; Maternal vitamin B12; Hematological biomarkers; Infant nutrition.*

INTRODUCTION

Vitamin B12 is an essential water-soluble micronutrient required for DNA synthesis, erythropoiesis, myelination and normal neurodevelopment during infancy. The first six months of life represent a period of rapid hematological maturation and brain growth, making adequate vitamin B12 availability especially important. In early infancy, vitamin B12 status is largely determined by maternal stores during pregnancy, placental transfer, breast milk vitamin B12 concentration and maternal dietary intake during lactation. Exclusively breastfed infants are therefore physiologically dependent on maternal micronutrient adequacy, and infants born to deficient mothers may develop vitamin B12 deficiency even when breastfeeding is otherwise appropriate [1]. This issue is clinically relevant in settings where vegetarian-predominant dietary patterns, limited animal-source food intake, inadequate antenatal supplementation and low awareness of vitamin B12 deficiency may reduce maternal stores [2]. Micronutrient deficiencies have been reported among exclusively breastfed infants when maternal nutritional status is suboptimal, reinforcing the importance of viewing infant deficiency through the lens of maternal nutrition [3]. Evidence from mother–infant studies has also shown a close relationship between maternal and infant vitamin B12 levels, supporting dyadic evaluation rather than isolated infant testing [4]. Breastfeeding-related biomarker studies have further demonstrated that infant cobalamin status is influenced by maternal biochemical status and feeding pattern [5]. Clinically, vitamin B12 deficiency in infancy may present with pallor, poor feeding, irritability, lethargy, hypotonia, vomiting, developmental delay, poor weight gain or recurrent illness. However, many symptoms are nonspecific, and neurological manifestations may appear before classical macrocytic anemia becomes evident. A systematic review of infant vitamin B12 deficiency has highlighted wide variability in biochemical, nutritional and clinical presentations, making early recognition challenging [6]. Maternal vitamin B12 status during pregnancy has been identified as an important determinant of infant deficiency, and early detection strategies have been suggested to prevent progression to severe neurological or hematological manifestations [7]. Dietary intake remains a key modifiable determinant of vitamin B12 status among pregnant women, lactating mothers and infants dependent on maternal stores [8]. Case-based evidence has repeatedly shown that severe deficiency may involve both neurological and hematological systems, emphasizing the need for early clinical suspicion [9]. Newborn and infant biochemical screening studies have also demonstrated that infant deficiency may reveal unrecognized maternal deficiency [10,11]. Among breastfed infants, maternal vitamin B12 status has been linked with neurodevelopmental outcomes, further supporting timely diagnosis and correction [12]. Indian pediatric evidence has documented vitamin B12 deficiency among exclusively breastfed infants, making this a relevant concern for routine pediatric practice [13]. Although serum vitamin B12 estimation remains central to diagnosis, it may not always be requested at first presentation. Routine complete blood count parameters such as hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and red cell distribution width may provide practical early clues, particularly in resource-constrained settings; however, biochemical deficiency may occur before overt hematological changes develop [14]. Feeding-pattern studies have also shown that exclusively breastfed infants may have different vitamin B12 profiles compared with infants receiving additional dietary sources [15]. The novelty of this study lies in its prospective assessment of exclusively breastfed infant–mother dyads, its focus on routine hematological biomarkers as accessible screening and monitoring tools, and its evaluation of maternal–infant vitamin B12 concordance in early infancy.

AIM

To prospectively evaluate routine hematological biomarkers and maternal–infant vitamin B12 status for early detection and monitoring of vitamin B12 deficiency in exclusively breastfed infants.

MATERIALS AND METHODS

This prospective descriptive hospital-based study was conducted among 150 exclusively breastfed infant–mother dyads attending the pediatric outpatient department, immunization clinic and pediatric inpatient services of a tertiary care hospital during the approved study period after institutional ethics approval. Infants aged 1–6 months who had been exclusively breastfed since birth and whose mothers provided written informed consent were included. Exclusive breastfeeding was defined as receipt of only breast milk without formula feeds, animal milk, semisolid feeds or complementary foods, except prescribed medicines or vitamin drops where applicable. Infants who had received prior therapeutic vitamin B12 supplementation, those already receiving formula or complementary feeding, and those with major congenital malformations, known hemolytic anemia, chronic liver disease, chronic kidney disease, severe acute infection requiring intensive care, history of blood transfusion within the preceding three months, or maternal refusal for participation were excluded.

After enrolment, maternal and infant details were recorded using a structured case record form. Infant variables included age, gender, birth order, birth weight, gestational maturity, feeding history, pallor, feeding difficulty, irritability, lethargy, vomiting, recurrent illness, poor weight gain and developmental concern. Maternal variables included dietary pattern, antenatal supplementation history and relevant socioeconomic background. All infants underwent general physical examination, anthropometric assessment and systemic examination, with specific attention to pallor, growth status, tone and developmental concerns.

Venous blood samples were collected under aseptic precautions from both mother and infant for serum vitamin B12 estimation. Infant complete blood count was performed to assess hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and red cell distribution width. Mean corpuscular hemoglobin concentration was not included in the final analysis to maintain consistency with the reported biomarker tables. Infant vitamin B12 status was categorized as deficient when serum vitamin B12 was <200 pg/mL, borderline when 200–300 pg/mL and sufficient when >300 pg/mL. Maternal vitamin B12 deficiency was defined as serum vitamin B12 <200 pg/mL. Anemia was interpreted using age-appropriate pediatric hemoglobin values, macrocytosis was considered when mean corpuscular volume exceeded the expected infant reference range, and raised red cell distribution width was considered suggestive of anisocytosis.

Infants identified as vitamin B12 deficient were managed according to institutional pediatric protocol. Clinical and hematological response was assessed at scheduled follow-up after initiation of treatment, where applicable. Clinical improvement, hemoglobin improvement and persistence of symptoms requiring further evaluation were documented.

Data were entered in Microsoft Excel and analyzed using appropriate statistical methods. Categorical variables were expressed as frequency and percentage. Continuous variables were expressed as mean and standard deviation. Association between maternal and infant vitamin B12 status was assessed using the chi-square test. Hematological parameters were compared across infant vitamin B12 categories using appropriate statistical tests. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 150 exclusively breastfed infant–mother dyads were analyzed. The results are presented across demographic and clinical characteristics, maternal–infant vitamin B12 status, hematological biomarkers and early monitoring outcomes after detection of deficiency.

Table 1: Demographic and Clinical Profile of Exclusively Breastfed Infants

Variable	Category	Frequency	Percentage
Age group	1–2 months	38	25.3%
	3–4 months	67	44.7%
	5–6 months	45	30.0%
Gender	Male	82	54.7%
	Female	68	45.3%
Birth order	First born	59	39.3%
	Second born	64	42.7%
	Third or higher	27	18.0%
Gestational status	Term	132	88.0%
	Late preterm	18	12.0%
Birth weight	≥2.5 kg	121	80.7%
	<2.5 kg	29	19.3%
Maternal diet	Vegetarian predominant	96	64.0%
	Mixed diet	54	36.0%

Most infants were aged 3–4 months (44.7%), followed by 5–6 months (30.0%) and 1–2 months (25.3%). Males constituted 54.7% of the cohort. Term infants formed the majority (88.0%), while 19.3% had birth weight below 2.5 kg. A vegetarian-predominant maternal dietary pattern was reported by 64.0% of mothers, a clinically relevant observation given the dependence of exclusively breastfed infants on maternal vitamin B12 stores.

Table 2: Clinical Manifestations Observed Among Infants

Clinical Feature	Frequency	Percentage
Pallor	42	28.0%
Irritability or lethargy	27	18.0%
Feeding difficulty	21	14.0%
Developmental concern	24	16.0%
Poor weight gain	31	20.7%
Hypotonia	16	10.7%
Recurrent vomiting	18	12.0%
No obvious clinical symptom	58	38.7%

Clinical manifestations were variable and often nonspecific. Pallor was the most frequent finding (28.0%), followed by poor weight gain (20.7%), irritability or lethargy (18.0%), developmental concern (16.0%) and feeding difficulty (14.0%). Importantly, 38.7% of infants had no obvious clinical symptom, indicating that clinically silent deficiency may occur during early infancy and may be missed without biochemical or hematological assessment.

Table 3: Maternal and Infant Vitamin B12 Status

Vitamin B12 Status	Infant Serum Vitamin B12	Maternal Serum Vitamin B12
Deficient, <200 pg/mL	68 (45.3%)	74 (49.3%)
Borderline, 200–300 pg/mL	36 (24.0%)	32 (21.3%)
Sufficient, >300 pg/mL	46 (30.7%)	44 (29.3%)
Mean ± SD, pg/mL	236.8 ± 91.4	228.5 ± 86.7

Vitamin B12 deficiency was identified in 45.3% of infants and 49.3% of mothers. Borderline vitamin B12 levels were present in 24.0% of infants and 21.3% of mothers. Mean infant serum vitamin B12 was 236.8 ± 91.4 pg/mL, while mean maternal serum vitamin B12 was 228.5 ± 86.7 pg/mL, reflecting a substantial burden of dyadic deficiency.

Table 4: Concordance Between Maternal and Infant Vitamin B12 Deficiency

Maternal Vitamin B12 Status	Infant Deficient	Infant Borderline	Infant Sufficient	Total
Maternal deficient	52 (70.3%)	15 (20.3%)	7 (9.5%)	74
Maternal borderline	11 (34.4%)	14 (43.8%)	7 (21.9%)	32
Maternal sufficient	5 (11.4%)	7 (15.9%)	32 (72.7%)	44
Total	68	36	46	150

Chi-square test: $\chi^2 = 51.86$, $p < 0.001$.

A strong and statistically significant concordance was observed between maternal and infant vitamin B12 status. Among 74 vitamin B12-deficient mothers, 52 infants (70.3%) were also deficient. In contrast, among 44 vitamin B12-sufficient mothers, only 5 infants (11.4%) were deficient and 32 (72.7%) were sufficient. This supports maternal vitamin B12 status as a major determinant of infant vitamin B12 stores during exclusive breastfeeding.

Table 5: Routine Hematological Biomarkers According to Infant Vitamin B12 Status

Hematological Parameter	Deficient Infants (n=68)	Borderline Infants (n=36)	Sufficient Infants (n=46)	p-value
Hemoglobin, g/dL	9.8 ± 1.2	10.5 ± 1.1	11.2 ± 1.0	<0.001
Mean corpuscular volume, fL	88.6 ± 8.4	83.7 ± 7.2	78.9 ± 6.8	<0.001
Mean corpuscular hemoglobin, pg	29.4 ± 3.6	27.8 ± 3.1	26.2 ± 2.8	0.002
Red cell distribution width, %	16.8 ± 2.4	15.2 ± 2.1	13.9 ± 1.8	<0.001
Platelet count, lakh/mm³	3.42 ± 0.91	3.58 ± 0.87	3.65 ± 0.82	0.312

Vitamin B12-deficient infants had significantly lower hemoglobin and higher mean corpuscular volume, mean corpuscular hemoglobin and red cell distribution width than sufficient infants. Platelet count did not differ significantly across vitamin B12 categories. These findings suggest that routine hematological biomarkers may support early suspicion of deficiency, although biochemical confirmation remains necessary.

Table 6: Frequency of Hematological Abnormalities and Early Monitoring After Vitamin B12 Detection

Parameter	Frequency	Percentage
Anemia	62	41.3%
Macrocytosis	43	28.7%
Raised red cell distribution width	57	38.0%
Combined anemia with macrocytosis	34	22.7%
Vitamin B12 deficient infants started on treatment	68	100.0%
Clinical improvement at follow-up among deficient infants	55/68	80.9%
Hemoglobin improvement at follow-up among deficient infants	49/68	72.1%
Persistent symptoms requiring further evaluation	9/68	13.2%

Anemia was present in 41.3% of infants, macrocytosis in 28.7% and raised red cell distribution width in 38.0%. Combined anemia with macrocytosis was observed in 22.7%. All vitamin B12-deficient infants were started on treatment. At scheduled follow-up, 80.9% showed clinical improvement and 72.1% showed hemoglobin improvement, while 13.2% had persistent symptoms requiring further evaluation.

DISCUSSION

Vitamin B12 deficiency in early infancy is clinically important because it may affect both hematological maturation and neurodevelopment during a period of rapid growth. The exclusive breastfeeding period is nutritionally sensitive, as infant vitamin B12 status depends heavily on maternal stores and breast milk concentration. The present prospective evaluation of 150 exclusively breastfed infant–mother dyads demonstrates three important findings: first, vitamin B12 deficiency was frequent among both infants and lactating mothers; second, maternal and infant vitamin B12 status showed strong concordance; and third, routine hematological biomarkers, particularly hemoglobin, mean corpuscular volume and red cell distribution width, showed meaningful differences across infant vitamin B12 categories.

The demographic profile provides important context for interpreting the biochemical findings. Most infants were aged 3–4 months, and nearly two-thirds of mothers reported a vegetarian-predominant dietary pattern. Since vitamin B12 is primarily derived from animal-source foods, vegetarian-predominant diets may increase the risk of depleted maternal stores, especially when antenatal or lactational supplementation is inadequate. Bjørkevoll et al. reported that infant vitamin B12 status is influenced by maternal and nutritional determinants [1]. Ata et al. similarly identified maternal vitamin B12 level during pregnancy as an important determinant of infant deficiency [2]. Dörtkardeşler et al. observed that micronutrient deficiencies may occur in exclusively breastfed infants when maternal nutrition is suboptimal [3]. Singhal et al. further supported the importance of mother–infant vitamin B12 assessment by demonstrating correlation between maternal and infant vitamin B12 levels [4]. These findings align with the dyadic pattern observed in the present study.

The burden of biochemical deficiency was substantial. Nearly half of infants were vitamin B12 deficient, and another one-fourth had borderline levels. Maternal deficiency was similarly frequent. This distribution suggests that vitamin B12 inadequacy in early infancy may be more common than clinically suspected, particularly among exclusively breastfed infants whose mothers have dietary or nutritional risk factors. Solvik et al. demonstrated that breastfeeding is closely linked with infant biomarkers of folate and cobalamin status [5]. Wirthensohn et al., in a systematic review, emphasized the heterogeneity of infant vitamin B12 deficiency, with wide variability in clinical and biochemical presentation [6]. Bjørkevoll et al. also described maternal–infant relationships in vitamin B12 and folate status [7], while Bärebring et al. highlighted dietary intake as a major determinant of vitamin B12 status in susceptible groups [8]. The present findings extend this evidence by focusing specifically on exclusively breastfed infant–mother dyads in a hospital-based pediatric setting.

Clinical manifestations were nonspecific. Pallor, poor weight gain, irritability or lethargy, developmental concern and feeding difficulty were observed, but more than one-third of infants had no obvious clinical symptom. This is a crucial finding because symptom-based recognition alone may miss early deficiency. Sharma et al. described combined neurological and hematological manifestations in infant vitamin B12 deficiency and emphasized that diagnosis may be delayed when early symptoms are subtle [9]. Reischl-Hajiabadi et al. showed that maternal vitamin B12 deficiency may be detected through infant biochemical abnormalities identified by newborn screening [10]. Pinto et al. highlighted the benefit of early detection in preventing progression to more severe manifestations [11]. The asymptomatic proportion in the current cohort reinforces the need for a lower diagnostic threshold in exclusively breastfed infants, particularly when maternal dietary risk is present.

The strongest finding was the statistically significant concordance between maternal and infant vitamin B12 status. Among vitamin B12-deficient mothers, 70.3% of infants were also deficient, whereas most infants of vitamin B12-sufficient mothers were sufficient. This finding supports a maternal–infant dyad model of assessment. Treating infant deficiency without identifying maternal deficiency may result in incomplete correction of the underlying nutritional problem. Keskin et al. reported an association between maternal vitamin B12 status and infant neurodevelopment in breastfed infants with deficiency [12]. Kadiyala et al. documented vitamin B12 deficiency among exclusively breastfed infants, highlighting the vulnerability of this feeding group [13]. El Hasbaoui et al. described the broad clinical spectrum of infant vitamin B12 deficiency [14], while Dağ et al. observed variation in infant vitamin B12 levels according to feeding patterns [15]. Taken together, these findings support simultaneous maternal and infant evaluation in suspected or confirmed cases.

Routine hematological biomarkers showed practical diagnostic relevance. Vitamin B12-deficient infants had lower hemoglobin and higher mean corpuscular volume, mean corpuscular hemoglobin and red cell distribution width compared with sufficient infants. The pattern is biologically plausible because impaired DNA synthesis due to vitamin B12 deficiency can lead to ineffective erythropoiesis, macrocytosis and anisocytosis. Anemia was present in 41.3%, macrocytosis in 28.7% and raised red cell distribution width in 38.0%. However, macrocytosis was not universal, indicating that absence of classical macrocytic anemia should not exclude deficiency. This is particularly important in early infancy, where hematological indices may be influenced by age, growth rate, iron status and evolving erythropoiesis. Routine complete blood count parameters are therefore best interpreted as supportive screening and monitoring tools rather than confirmatory diagnostic markers.

The monitoring findings add clinical value to the study. All deficient infants received treatment as per institutional protocol. Most showed clinical improvement, and nearly three-fourths demonstrated hemoglobin improvement at follow-up. Persistent symptoms in a minority highlight the need for continued clinical surveillance, assessment of compliance, evaluation for coexisting nutritional deficiencies and developmental monitoring where indicated. The prospective design strengthens this observation because it links baseline detection with early post-treatment monitoring rather than limiting the study to cross-sectional biochemical description.

The study has several practical implications. Exclusive breastfeeding should continue to be promoted as the optimal infant feeding practice, but clinicians must recognize that exclusively breastfed infants can still develop vitamin B12 deficiency if maternal stores are inadequate. Maternal dietary history, antenatal supplementation history and subtle infant symptoms

should be incorporated into routine pediatric assessment. In settings where serum vitamin B12 testing is not immediately available, hemoglobin, mean corpuscular volume and red cell distribution width may help identify infants requiring biochemical evaluation. The most useful clinical approach is not infant-only screening, but maternal–infant dyad assessment with correction of deficiency in both when required.

The strengths of the study include its prospective design, exclusive focus on breastfed infants, simultaneous maternal–infant biochemical assessment and evaluation of routine hematological biomarkers that are widely available in pediatric practice. The dyad-based approach is particularly important because it reflects the biological dependence of infant vitamin B12 status on maternal nutrition during pregnancy and lactation.

The study also has limitations. Being hospital-based, the findings may not reflect community prevalence. Functional biomarkers such as methylmalonic acid and homocysteine were not assessed. Neurodevelopmental evaluation was based on clinical concern rather than standardized developmental testing. Follow-up outcomes were limited to early clinical and hematological response and did not include long-term developmental assessment. Despite these limitations, the findings provide clinically useful evidence supporting early recognition of vitamin B12 deficiency among exclusively breastfed infants using combined maternal, biochemical and hematological evaluation.

CONCLUSION

Vitamin B12 deficiency was common among exclusively breastfed infants and showed strong concordance with maternal vitamin B12 deficiency, emphasizing the importance of maternal–infant dyad assessment. Routine hematological biomarkers, particularly hemoglobin, mean corpuscular volume and red cell distribution width, may support early suspicion and monitoring, but serum vitamin B12 estimation remains essential for confirmation and treatment planning.

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