

Original Research Article

Vitamin B12 Status in Anaemic Children in a Tertiary Care Institution, Trichy

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Received: 29.06.2026 Revised: 23.07.2026 Accepted: 08.09.2026 Published: 13.09.2026

ABSTRACT

Background: Vitamin B12 deficiency is an important yet under-recognized cause of anemia in children and is associated with significant hematological, neurological, and neurocognitive complications. Early identification of vitamin B12 deficiency in anaemic children is essential to facilitate timely intervention and prevent irreversible adverse outcomes. The present study aimed to determine the prevalence of vitamin B12 deficiency among children with anemia and to evaluate its association with clinical and demographic factors.

Methods: A hospital-based cross-sectional observational study was conducted from October 2022 to September 2023 at KAPV Government Medical College, Trichy. A total of 185 children aged 1 month to 12 years admitted with anemia were enrolled. Anemia was diagnosed according to World Health Organization age-specific criteria. Detailed clinical evaluation, socio-demographic information, red blood cell indices, peripheral smear examination, and serum vitamin B12 estimation were performed using a structured case record form. Statistical analysis was carried out using Jamovi software after obtaining institutional ethics committee approval.

Results: The mean age of the participants was 3.39 years, and 57.8% were males. The prevalence of vitamin B12 deficiency was 49.7%, with a mean serum vitamin B12 level of 301 pg/mL. Although age, gender, socioeconomic status, nutritional status, and exclusive breastfeeding did not show statistically significant associations with vitamin B12 deficiency, a significant association was observed with vegetarian dietary habits. Clinical manifestations of anemia were significantly associated with vitamin B12 deficiency, whereas developmental delay and knuckle pigmentation were not significantly associated.

Conclusion: Vitamin B12 deficiency is highly prevalent among children with anemia. Routine assessment of vitamin B12 status, along with peripheral smear examination, should be incorporated into the evaluation of anaemic children to enable early diagnosis and prompt treatment, thereby reducing hematological and neurological complications.

Keywords: Anaemia, Vitamin B12, Children, Prevalence, Nutritional Deficiency, Peripheral Smear, Risk Factors.

INTRODUCTION

Anaemia is a condition characterized by a reduced number of red blood cells or a decreased haemoglobin concentration, resulting in impaired oxygen transport to body tissues. It commonly presents with symptoms such as fatigue, weakness, dizziness and dyspnoea. The condition has a multifactorial etiology, including nutritional deficiencies,

infections, chronic inflammatory diseases, inherited red blood cell disorders, and obstetric or gynaecological conditions. Among nutritional causes, iron deficiency is the most common, while deficiencies of vitamin B12, folate and vitamin A also contribute significantly to anaemia. Appropriate management requires identification and treatment of the underlying cause.

Anaemia remains a major global public health problem, particularly among young children, adolescent girls and women of reproductive age. According to the World Health Organization (WHO), approximately 40% of children aged 6–59 months, 37% of pregnant women and 30% of women aged 15–49 years are affected by anaemia worldwide.^[1]

Vitamin B12 deficiency is an important public health concern in developing countries, including India, where vegetarian dietary practices limit intake of animal-based foods, the primary source of vitamin B12. The Comprehensive National Nutrition Survey reported vitamin B12 deficiency in 14% of preschool children, 17% of school-age children and 31% of adolescents.^[2] Vitamin B12 and folate are essential water-soluble vitamins involved in one-carbon metabolism, DNA synthesis, red blood cell production and normal neurological development.^[3,4] Deficiency of these vitamins disrupts cell division and methylation processes, leading to megaloblastic anaemia, impaired growth and irreversible neurological damage in severe cases.^[5,6] Furthermore, deficiencies during pregnancy are associated with neural tube defects, low birth weight, intrauterine growth restriction, miscarriage and pre-eclampsia. Hyperhomocysteinaemia resulting from vitamin B12 and folate deficiency also increases the risk of cardiovascular disease.^[7,8]

Although iron supplementation has been implemented in India for several decades, the reduction in anaemia prevalence has been modest, suggesting that deficiencies of vitamin B12 and folate may contribute to persistent anaemia. Since vitamin B12 deficiency is common, associated with significant morbidity and amenable to treatment, the present study was undertaken to determine the prevalence of vitamin B12 deficiency among anaemic children.

Aims and Objectives

To determine the prevalence of vitamin B12 deficiency among anaemic children aged 1 month to 12 years.

MATERIALS AND METHODS

Study Design

This hospital-based cross-sectional study was conducted over a period of one year, from October 2022 to September 2023, at KAPV Medical College and Hospital, Tiruchirappalli.

Inclusion and Exclusion Criteria

The study included all anaemic children aged 1 month to 12 years who were admitted as inpatients and whose parents or guardians provided informed consent to participate in the study. Anaemia was defined according to the WHO (World Health Organization) criteria, with haemoglobin (Hb) levels of <11 g/dL for children aged 1 month to 59 months and <11.5 g/dL for children aged 5–12 years. Children who had received vitamin B12 supplementation within the preceding three months were excluded from the study to avoid the influence of recent supplementation on serum vitamin B12 levels and study outcomes.

Sample Size Calculation

The calculated sample size was 185 based on a previous study by Kalyan et al.^[9]

Data Collection Procedure

All children aged 1 month to 12 years diagnosed with anaemia and admitted to the IPD (In-Patient Department) of the Department of Paediatrics at K.A.P.V. Medical College Hospital during the study period were enrolled after obtaining informed consent from their parents or guardians. Data were collected using a semi-structured proforma, which included demographic details, socioeconomic status, developmental milestones, dietary habits, clinical and neurological examination findings, and anthropometric measurements. The socioeconomic status of each participant was assessed using the Modified Kuppuswamy Scale. Fresh venous blood samples were collected under aseptic precautions for laboratory investigations, including complete blood count with RBC (Red Blood Cell) indices, peripheral smear examination, and estimation of serum vitamin B12 levels. The collected data were systematically recorded and used for subsequent statistical analysis to determine the prevalence of vitamin B12 deficiency among anaemic children.

Statistical Analysis

The collected data were entered into Microsoft Excel and analysed using Jamovi statistical software. Descriptive statistics were used to summarize the data, with continuous variables expressed as mean $\pm$ standard deviation (SD) and categorical variables presented as frequency and percentage. The associations between various study parameters were assessed using the Independent Samples t-test, Pearson's correlation test, and the chi-square

test, as appropriate. A p-value of <0.05 was considered statistically significant.

RESULTS

Variable	Category	Number	Percentage
Age	1–6 months	24	13.0
	>6 months–1 year	18	9.7
	1–5 years	104	56.2
	6–9 years	19	10.3
	≥10 years	20	10.8
Gender	Male	107	57.8
	Female	78	42.2
Food Habit	Non-vegetarian	170	91.9
	Vegetarian	15	8.1

Table 1. Demographic Characteristics of the Study Children (N = 185)

Table 1 shows the demographic characteristics of the study participants. Most children (56.2%) belonged to the 1–5 years age group. Male

children constituted 57.8% of the study population, and 91.9% of the participants consumed a non-vegetarian diet.

Variable	Category	Number	Percentage
Parental Occupation	Skilled	139	75.1
	Unskilled	46	24.9
Socioeconomic Status	Upper	4	2.2
	Upper Middle	66	35.7
	Lower Middle	67	36.2
	Upper Lower	37	20.0
Developmental Delay	Lower	11	5.9
	Present	17	9.2
	Absent	168	90.8

Table 2. Socioeconomic and Developmental Characteristics (N = 185)

Table 2 illustrates the socioeconomic profile of the study children. Most parents (75.1%) had skilled occupations, while the majority of families belonged to the lower middle (36.2%)

and upper middle (35.7%) socioeconomic classes. Developmental delay was observed in 9.2% of children.

Variable	Category	Number	Percentage
Comorbidity	Nil	144	77.8
	Seizure disorder	8	4.3
	Heart disease	5	2.7
	Renal disorders	4	2.2
	Respiratory illness	3	1.6
	Others	21	11.4
Weight for Age	Normal	103	55.7
	Underweight	31	16.8
	Severe underweight	51	27.6
Height for Age	Normal	127	68.6
	Stunting	22	11.9
	Severe stunting	36	19.5

Table 3. Clinical and Nutritional Characteristics (N = 185)

Table 3 observes that 77.8% of children had no associated comorbidity. More than half (55.7%) had normal weight-for-age, whereas 68.6%

had normal height-for-age. Severe underweight and severe stunting were present in 27.6% and 19.5% of children, respectively.

Variable	Category	Number	Percentage
Head-to-foot examination	Normal	177	95.7
	Knuckle hyperpigmentation	3	1.6
	Others	5	2.7
Neurological examination	Normal	179	96.8
	Hypotonia	2	1.1
	Microcephaly	2	1.1
	Neuroregression	1	0.5
	Poor appetite	1	0.5
Anemia Symptoms	Present	15	8.1
	Absent	170	91.9
Anemia Signs	Present	28	15.1
	Absent	157	84.9

Table 4. Clinical Examination Findings (N = 185)

Table 4 shows that almost all children had normal findings on head-to-foot (95.7%) and neurological (96.8%) examinations. Signs and

symptoms of anemia were present in 15.1% and 8.1% of children, respectively.

Parameter	Result	
Mean Hemoglobin	8.31 ± 1.82 g/dL	
Mean MCV	70.9 ± 12.3 fL	
Mean MCH	22.6 ± 3.31 pg	
Mean MCHC	30.3 ± 3.03 g/dL	
Peripheral Smear	Number	Percentage
Normal	148	80.0
Microcytic hypochromic anemia	31	16.8
Pancytopenia	2	1.1
Others	4	2.1

Table 5. Laboratory Characteristics (N = 185)

Table 5 demonstrates the hematological profile of the study population. The mean hemoglobin level was 8.31 ± 1.82 g/dL. Most peripheral

smears (80%) were normal, while 16.8% showed microcytic hypochromic anemia.

Variable	Category	Number	Percentage
MCV	Low	105	56.8
	Normal	77	41.6
	High	3	1.6
MCH	Low	121	65.4
	Normal	63	34.1
	High	1	0.5
MCHC	Low	122	65.9
	Normal	61	33.0
	High	2	1.1
Vitamin B12	Low	92	49.7
	Normal	85	46.0
	High	8	4.3

Table 6. Red Cell Indices and Vitamin B12 Status (N = 185)

Table 6 reveals that low MCV, MCH, and MCHC values were common among study participants.

Nearly half of the children (49.7%) had vitamin B12 deficiency, whereas 46% had normal vitamin B12 levels.

Variable	p-value	Significance
Age	0.96	Not Significant
Gender	0.36	Not Significant
Socioeconomic status	0.07	Not Significant
Food habit	0.03	Significant
Weight-for-age	0.11	Not Significant
Height-for-age	0.35	Not Significant
Head-to-foot findings	0.25	Not Significant
Neurological findings	0.43	Not Significant
Developmental delay	0.84	Not Significant
Table 7. Association Between Vitamin B12 Deficiency and Baseline Characteristics		

Table 7 indicates that among all baseline characteristics, only food habit showed a **statistically** significant association with vitamin B12 levels ($p = 0.03$). No significant

association was observed with age, gender, socioeconomic status, nutritional status, developmental delay, or examination findings.

Variable	p-value	Significance
MCV	0.14	Not Significant
MCH	0.39	Not Significant
MCHC	0.96	Not Significant
Hemoglobin	0.93	Not Significant
Signs of anemia	0.005	Significant
Symptoms of anemia	0.008	Significant
Comorbidity	0.37	Not Significant
Exclusive breastfeeding	0.25	Not Significant
Table 8. Association Between Vitamin B12 Deficiency and Hematological Parameters		

Table 8 shows that vitamin B12 deficiency was significantly associated with both clinical signs of anemia ($p = 0.005$) and symptoms of anemia ($p = 0.008$). However, no statistically significant association was observed with haemoglobin concentration, red cell indices (MCV, MCH, MCHC), comorbidities, or exclusive breastfeeding.

DISCUSSION

The mean age of children in the present study was 3.39 ± 3.4 years, with the majority (56.2%) belonging to the 1–5-year age group. More than half of the children were underweight (56.4%), while only 10.3% were overweight. This prevalence of overweight was lower than that reported by Cobayashi et al.^[10] (15%). Stunting and severe stunting were observed in 11.9% and 19.5% of children, respectively, whereas Cobayashi et al.^[10] reported only 5.4% stunting. Verma et al.^[11] reported kwashiorkor and marasmus in 15% and 29% of children with severe acute malnutrition, respectively.

The mean serum vitamin B12 level in the present study was 301 pg/mL (range: 2.02–2000 pg/mL). This finding is comparable to Verma et al.^[11] who reported a mean vitamin

B12 level of 250.1 pg/mL, and Christian et al.^[12] who reported 312 pmol/L. Similarly, Masalha et al.^[13] observed a mean vitamin B12 level of 280.4 pg/mL, while Cobayashi et al.^[10] reported 257.5 pmol/L. In contrast, Cuevas-Nasu et al.^[14] reported a higher mean vitamin B12 concentration of 510 pg/mL. These differences may be attributed to variations in age groups and study populations.

Vitamin B12 deficiency was identified in 49.7% of children in the present study. This prevalence is comparable to the 41.8% reported by Kumar et al.^[15] but considerably higher than the prevalence reported by Verma et al.^[11] (26%), Christian et al.^[12] (2.5%), Azad et al.^[16] (22% among infants), Masalha et al.^[13] (25.4%), Cuevas-Nasu et al.^[14] (7.7%), and Kaur et al.^[17] (30.5%). The higher prevalence in the present study may be due to inclusion of only anaemic hospitalized children and the use of a higher diagnostic cut-off (<200 pg/mL).

Clinical signs of anaemia were present in 15.1% of children, which was lower than the 20.9% reported by Masalha et al.^[13] No significant association was observed between age and vitamin B12 deficiency, which is consistent with the findings of Cuevas-Nasu et al.^[14] However, Cobayashi et al.^[10] demonstrated a significant

negative association between age and serum vitamin B12 levels.

Although vitamin B12 deficiency was more common among male children, the association with gender was not statistically significant. Similar findings were reported by Christian et al.^[12] Masalha et al.^[13] and Cuevas-Nasu et al.^[14] In contrast, Masalha et al.^[13] observed a significantly higher prevalence of anaemia among female children.

Dietary pattern had a significant influence on vitamin B12 status. Children consuming a vegetarian diet had significantly lower mean vitamin B12 levels (172 vs. 262 pg/mL) than non-vegetarians. Similar observations were reported by Christian et al.^[12] Cobayashi et al.^[10] and Masalha et al.^[13] who demonstrated better vitamin B12 status with higher intake of animal-source foods. However, Kumar et al.^[15] found no significant association between dietary pattern and vitamin B12 deficiency.

No significant association was observed between socioeconomic status and vitamin B12 deficiency, which agrees with the findings of Kalyan et al.^[9] However, Cobayashi et al.^[10] and Cuevas-Nasu et al.^[14] reported a positive association between higher socioeconomic status and better vitamin B12 status.

Similarly, no significant association was found between vitamin B12 deficiency and developmental delay or nutritional status, consistent with the observations of Kumar et al.^[15] In contrast, Azad et al.^[16] reported a significant association between vitamin B12 deficiency and developmental delay among infants.

Macrocytosis was observed in only 1% of children in the present study, compared with 21.4% reported by Kumar et al.^[15] who also found that 95.2% of adolescents with macrocytosis had vitamin B12 deficiency. Likewise, no significant association was observed between vitamin B12 deficiency and MCV or knuckle pigmentation, which is in agreement with the findings of Kumar et al.^[15] Children who had not received exclusive breastfeeding had a higher prevalence of vitamin B12 deficiency (70% vs. 46.2%). These findings are supported by Ljungblad et al.^[18] who reported a significant relationship between breastfeeding practices and serum vitamin B12 levels.

The present study demonstrates a high prevalence of vitamin B12 deficiency among anaemic children, highlighting the importance of routine screening, especially in hospitalized children and those consuming vegetarian diets.

Early diagnosis and appropriate nutritional intervention can help prevent the haematological and neurological complications associated with vitamin B12 deficiency.

Limitations

The present study has certain limitations that should be considered while interpreting the findings. The assessment of vitamin B12 deficiency was based solely on serum vitamin B12 levels, which is a screening test and not a definitive confirmatory test. Due to financial constraints, serum ferritin levels and maternal vitamin B12 levels could not be estimated. In addition, confirmatory biomarkers such as methylmalonic acid and homocysteine levels were not measured because of resource limitations. Since the study included only children with anaemia, the observed prevalence of vitamin B12 deficiency may not reflect its prevalence in the general paediatric population. Furthermore, the absence of confirmatory biochemical tests limited the ability to establish population-specific cut-off values for vitamin B12 deficiency in the paediatric age group.

CONCLUSION

The present study demonstrates that vitamin B12 deficiency is common among children with anaemia and is significantly associated with the clinical manifestations of anaemia, highlighting the importance of routine evaluation of vitamin B12 status in these patients. The observed association between vitamin B12 deficiency and developmental delay underscores the need for adequate maternal and childhood vitamin B12 nutrition to ensure normal growth and neurodevelopment. Early detection and timely treatment are essential to prevent irreversible neurological sequelae, as haematological manifestations often precede neurological symptoms. Promotion of micronutrient-rich diets, food fortification, vitamin B-complex supplementation, and public awareness, particularly among pregnant women and children, should be prioritised as part of anaemia control programmes. Further large-scale studies are warranted to better understand the burden of vitamin B12 deficiency and to guide effective public health policies.

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