

Original Research Article

A COMPARATIVE STUDY ON THE EFFECT OF EXCLUSIVE BREASTFEEDING VERSUS MIXED FEEDING ON THE DEVELOPMENT OF VITAMIN B12 DEFICIENCY AMONG THE INFANTS AGED 3 TO 6 MONTHS

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Received : 06/05/2026
Received in revised form : 22/06/2026
Accepted : 08/07/2026

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DOI: 10.70034/ijmedph.2026.3.134

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2026; 16 (3); 827-831

ABSTRACT

Background: Vitamin B12 is an important vitamin for the body and plays vital roles in DNA synthesis, blood cell formation, neurological development, and myelin formation. Its deficiency leads to failure to thrive, lethargy, hypotonia, and delays in cognitive and motor development. And the recent studies had shown that a significant number of patients who had exclusive breastfeeding during the early part of their lives were found to be affected with the vitamin B12 deficiency. The aim is to compare the effect of exclusive breastfeeding versus mixed feeding on the development of Vitamin B12 deficiency among the infants aged 3 to 6 months.

Materials and Methods: This prospective comparative study was conducted among infants aged 3 to 5 months at a tertiary care centre in Puducherry, India. After the initial assessment, all infants were divided into two groups: Group I was exclusively breastfed, whereas Group 2 received mixed feeding, including breast milk along with formula or animal milk. And all the infants had been subjected to the general physical examination and systemic examination, followed by the serum vitamin B12 estimation. All the data were entered into Microsoft Excel and analysed using SPSS version 25.

Results: A total of 120 infants participated in the study; 60 were assigned to group 1 and 60 to group 2. 43.3% of infants in group 1 and 41.6% in group 2 were 5 to 6 months old. In both groups, male infants outnumbered female infants. The prevalence of vitamin B12 deficiency was 15% in exclusively breastfed infants and 3.3% in mixed-fed infants. Mothers with associated comorbidities and vegetarian consumers were found to be associated with very low vitamin B12 levels.

Conclusion: The study recommends that maternal supplementation during pregnancy and lactation helps reduce vitamin B12 deficiency, and that early screening of infants also helps prevent later complications.

Keywords: breastfeeding; mixed-fed; infants; vitamin B12 deficiency.

INTRODUCTION

Serum Vitamin B12, also called cobalamin, is a water-soluble vitamin. This vitamin cannot be produced in the body and has been found to play a critical role in DNA synthesis, blood cell formation, neurological development, and myelin formation.^[1,2]

Vitamin B12 has been found to play a vital role in cell division and helps in the maturation of the nervous system. During infancy, vitamin B12 levels are particularly important due to rapid brain growth and development.^[3] Since the body does not synthesise vitamin B12, infants are primarily dependent on dietary intake of the vitamin, which comes from breast milk. The recommended daily dose of vitamin

B12 for infants aged 0 to 6 months was 0.4 µg, and for 7- to 12-month-old children, 0.5 µg.^[4]

Although exclusive breastfeeding is highly recommended and indicated for the initial 4–6 months of life, breast milk lacks nutritional completeness owing to insufficient levels of vitamins D and K. Breast milk is regarded as a beneficial source of folate; nonetheless, apprehensions have been expressed over diminished vitamin B12 levels in breast milk, particularly in the context of exclusive and extended nursing.^[5,6] The vitamin B12 concentration in breast milk was found to depend mainly on maternal status and maternal vitamin B12 intake during the antenatal period. Studies also show that the concentration of vitamin B12 in breast milk decreases during the first 4 to 6 months of life.^[7] The symptoms noted in infants with vitamin B12 deficiency were failure to thrive, development of lethargy, hypotonia, and cognitive and motor development. In most cases, a lower level of serum vitamin B12 deficiency was asymptomatic. Still, it was found to have long-term effects on the infant's growth and development. And studies have shown that vitamin B12 deficiency during infancy is associated with impaired growth and poor performance on neuropsychological tests at 5 years of age.^[8,9]

And in recent years, studies have shown that mixed-fed infants receive additional vitamin B12 from animal milk or fortified formula, which helps protect them against deficiency.^[10] But until now, only a few studies have examined the effects of exclusive breastfeeding and mixed feeding among infants, so this comparative study was designed to explore the effects of exclusive breastfeeding versus mixed feeding among infants aged 3 to 6 months on the development of vitamin B12 deficiency.

MATERIALS AND METHODS

Study design: This prospective comparative study was conducted among infants aged between 3 and 5 months who attended the outpatient department at the Sri Lakshmi Naraya Institute of Medical Sciences in Puducherry, India. The study was conducted for 12 months, from May 2025 to May 2026.

Sampling: According to the study by Kaur A et al,^[11] The prevalence of Vitamin B12 deficiency among breastfed infants was 22.5%, and 5% in mixed-fed infants. Considering a 95% confidence interval, a 5% alpha level, and 80% power, the sample size in each group was calculated to be 60. And so, the total sample size was 120.

Inclusion and Exclusion criteria

All infants aged 3 to 5 months who attended the outpatient department of Sri Lakshmi Narayana Institute of Medical Sciences, as well as those younger than 6 months, were included in the study. And the infants born with prematurity, low birth weight, congenital malformation and infants with

congenital malformations and with systemic illness and the infants with severe acute malnutrition were excluded from the study.

Data collection: After obtaining approval from the Institute Ethics Committee and informed written consent, all the infants aged between 3 and 6 months were included in the study. Infants were divided into two groups: Group I was exclusively breastfed, whereas Group 2 received mixed feeding, including breast milk along with formula or animal milk. After the initial assessment, all basic demographic details including the name, gender, socio economic status,^[12] were obtained from the patient, followed by the feeding history, the mother's diet, and the patient's socio-economic status. The systemic examination was then performed. After that, all patients had their serum vitamin B12 levels measured, along with other routine investigations.

The serum vitamin B12 level was divided into three categories: patients with levels greater than 300 pg/ml were considered normal; those between 200 and 300 pg/ml were considered borderline; and those less than 200 pg/ml were considered deficient. The serum vitamin B12 level, along with the patient's breastfed status, had been compared. All adverse events occurring during the study period were recorded.

Data analysis: All the data were entered into Microsoft Excel, and the analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 25 (IBM Corp., Armonk, NY, USA).

Ethical Approval: The study procedure was approved by the Internal Human Ethics Committee of Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry, under reference number 10/SLIMS/IEC/0843.

RESULTS

A total of 120 infants had been included in the study. About 60 infants had been included in Group 1, who had been exclusively breastfed, and another 60 infants had been included in Group 2, who had been mixed-fed. In group 1, about 56.6% of patients were between 3 and 4 months old, whereas in group 2, about 58.3% were between 3 and 4 months old. In both groups, male infants outnumbered female infants. Male infants in group 1 account for 60%, and those in group 2 for 51.7%. and 31.7% of the infants in group 1 and 33.3% in group 2 were in class V socioeconomic status. And the majority of the infants were from the rural areas, as in [Table 1].

Upon exploring maternal factors among the study participants, the study noted that 36.7% of infants' mothers in group 1 and 33.3% in group 2 had maternal comorbidities during pregnancy. And 68.3% of the mothers in group 1 and 61.7% in group 2 were found to have a non-vegetarian diet. And on exploring the birth weight of the infants, as in [Table 2].

Table 1: Socio-demographic characteristics of the study participants

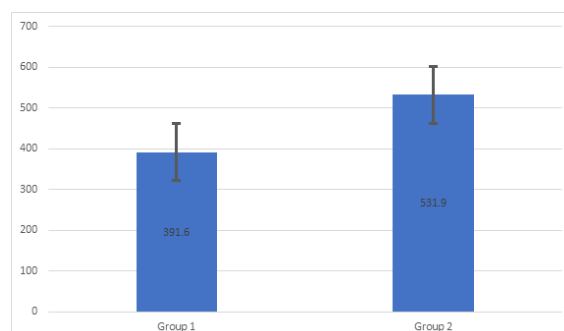
Variable	Group 1 N (%)	Group 2 N (%)
Age (in months)		
3-4	34 (56.6)	35 (58.3)
5-6	26 (43.3)	25 (41.6)
Gender		
Male	36 (60)	31 (51.7)
Female	26 (40)	29 (48.3)
Socio-Economic Status		
Class I	5 (8.3)	3 (5)
Class II	9 (15)	8 (13.3)
Class III	12 (20)	13 (21.7)
Class IV	15 (25)	16 (26.7)
Class V	19 (31.7)	20 (33.3)
Residence		
Rural	40 (66.7)	36 (60)
Urban	20 (33.3)	24 (40)

Table 2: Maternal and Fetal Factors among the study participants

Factors	Group 1 N (%)	Group 2 N (%)
Maternal comorbidities		
Present	22 (36.7)	20 (33.3)
Absent	38 (63.3)	40 (66.7)
Maternal diet		
Vegetarian	19 (31.7)	23 (38.3)
Non-vegetarian	41 (68.3)	37 (61.7)

When comparing mean serum vitamin B12 levels between the groups, the study found a significant difference ($p=0.001$). The mean serum vitamin B12 levels were 391.6 ± 211.6 pg/ml in group 1 and 531.9 ± 228.8 pg/ml in group 2, as shown in [Figure 1].

And no noticing the status of the serum vitamin B12 level between the groups, the study had noted that among group 1 infants about 15% of the infants were found to have deficient serum vitamin B12 level and borderline level had been noted in 33.3% of the infants and in Group 2, only 3.3% of the infants were found to have deficient level and the borderline levels had been noted in 25% of the infants, as in [Table 3].

**Figure 1: Comparison of mean serum vitamin B12 level between the groups.****Table 3: Level of serum vitamin B 12 between the groups**

Vitamin B12 level	Group 1	Group 2	p-value
Normal (>300 pg/ml)	31 (51.7)	43 (71.7)	0.028
Borderline (200-300 pg/ml)	20 (33.3)	15 (25)	
Deficient (<200 pg/ml)	9 (15)	2 (3.3)	

When comparing serum vitamin B12 levels across maternal factors, the study found a significant difference in patients with maternal comorbidities ($p < 0.001$). and the study noted that mothers who had both a vegetarian ($p < 0.001$) and a non-vegetarian (p

< 0.001) diet also showed significant differences in serum vitamin B12 levels. However, the level difference was more pronounced among vegetarian mothers, as shown in [Table 4].

Table 4: comparison of serum vitamin B12 level with respect to the various maternal and infant factors

Parameters	Group 1	Group 2	t-value	p-value
Maternal comorbidities				
Present	289.9 + 155.9	502.7 + 129.5	8.133	<0.001
Absent	412.9 + 211.9	440.9 + 201.9	0.741	0.460
Maternal diet				
Vegetarian	320.7 + 154.3	522.7 + 220.9	5.807	<0.001
Non-vegetarian	411.9 + 211.9	530.8 + 189.9	3.237	0.001

When comparing serum vitamin B12 levels across the types of mixed feeding, the study noted a significant difference between the two ($p=0.001$). In the study, infants who had breastfed and received

animal milk had vitamin B12 levels of 399.8 ± 199.5 pg/ml, and those who had breastfed and received formula milk had vitamin B12 levels of 589.7 ± 201.2 pg/ml, as shown in [Table 5].

Table 5: Comparison of serum vitamin B12 levels based on the type of mixed feeding

Type of Mixed Feeding	N (%)	Mean serum Vitamin B12 level (pg/ml)	t-value	p-value
Breastfeeding + animal milk	21 (35)	399.8 + 199.5	3.497	0.001
Breastfeeding + formula milk	39 (65)	589.7 + 201.2		

DISCUSSION

Exclusive breastfeeding was an important practice for 6-month-old infants. But due to lower levels of vitamin B12 in breast milk, many infants were found to have a higher incidence of vitamin B12 deficiency-related consequences. In our study, the prevalence of vitamin B12 deficiency was 15% among exclusively breastfed infants and 3.3% among mixed-fed infants. and the study by Kadiyala A et al,^[13] had shown that the prevalence of vitamin B12 deficiency among exclusively breastfed infants was 63.7%. and the study by Kaur A et al,^[11] had noted that vitamin B12 deficiency among exclusively breastfed infants was 22.5%, whereas among mixed-fed infants it was 5%, which was similar to our study results. In our study, the mean serum vitamin B12 among exclusively breastfed infants was 391.6 ± 211.6 , and among mixed-fed infants, 531.9 ± 228.8 , indicating a significant difference between the two groups—the study by Kadiyala A et al,^[13] had shown that the mean serum vitamin B12 level among exclusively breastfed patients was 199.91 ± 112.52 , which was similar to our study results. and the study by Kaur A et al,^[11] had shown that the mean vitamin B12 level among exclusively breastfed infants was 379.90 ± 205.60 pg/ml, and among mixed-fed infants was 514.85 ± 227.73 pg/ml.

Our study showed that patients with maternal comorbidities, such as anaemia and GDM, had significantly lower vitamin B12 levels than mixed-fed infants. And the study by Idris N et al,^[14] had shown that maternal anaemia is often associated with serum vitamin B12 deficiency in children. And this is because due to the presence of anaemia, the healthy RBC cannot be produced, and this inadequate level during pregnancy was found to lead to the development of megaloblastic anaemia, which affects the placental health, and further increases the risk of the development of neurological complications in the baby—the study by Kadiyala A et al,^[13] About 76.9% of infants with vitamin B12 deficiency were found to be associated with maternal anaemia at the time of delivery. Additionally, the study by Wang Li et al,^[4] found that gestational diabetes mellitus was associated with an increased risk of vitamin B12 deficiency in infants.

Our study also showed a significant difference in maternal diet among the groups. In our study, both vegetarian and non-vegetarian diets showed differences in vitamin B12 levels. But the mean serum vitamin B12 level was significantly lower in infants on a maternal vegetarian diet and in exclusively breastfed infants. The study by Kadiyala et al,^[2] also showed a significant association between mothers' vegetarian diet and serum vitamin B12 deficiency in infants. In our study, all mothers who

followed a vegetarian diet were found to have a vitamin B12 deficiency in their infants. And the study by Kaur A et al,^[11] Also found that maternal vegetarianism was significantly associated with vitamin B12 deficiency in infants. The mean Vitamin B12 level among mothers who had a vegetarian diet was 327.4 ± 174.1 pg/ml, and among those with a non-vegetarian diet, it was 537.3 ± 220.9 pg/ml—the study by Jathar VS et al,^[15] also showed that the vegetarian diet was associated with lower serum vitamin B12 levels, because vitamin B12 is found primarily in animal-sourced foods. So, the pregnant and lactating vegetarian mothers were found to have an increased risk of developing vitamin B12 deficiency. And so, the studies recommend vitamin B12 supplementation and fortified foods to prevent irreversible neurological complications in both mothers and infants.^[16]

Limitations of the study: The study had been done in a single centre, so if the study had been done in different regions, the effect of exclusive breastfeeding versus mixed feeding on the development of vitamin B12 deficiency across different populations could be assessed.

CONCLUSION

The study had shown that exclusive breastfeeding was found to be associated with the development of vitamin B12 deficiency more than mixed-fed infants. And so the study recommends that maternal supplementation during pregnancy and lactation helps reduce vitamin B12 deficiency, and that early screening of infants is also helpful in preventing complications later. The study also recommends further research with a larger sample size to confirm its findings.

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