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Original Research Article

Comparison of serum vitamin B12 levels in exclusively breastfed and mixed-fed term infants aged 3-6 months: a hospital-based observational study

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ABSTRACT

Background: Vitamin B12 is essential for DNA synthesis, neurological development, red blood cell productio- and myelination during infancy. Infants depend mainly on maternal stores and breast milk during the first few months of life. Maternal deficiency is common in vegetarian populations and may predispose infants to deficiency.
Methods: This hospital-based observational study was conducted in the Department of Paediatrics, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, from July 2024 to December 2025. A total of 80 term infants aged 3-6 months were enrolled. Forty infants were exclusively breastfed and forty infants were mixed-fed. Serum vitamin B12 levels, complete blood count parameters, feeding history and maternal dietary practices were assessed.
Results: Mean serum vitamin B12 was significantly lower in exclusively breastfed infants (379.90±205.60 pg/ml) compared with mixed-fed infants (514.85±227.73 pg/ml). Vitamin B12 deficiency (<200 pg/ml) was found in 22.5% of exclusively breastfed infants and 5% of mixed-fed infants. Maternal vegetarian diet was significantly associated with lower infant vitamin B12 levels. Exclusive breastfeeding was identified as an independent predictor of vitamin B12 deficiency.
Conclusions: Exclusively breastfed infants, particularly those born to vegetarian mothers, are at increased risk of vitamin B12 deficiency. Early screening, maternal nutritional counselling and timely supplementation may help prevent long-term hematological and neurological complications.
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Keywords: Breastfeeding, Infant nutrition, Mixed feeding, Neurodevelopment, Serum vitamin B12, Vitamin B12 deficiency

INTRODUCTION

Vitamin B12 is a water-soluble vitamin that is essential for normal hematopoiesis, DNA synthesis, neurological development and myelin formation. It acts as a cofactor in important metabolic pathways involved in cell division and nervous system maturation. During infancy, adequate vitamin B12 levels are crucial because the brain is undergoing rapid growth and development.[1] Since it is not synthesized in the human body, infants primarily rely on dietary intake, including breast milk, for their Vitamin B12 requirements. World-wide, exclusive breast feeding is being vigorously promoted in view of its tremendous benefits. However, in low to middle income countries the milk of mothers may be deficient in Vitamin B12.[7,8] If their infants are exclusively breast fed (EBF), they may develop B12 deficiency.[9] The recommended dietary allowance (RDA) of vitamin B12 varies by age. For infants aged 0-6 months, the requirement is 0.4µg per day, while for those aged 7-12 months it is 0.5µg per day.

In early childhood, the RDA increases to 0.9 µg per day for ages 1-3 years and 1.2µg per day for ages 4-8 years. For older children, the requirement rises to 1.8µg per day for ages 9-13 years, and during adolescence (14-18 years), the recommended intake reaches 2.4µg per day.

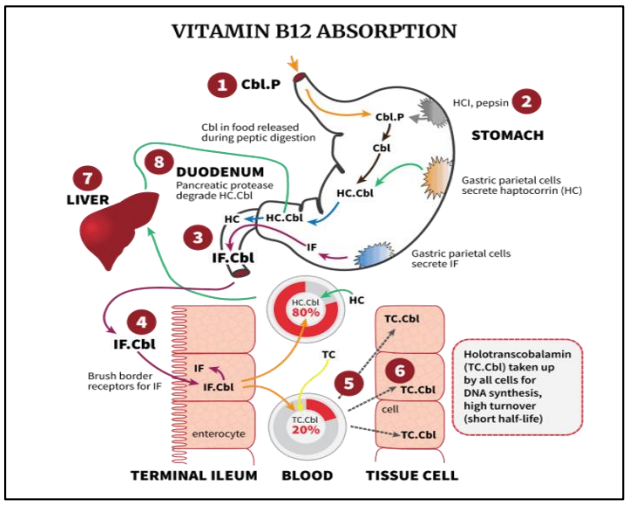

Figure 1: Absorption of vitamin B12.[10]

Infant feeding only from breast milk is defined as "exclusive breastfeeding", except any syrup or drops consisting of any vitamin, medicine or mineral supplements.[10] Infants are born with limited vitamin B12 stores and depend on maternal transfer during pregnancy and breast milk after birth. Therefore, maternal vitamin B12 deficiency may directly influence infant vitamin B12 status. This issue is especially important in countries like India, where vegetarian diets are common and intake of animal-source foods may be inadequate.[2]

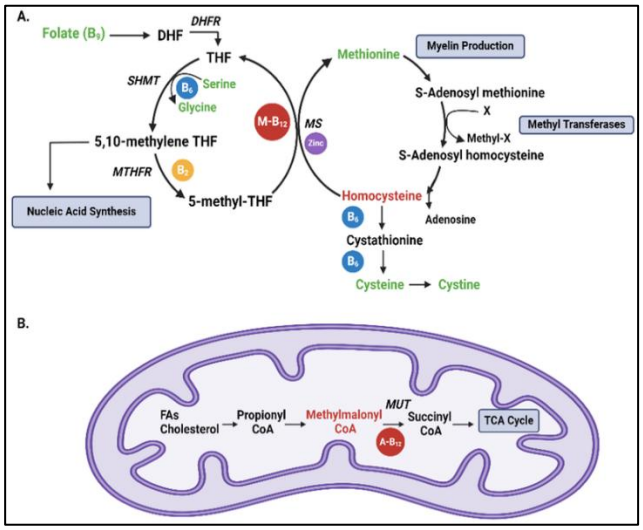

Figure 2: Vitamin B12 dependent reactions in body.[12]

Vitamin B12 deficiency during infancy may present with a wide spectrum of manifestations, including megaloblastic anemia, pallor, poor feeding, developmental delay, apathy, hypotonia, tremors, seizures, and delayed milestones. In severe cases, neurological impairment may become irreversible.[3] Although exclusive breastfeeding is the recommended feeding practice during the first six months of life, the vitamin B12 content of breast milk depends on maternal nutritional status. Mixed-fed infants may receive additional vitamin B12 through animal milk or fortified formula milk, which may protect them against deficiency.[4] The present study was conducted to compare serum vitamin B12 levels between exclusively breastfed and mixed-fed term infants aged 3-6 months and to evaluate the role of maternal dietary practices in determining infant vitamin B12 status.

METHODS

This hospital-based observational study was carried out in the Department of Paediatrics, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, over a period extending from July 2024 to December 2025.

A total of 80 term infants aged between 3 and 6 months were included in the study. The infants were divided into two groups. Group 1 consisted of 40 exclusively breastfed infants, whereas Group 2 consisted of 40 mixed-fed infants receiving breast milk along with formula milk or animal milk. Infants with prematurity, low birth weight, congenital malformations, chronic systemic illnesses, severe acute malnutrition, serious infections, or previous vitamin supplementation were excluded from the study. Detailed demographic information, feeding history, maternal diet, anthropometric measurements, socioeconomic status and clinical examination findings were recorded using a predesigned proforma. Blood samples were collected under aseptic precautions. Serum vitamin B12 levels were measured along with complete blood counts and peripheral smear examination.

Serum vitamin B12 levels were categorized as normal (>300 pg/ml), borderline (200-300 pg/ml), and deficient (<200 pg/ml). Statistical analysis was performed using SPSS version 30.0.0. Continuous variables were expressed as mean±standard deviation and compared using independent sample t-test. Categorical variables were compared using Chi-square test or Fisher's exact test. Logistic regression analysis was performed to identify independent predictors of vitamin B12 deficiency. A p value of less than 0.05 was considered statistically significant.

RESULTS

The present study included 80 term infants aged between 3 and 6 months, with 40 infants in each feeding group. The age distribution was comparable between the two groups. In Group 1, 45% of infants were aged 3-4 months and 55% were aged 5-6 months. In Group 2, 40% of

infants were aged 3-4 months and 60% were aged 5–6 months.

Table 1: Demographic characteristics of study population.

Variables	Group 1 (n=40) N (%)	Group 2 (n=40) N (%)	P value
Age 3-4 months	18 (45)	16 (40)	
Age 5-6 months	22 (55)	24 (60)	0.65
Male	22 (55)	21 (52.5)	
Female	18 (45)	19 (47.5)	0.82
Rural residence	28 (70)	26 (65)	0.63

*p value <0.05 considered significant.

The age distribution was comparable between the two groups, with most infants belonging to the 5-6 months age group. Male predominance was observed in both groups, and the majority of infants belonged to rural areas. No statistically significant difference was observed in demographic characteristics between the two groups.

Table 2: Comparison of mean serum vitamin B12 levels between groups.

Parameters	Group 1	Group 2	T value	P value
Mean serum vitamin B12 (pg/ml)	379.90± 205.60	514.85± 227.73	2.79	0.0066

*p value <0.05 considered significant.

Exclusively breastfed infants had significantly lower mean serum vitamin B12 levels compared to mixed-fed infants. The difference between the two groups was statistically significant. Gender distribution was also comparable between the groups. Male infants constituted 55% of group 1 and 52.5% of group 2. Most infants belonged to rural areas. Mean serum vitamin B12 level was significantly lower in exclusively breastfed infants compared with mixed-fed infants.

Vitamin B12 deficiency was more common among exclusively breastfed infants. Among mixed-fed infants, those receiving formula milk had significantly higher serum vitamin B12 levels than infants receiving animal milk.

Maternal vegetarian diet was associated with significantly lower infant vitamin B12 levels. Vitamin B12 deficiency was more common among exclusively breastfed infants, whereas most mixed-fed infants had normal Vitamin B12 levels. The difference in deficiency status between the groups was statistically significant.

Table 3: Vitamin B12 deficiency status in both groups.

Vitamin B12 status	Group 1 N (%)	Group 2 N (%)	P value
Normal (>300 pg/ml)	22 (55)	30 (75)	0.048
Borderline (200-300 pg/ml)	9 (22.5)	8 (20)	
Deficient (<200 pg/ml)	9 (22.5)	2 (5)	

*p value <0.05 considered significant.

Table 4: Comparison of vitamin B12 levels according to maternal diet.

Maternal diet	Mean vitamin B12 (pg/ml)	T value	P value
Vegetarian	327.4±174.1	2.73	0.017
Non-vegetarian	537.3±220.9		

*p value <0.05 considered significant.

Infants born to vegetarian mothers had significantly lower mean serum vitamin B12 levels compared to infants born to non-vegetarian mothers. This association was statistically significant.

Table 5: Comparison of vitamin B12 levels according to type of mixed feeding.

Type of feeding	Mean vitamin B12 (pg/ml)	T value	P value
Breastfeeding+ animal milk	446.68±210.62	2.62	0.012
Breastfeeding+ formula milk	628.46±215.15		

*p value <0.05 considered significant.

Among mixed-fed infants, those receiving formula milk in addition to breastfeeding had significantly higher vitamin B12 levels compared to those receiving animal milk along with breastfeeding.

Table 6: Logistic regression analysis for vitamin B12 deficiency.

Variable	Odds ratio	95% confidence interval	P value
Exclusive feeding	5.52	1.11-27.44	0.037

*p value <0.05 considered significant.

Logistic regression analysis showed that exclusive breastfeeding was an independent predictor of vitamin B12 deficiency, with exclusively breastfed infants having higher odds of deficiency compared to mixed-fed infants.

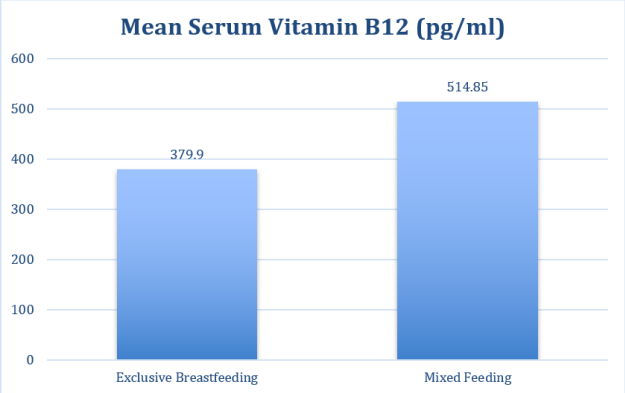

Figure 1: Comparison of mean serum vitamin B12 levels in both groups.

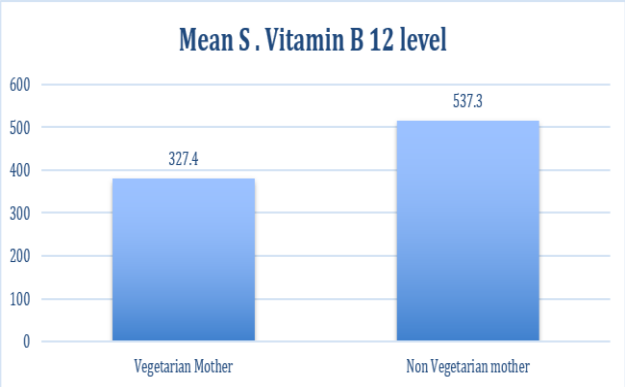

Figure 2: Association of maternal dietary pattern with serum vitamin B12 levels in exclusively breastfed infants (group 1).

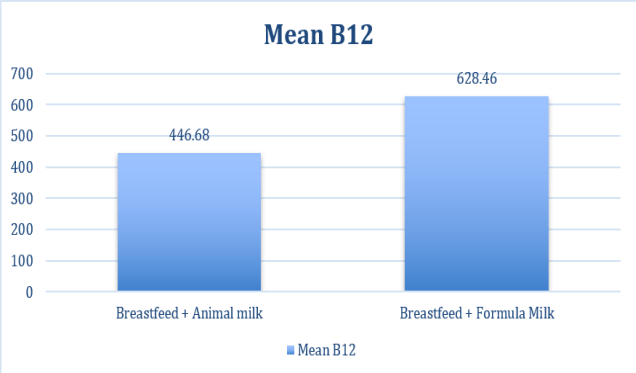

Figure 3. Comparison of mean serum vitamin B12 levels in group 2 infants.

Logistic regression analysis showed that exclusive breastfeeding was an independent predictor of vitamin B12 deficiency with an odds ratio of 5.52.

DISCUSSION

The present study demonstrated that exclusively breastfed infants had significantly lower serum vitamin B12 levels than mixed-fed infants. The mean serum vitamin B12 concentration was 379.90±205.60 pg/ml in exclusively breastfed infants compared with 514.85±227.73 pg/ml in mixed-fed infants. Vitamin B12 deficiency was more common among exclusively breastfed infants. These findings suggest that infants who depend solely on breast milk may be more vulnerable to deficiency if maternal vitamin B12 intake is inadequate.

Similar observations have been reported in studies from India, Nepal, Pakistan and Norway. Kadiyala et al reported a high prevalence of vitamin B12 deficiency among exclusively breastfed infants in South India.[5] Chandyo et al, also reported lower cobalamin levels among breastfed infants in Nepal.[2] The present study further showed that formula-fed infants had higher serum vitamin B12 levels than infants receiving animal milk in addition to breastfeeding. This may be due to fortification of formula milk with vitamin B12.

Maternal vegetarian diet was significantly associated with lower infant vitamin B12 levels. Similar findings were reported by Specker et al, who observed that low milk vitamin B12 concentrations in vegetarian mothers were associated with low serum vitamin B12 levels and higher methylmalonic acid concentrations in their infants.[6] This finding highlights the importance of maternal nutritional counselling during pregnancy and lactation.

Clinical significance

Vitamin B12 deficiency during infancy can lead to significant hematological and neurological complications. Hematological manifestations include macrocytic anemia, leukopenia, thrombocytopenia and pancytopenia. Neurological manifestations include hypotonia, developmental delay, apathy, irritability, tremors, seizures, feeding refusal and delayed milestones. If not identified early, deficiency may result in long-term cognitive impairment and irreversible neurological damage. Therefore, early screening of high-risk infants and mothers is important, especially in populations where vegetarian dietary practices are common.

Strengths and limitations

The strengths of the present study include direct comparison between exclusively breastfed and mixed-fed infants, evaluation of maternal dietary practices and assessment of different types of mixed feeding.

However, the study also had certain limitations. Maternal serum vitamin B12 levels were not measured. Breast milk vitamin B12 concentration could not be assessed.

The sample size was relatively small and the quantity of formula milk or animal milk intake could not be measured accurately. Biochemical markers such as homocysteine and methylmalonic acid were not included in the study.

CONCLUSION

Exclusively breastfed infants aged 3-6 months had significantly lower serum vitamin B12 levels than mixed-fed infants. Vitamin B12 deficiency was more common in exclusively breastfed infants. Maternal vegetarian diet was associated with lower infant vitamin B12 levels. Exclusive breastfeeding was identified as an independent predictor of vitamin B12 deficiency.

Recommendations

Future studies should include larger sample sizes and multicentric data collection. Maternal serum vitamin B12 estimation, breast milk vitamin B12 concentration, homocysteine levels and methylmalonic acid levels should be evaluated. Maternal supplementation during pregnancy and lactation, routine infant screening and public health awareness programs may help reduce the burden of vitamin B12 deficiency in infancy.

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Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Bjørke-Monsen AL. Defining optimal cobalamin status for neonates and infants. Food Nutr Bull. 2024;45(1):S16-22.
2. Chandyo RK, Ulak M, Kvestad I, Hysing M, Shrestha M, Ranjitkar S, et al. Cobalamin and folate status among breastfed infants in Bhaktapur, Nepal. Nutrients. 2018;10(5):597.
3. Jain S, Beg S, Verma A. To study the relationship between vitamin B12 deficiency in breast-fed infants and their mothers and its clinico-hematological manifestations: a cross-sectional study. Int J Life Sci Biotechnol Pharma Res. 2023;12:671-7.
4. Solvik BS, Bakken KS, McCann A, Ueland PM, Henjum S, Strand TA. Breastfeeding and biomarkers of folate and cobalamin status in Norwegian infants: a cross-sectional study. J Nutr Sci. 2024;13:e40.
5. Kadiyala A, Palani A, Rajendraprasath S, Venkatramanan P. Prevalence of vitamin B12 deficiency among exclusively breast-fed term infants in South India. J Trop Pediatr. 2021;67(1):114.
6. Specker BL, Black A, Allen L, Morrow F. Vitamin B12: low milk concentrations are related to low serum concentrations in vegetarian women and to methylmalonic acid concentrations in their infants. Am J Clin Nutr. 1990;52(6):1073-6.
7. Daniels L, Gibson RS, Diana A, Haszard JJ, Rahmannia S, Luftimas DE, et al. Micronutrient intakes of lactating mothers and their association with breast milk concentrations and micronutrient adequacy of exclusively breastfed Indonesian infants. Am J Clin Nutr. 2019;110(2):391-400.
8. Ask TF, Ranjitkar S, Ulak M, Chandyo RK, Hysing M, Strand TA, et al. The association between heart rate variability and neurocognitive and socio-emotional development in Nepalese infants. Front Neurosci. 2019;13:411.
9. Pawlak R, Vos P, Shahab-Ferdows S, Hampel D, Allen LH, Perrin MT. Vitamin B-12 content in breast milk of vegan, vegetarian, and nonvegetarian lactating women in the United States. Am J Clin Nutr. 2018;108(3):525-31.
10. WHO. United Nations Children's Fund. Indicators for assessing infant and young child feeding practices: definitions and measurement methods. Geneva: World Health Organization and UNICEF, 2021. Available at: https://www.who.int/publications/i/item/9789240018389. Accessed on 20 March 2026.
11. Pernicious Anaemia Society. Vitamin B12 absorption. Available at: https://perniciousanemia.org/b12/absorption/. Accessed on 20 March 2026.
12. Hatabah D, Krieger R, Brown LA, Harris F, Korman R, Reyes L, et al. Cobalamin deficiency in children and adolescents with sickle cell disease. Nutrients. 2025;17(3):597.
13. Saint-Cyr M, Waldrop SW, Krebs NF. Nutritional requirements. In: Kliegman RM, St Geme JW III, Blum NJ, Tasker RC, Wilson KM, Schuh AM, Mack CL, editors. Nelson Textbook of Pediatrics. 22nd ed. Philadelphia: Elsevier; 2023:388.

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