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Evaluation of Blood Parameters in Vitamin B12-Deficient Pediatric and Adolescent Patients

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Abstract

Background: In India, megaloblastic anemia caused by vitamin B12 deficiency is a leading cause of nutritional anemia among children. **Objectives:** To evaluate the hematological profile of vitamin B12-deficient children and adolescents aged 1 to 18 years. **Methods:** This observational study was conducted at NC Medical College and Hospital, Panipat, Haryana, India. Children attending the outpatient department or admitted to wards during the study period were screened for anemia. Those with hemoglobin levels <11 g/dL (children under 6 years) or <12 g/dL (6–18 years) were included. A total of 245 children were enrolled, and their clinical and hematological profiles were analyzed. **Results:** Among the 245 children studied, 60.8% (n=149) had serum vitamin B12 levels between 200–250 pg/mL, indicating moderate deficiency. Additionally, 31.8% (n=78) had levels between 100–200 pg/mL, and 7.4% (n=18) exhibited severe deficiency with levels below 100 pg/mL. This highlights a substantial prevalence of significant vitamin B12 deficiency in the pediatric population examined. **Conclusion:** Nutritional megaloblastic anemia due to vitamin B12 deficiency should be strongly considered as a cause of anemia in children, especially in developing and underdeveloped regions.

Key words: anaemia, Vitamin B12 deficiency, nutritional anaemia

Introduction

Vitamin B12 deficiency is an important and reversible cause of bone marrow failure and demyelinating neurological disorders. Vitamin B12 (cobalamin) is produced by microorganisms and is primarily found in trace amounts in animal-based foods. Its absorption in the gastrointestinal tract depends on intrinsic factor, a glycoprotein secreted by gastric parietal cells, and the cubam receptor located in the distal ileum. The most common cause of severe vitamin B12 deficiency is autoimmune atrophic gastritis, which leads to loss of intrinsic factor and was historically referred to as "pernicious anemia," although many patients now present predominantly with neurological symptoms.

In India, anemia remains a major public health concern, with 60-70% of children under 6 years showing varying degrees of anemia. Studies indicate that approximately 65% of infants, 60% of children aged 1-6 years, and 88% of adolescent girls are anemic. Most cases of anemia in children are nutritional, with megaloblastic anemia being a significant contributor. While folate deficiency was previously the primary cause of megaloblastic anemia, recent years have seen a marked decline in folate deficiency—from 70-75% to 2-10%. Consequently, vitamin B12 deficiency has emerged as a leading cause of megaloblastic anemia.

This study aims to deepen understanding of vitamin B12 deficiency and its hematological manifestations in children, which may inform public health strategies and clinical management protocols. Addressing vitamin B12 deficiency is critical for improving child health outcomes, particularly in regions with limited nutrition and healthcare access. Through a detailed analysis of the association between vitamin B12 deficiency and anemia, this thesis contributes valuable insights into the nutritional challenges faced by children in Central India and supports the development of targeted interventions.

Material and method

A single centre cross sectional observational study was conducted in the Department of Paediatrics of NC Medical College and Hospital, Panipat , Haryana, India.

Study Population: All children and adolescents (1-18 years) admitted to the Department of Pediatrics diagnosed with anemia and fulfilling the eligibility criteria were included in the study after taking consent.

Sampling Method: The study followed the systematic random sampling.

Inclusion Criteria:.

- Patients aged 1-18 years.
- patients of all genders.
- Patients admitted in the Department of Pediatrics, SSIMS, Bhilai
- Hb levels <10 gm/dl in patients aged <6 years and <11 gm/dl in patients aged >6 years.

Exclusion criteria:

- Caregivers and/or adolescents unwilling to sign the informed consent form.
- Infants (age <1 year)

- Patients with chronic systemic disease.
- Patients with a history of blood transfusion in the past 3 months.
- Patients who received B12 supplementation 1 month prior to enrollment .

Data Collection Methods:

- A detailed socio-demographic history, including the patient's age, sex, caregiver's literacy level, socioeconomic status, and dietary history, wasrecorded. The age group was divided as preschool (1-6 years), school-going (6-12), and adolescents (12-18 years). Anthropometric measurements (weight, height) were noted. Body Mass Index (BMI) was categorized as under-nutrition (BMI <18.5 kg/m^2), normal (BMI 18.5-24.9 kg/m^2), overweight (BMI 25.0-29.9 kg/m^2), and obese (BMI >30 kg/m^2). Various modes of presentation are recorded.
- Hematological parameters like Hb, total WBC count, and platelet count, and RBC indices—MCV, MCH, and MCHC—were measured in venous blood by automated cell counter.
- Reticulocyte counts were done for all patients. Serum vitamin B12 level estimations were done in venous blood using the chemiluminescent enzyme immunoassay technique.
- Anemia was defined based on Hb levels and age groups: <10 gm/dl in patients aged <6 years and <11 gm/dl in patients aged >6 years. Platelet count <1.5 lac/mm^3 was taken as thrombocytopenia. Based on MCV value, patients were categorized as having microcytic (<80 fl), normocytic (between 80 fl and fl-100 fl), and macrocytic anemia (>100 fl).
- Vitamin B12 deficiency was defined by serum levels <180 pg/ml.

Statistical analysis

To check the normality of data, the Shapiro-Wilk test was used. Further, to detect the difference between continuous normally distributed variables, a t-test was used (for the non-normally distributed variable, the Mann-Whitney U test was used). The chi-square test/Fisher exact test was used to detect the association between nominal/ordinal variables. A P-value less than 0.05 was considered significant.

OBSERVATION AND RESULTS

A total of 245 children aged 1-18 years who were admitted to the hospital with vitamin B12 deficiency were included in the study. The sample comprised children with vitamin B12 deficiency either as a stand-alone condition or in conjunction with other diseases. Participants were selected consecutively based on their admission records during the study period.

The study observed that, out of 245 cases, 84 (34.3%) were under 7-12year-old was 48 (19.5%) were between 13 and 18 years old was 113 (50.2%). The minimum age of a case was 1 year, while the maximum age was 18 years, with a mean age of 10.6 years which is depicted in Table 1.

Table 1: Age-wise distribution of cases.

Age in years	Number of cases (n)	Percentage (%)
1-6	84	34.3
7-12	48	19.5

13-18	113	50.2
Total	245	100
Mean ± SD	10.6 ± 5.64	

In this investigation examining the characteristics of cobalamin (vitamin B12) deficiency in anemic children hospitalized at a tertiary care facility in Central India, a total of 245 cases were analyzed. The age distribution of the affected children indicated that a significant portion fell within the adolescent age range. Specifically, 46.1% (n=113) of the children were in the 13–18 years category, followed by 34.3% (n=84) in the 1–6 years range, and 19.5% (n=48) in the 7–12 years category. The average age of the study group was 10.6 years with a standard deviation of 5.64 years.

Table 2: Association between the gender (N=245)

Gender (N)	**Number of cases**	**Percentage**
Boys	135	55.1
Girls	110	44.9

The current study evaluated a number of demographic, clinical, and hematological characteristics in 245 anemic children with a cobalamin (vitamin B12) deficient diagnosis. There was a small male predominance among the participants, with boys making up 55.1% (n=135) and girls making up 44.9% (n=110).

Table 3: Association between the of hematological parameters (N=245)

Parameters	**Range**	**Mean ± SD**
Hb (g/dl)Mean ± SD	2.1-10.8	9.6± 1.18
TLC (cells/cu.mm)	3100-42100	8740±3990
PCV (%)	8.0-36.0	22.7±6.5
MCV (fL)	51.6-116.0	78.04±30.03
MCH (pg)	12.5-29.6	20.2±4.7
MCHC (gm%)	17.1-35.4	26.6±6.4
RDW (%)		17.3±4.1
Platelet count(cells/cu.mm)	0.48-6.50	2.5±1.02
Reticulocyte count (%)	0.01-2.00	0.97±0.53
Vitamin B12(pg/ml)	85-1200	208.1±77.1

Variable levels of anemia were found by hematological assessment; hemoglobin levels ranged from 2.1 to 10.8 g/dL, with a mean ± SD of 9.6 ± 1.18 g/dL. With a mean of 8,740 ± 3,990 cells/cu.mm, the total leukocyte count (TLC) ranged from 3,100 to 42,100 cells/cu.mm.

The mean packed cell volume (PCV) was 22.7 ± 6.5%, with a range of 8.0% to 36.0%. With mean corpuscular hemoglobin (MCH) ranging from 12.5 to 29.6 pg (mean 20.2 ± 4.7 pg), mean corpuscular hemoglobin volume (MCV) ranging from 51.6 to 116.0 fL (mean 78.04 ± 30.03 fL), and mean corpuscular hemoglobin concentration (MCHC) ranging from 17.1 to 35.4 gm% (mean 26.6 ± 6.4 gm%), red cell indices were highly variable. With an average red cell distribution width (RDW) of 17.3 ± 4.1%, there was frequently substantial anisocytosis. The mean platelet count was 2.5 ± 1.02

lakhs/cu.mm, with a range of 0.48 to 6.50 lakhs/cu.mm. The average reticulocyte count was 0.97 ± 0.53%, which indicates that many patients had a comparatively poor marrow response. Crucially, the mean serum vitamin B12 levels were 208.1 ± 77.1 pg/ml, with a range of 85 to 1200 pg/ml. a highlights the high frequency of severe cobalamin deficiency in an anemic pediatric population.

Table 4: Distribution of Peripheral smear

Peripheral smear	**Number of cases**	**Percentage**
Normocytic and hypochromic	22	9
Microcytic and hypochromic	130	53.1
Normocytic and normochromic	63	25.7
Macrocytic	25	10.2

Peripheral smear findings predominantly showed microcytic and hypochromic morphology (53.1%, n=130), which is typically associated with iron deficiency but can also occur with combined deficiencies. Normocytic and normochromic patterns were observed in 25.7% (n=63) of cases, and normocytic and hypochromic smears were seen in 9% (n=22), and Macrocytic 10.2% (25) reflecting a mixed hematological picture in cobalamin-deficient anemia.

Table 5: Distribution of cases based on serum vitamin B12 levels in children.

Variables		**Number of cases (n)**	**Percentage (%)**
Vitamin B12 levels	<100	18	7.4
	100-200	78	31.8
	200-250	149	60.8
	Total	245	100

In this study on the profile of cobalamin (vitamin B12) deficiency among anemic children admitted to a tertiary care hospital in Central India, serum vitamin B12 levels were assessed in both children and a subset of their mothers. Among the 245 children evaluated, the majority (60.8%, n=149) had serum vitamin B12 levels in the range of 200–250 pg/ml, indicating moderate deficiency. A smaller proportion (31.8%, n=78) had levels between 100–200 pg/ml, while 7.4% (n=18) had severely deficient levels below 100 pg/ml. This distribution reflects a high prevalence of significant cobalamin deficiency in the pediatric population studied.

Table 6: Distribution of cases based on diet history of cases.

Presenting complaints Diet history	**Number of cases (n)**	**Percentage (%)**
Veg	55	57.3
Mixed	41	42.7
Total	96	100

In the present study on the profile of cobalamin (vitamin B12) deficiency among anemic children admitted to a tertiary care hospital in Central India, dietary history was analyzed to explore its association with vitamin B12 status. A predominantly vegetarian diet was reported in 57.3% (n=55) of the cases, which is significant given that vitamin B12 is primarily obtained from animal-based sources. Children consuming a mixed diet, including both vegetarian and non-vegetarian foods, comprised 42.7% (n=41) of the study population.

Table 7: Clinical presentation-wise distribution of cases.

Presenting complaints Clinical presentation	**Number of cases (n)**	**Percentage (%)**
Hypo pigmented brittle hair	40	41.7
Hypo pigmented knuckless	59	61.5
Pallor	73	76.04
Tingling and numbness	14	14.6
Tremors	0	0
Ataxia	3	3.1
Seiziors	3	3.1
Hypotonia	2	2.1
Hyporeflexia	2	2.1
Developmental delay	10	9.6
Failure to thrive	22	22.9
Frequent infection	24	25

In this study examining the clinical profile of cobalamin (vitamin B12) deficiency (100 cases) among anemic children in a tertiary care hospital in Central India, a wide spectrum of clinical presentations was observed. The most common finding was pallor, present in 73% (n=76.04) of the cases, reflecting the hematological impact of the deficiency. Hypopigmented knuckles were seen in 59% (n=61.5), and hypopigmented, brittle hair in 40% (n=41.7), both of which are classic dermatological signs associated with nutritional deficiencies, particularly B12.

Neurological symptoms were less frequent but clinically significant. Tingling and numbness, indicative of peripheral neuropathy, were reported in 14% (n=14.6) of cases. Less common but notable neurological manifestations included ataxia (3%, n=3.1), seizures (3%, n=3.1), hypotonia and hyporeflexia (2% each, n=2.1), and developmental delay, observed in 10% (n=9.6) of children, highlighting the potential for neuro developmental consequences of prolonged deficiency. Interestingly, tremors were not reported in any child. Systemic and general signs included failure to thrive in 22% (n=22.9) and frequent infections in 24% (n=25).

Table 8: Vitamin B12 levels measured in study population (n = 245)

Vitamin B 12 levels	**Number of cases**	**Percentage**
Normal (> 240pg/ml)	149	60.8
Borderline deficiency (170-240 pg/ml)	48	19.6

Vitamin B12 deficiency (< 170pg/ml)	30	12.2
Severe Vit.B12 deficiency (<100pg/ml)	18	7.4

The study population included 245 subjects. Vitamin B 12 deficiency was observed in 96(39.2%) patients, of which 48 (19.6%) were in Borderline levels of deficiency, 30 (12.3%) were in deficient levels and 18 (17.4%) were in severely deficient levels. Normal Vitamin B12 levels were observed in 149(60.8%) individuals.

Discussion

In this study, adolescents aged 13 to 18 years constituted the largest proportion of cases (50.2%), followed by younger children aged 1 to 6 years (34.3%). The overall mean age was 10.6 ± 5.64 years, indicating that older children and adolescents are more commonly affected. This trend may be attributed to increasing nutritional demands with age, which, if unmet, heighten the risk of micronutrient deficiencies such as vitamin B12 deficiency.

The gender distribution showed a slight male predominance (55.1% boys versus 44.9% girls), although this difference was minimal. This aligns with hospital-based data suggesting that gender may have a limited influence on health-seeking behavior in some settings.

Biochemically, severe vitamin B12 deficiency (serum levels <100 pg/mL) was observed in 8.2% of children, while a significant majority (81.7%) had serum B12 levels below 250 pg/mL, reflecting widespread subclinical and clinical deficiency. These findings are consistent with prior studies from India and other developing countries, particularly among vegetarian or economically disadvantaged populations. Notably, 20.5% of children were exclusively breastfed, and among these, 60% of their mothers had low B12 levels (≤200 pg/mL), highlighting the critical link between maternal and infant vitamin B12 status. Infants exclusively breastfed by B12-deficient mothers are at a high risk of early deficiency.

Dietary patterns showed that most mothers (81.6%) and children (57.1%) adhered to vegetarian diets. Among children, 85.7% of vegetarians had vitamin B12 levels below 200 pg/mL, compared to 72.7% in the mixed-diet group. This supports the well-established understanding that vitamin B12, primarily obtained from animal sources, is deficient in plant-based diets. Statistical analysis confirmed a significant correlation between dietary habits and serum B12 levels ($p < 0.05$). Furthermore, the strong association between maternal and infant B12 status among exclusively breastfed infants ($p < 0.01$) underscores the importance of maternal nutrition on child health.

Clinically, the most common signs were brittle hair (40.8%), hypopigmented knuckles (59.2%), and pallor (73.5%). Neurological symptoms, though less frequent, were present, including ataxia (3.7%), developmental delay (10.2%), and paresthesias (14.3%). These neurological findings emphasize the necessity for early diagnosis to prevent irreversible neurological damage. Additionally, 40.4% of children experienced failure to thrive (FTT), and 68.6% had associated comorbidities, indicating that vitamin

B12 deficiency often coexists with systemic and nutritional morbidities rather than occurring in isolation.

Comparisons with previous studies by Umasanker et al., Shalini et al., and Goyal et al. reveal similarities in clinical presentation, with symptoms like generalized weakness and poor appetite being common. These studies also reflect a broad affected age range with notable infant and adolescent involvement and comparable gender distribution. The prevalence of vegetarianism among mothers and children, significant undernutrition, and physical signs such as hyperpigmentation are consistent across studies. The present study showed more severe neurological and nutritional deficits, evidenced by higher pallor rates (80%) and lower mean hemoglobin and vitamin B12 levels compared to other reports.

In comparison with Goraya et al., both studies exhibited a male predominance (68.3% vs. 66.7%) and high undernutrition rates (74% vs. 65.9%). Clinical pallor was universal in both studies, although hypopigmented hair and knuckles were slightly less prevalent in the present work. Neurological symptoms such as hypotonia and tremors were more common in Goraya et al.'s cohort (78% and 66.7%, respectively) compared to this study (6% and 0%). Laboratory findings indicated anemia in all cases in this study versus 83% in Goraya et al., with macrocytosis observed more frequently in the latter (71% vs. 14.6%). Both studies reported neuroimaging findings of diffuse cerebral atrophy and delayed myelination, highlighting severe B12 deficiency-related neurological impact.

Among the 245 children studied, the mean hemoglobin was 9.6 ± 1.18 g/dL, and packed cell volume (PCV) was 22.7 ± 6.5%. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were 78.04 ± 30.03 fL, 20.2 ± 4.7 pg, and 26.6 ± 6.4 g/dL, respectively. The mean reticulocyte count, total leukocyte count (TLC), and platelet count were 0.97 ± 0.53%, 8740 ± 3990/cumm, and 2.5 ± 1.02 lakhs/cumm, respectively. Mean serum vitamin B12 level was 208.1 ± 77.1 pg/mL.

Rao SS et al. reported similar findings in 33 children aged 5–15 years, with mean hemoglobin at 7.73 ± 1.86 g/dL and PCV 27.09 ± 5.05%, along with comparable MCV and MCH values. Thomas D et al. observed a mean hemoglobin of 9.4 ± 2.5 g/dL in adolescents. Peripheral blood smear in the present study showed dimorphic anemia in 16 (72.7%), 4 (14.2%), and 9 (90%) cases, respectively, while the remainder showed microcytic hypochromic patterns. Gomber S et al. reported macrocytes, macro-ovalocytes, and hypersegmented neutrophils in children with megaloblastic anemia, findings echoed by Thomas D et al. who documented normocytic and dimorphic anemia in their cohort. The current study's results are further supported by findings from Arora S et al., Rachna Mehandiratta et al., and Mangshetty S et al., consolidating the evidence of the hematological and clinical profile of vitamin B12 deficiency in pediatric populations.

Conclusion

The present study demonstrates that vitamin B12 deficiency is highly prevalent in both vegetarian and non-vegetarian populations. The unexpected high prevalence among non-vegetarians may be attributed to factors such as irregular consumption of vitamin B12-rich foods like milk and fish, common cooking practices like frying that may

reduce vitamin availability, and lifestyle factors including increased intake of coffee, smoking, alcoholism, and higher rates of diabetes and hypertension. These findings highlight that dietary habits alone may not fully protect against vitamin B12 deficiency. Therefore, awareness, early diagnosis, and appropriate nutritional interventions are essential across all population groups to prevent the adverse health effects associated with vitamin B12 deficiency.

Reference

1. Stabler SP, Allen RH. Vitamin B12 deficiency as a worldwide problem. Annu Rev Nutr 2004;24:299-326.
2. Nielsen MJ, Rasmussen MR, Andersen CB, Nexø E, Moestrup SK. Vitamin B(12) transport from food to the body's cells-a sophisticated, multistep pathway. Nat Rev Gastroenterol Hepatol 2012;9:345-54.
3. Toh BH, Chan J, Kyaw T, Alderuccio F. Cutting edge issues in autoimmune gastritis. Clin Rev Allergy Immunol 2012; 42:269-78.
4. Lindenbaum J, Healton EB, Savage DG, et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. N Engl J Med 1988;318:1720-8.
5. Healton EB, Savage DG, Brust JC, Garrett TJ, Lindenbaum J. Neurologic aspects of cobalamin deficiency. Medicine 1991;70:229-45.
6. Kapur D, Agarwal KN, Agarwal DK. Nutritional anaemia and its control. Indian Journal of Pediatrics 2002; 69(7): 607-16.
7. Fazilli F. Nutritional anaemia in children. Indian Journal for the Practicing Doctor 2005; 2(5):5.
8. Gomber S, Kumar S, Rusia S, et al. Prevalence and aetiology of nutritional anaemias in early childhood in an urban slum. Indian Journal of Medical Research 1998; 107:269-73.
9. Modood-ul-Mannan, Anwar M, Saleem M, Wiqar A, Ahmad S. A study of serum vitamin B12 and folate levels in patients of megaloblastic anaemia in northern Pakistan. Journal of Pakistan Medical Association 1995; 45:187-8.
10. Sarode R, Garewal G, Marwaha N, Marwaha RK, Varma S, Ghosh K. Pancytopenia in nutritional megaloblastic anaemia. A study from north-west India. Tropical and Geographical Medicine 1989; 41:331-6.
11. Umasanker S, Bhakat R, Mehta S, Rathaur VK, Verma PK, Bhat NK, et al. Vitamin B12 deficiency in children from Northern India: Time to reconsider nutritional handicaps. J Fam Med Prim Care. 2020;9(9):4985.
12. Shalini T, Pullakhandam R, Ghosh S, Kulkarni B, Rajkumar H, Sachdev HS, et al. Prevalence of Vitamin B12 and Folate Deficiencies in Indian Children and Adolescents. Nutrients. 2023;15(13):3026.
13. Goyal S, Tiwari K, Meena P, Malviya S, Mohd A. Cobalamin and folate status in malnourished children. Int J Contemp Pediatr. 2017;4:1480-4.
14. Goraya JS, Kaur S. Infantile tremor syndrome -- down but not out. Indian Pediatr. 2015;52(3):249-50.
15. Ministry of Health and Family Welfare - Government of India. Comprehensive national nutritional survey, 2016-2018. Available at: https://nhm.gov.in/WriteReadData/l892s/140579603 1571201348.pdf. Accessed on 25 January 2025.

16. Rao SS, Goyal JP, Raghunath SV, Shah VB. Hematological profile of sickle cell disease from south Gujarat, India. Hematop Rep.2012;4(2):e8.
17. Thomas D, Chandra J, Sharma S, Jatin A, Pemde HK. Determinants of nutritional anaemia in adolescents. Indian Pediatr. 2015;52(10):867-9
18. Gomber S, Kumar S, Rusia U, Gupta P, Agarwal KN, Sharma S. Prevalence and etiology of nutritional anaemias in early childhood in an urban slum. Indian J Med Res.1998;107:269-73.
19. Arora S, Sheemar PS, Khurana MS, Kaur J, Kumar A. Serum folate, vitamin B12 levels and clinicohematological profile in 200 anaemic children aged 1-5 years. Int J Contemp Pediatr 2018;5:2167-72.
20. Rachna Mehandiratta et al. Prevalence and Clinical Hematological Profile of Vitamin B12 Deficiency Associated Megaloblastic Anemia in Children - A Hospital-Based Observational Study, International Journal of Pharmaceutical and Clinical Research 2024; 16(12); 1805-1808
21. Mangshetty S, Patil A, Mangshetty R. A study of clinical spectrum of vitamin B12 deficiency in pediatric age group in a tertiary care hospital. Int J Contemp Pediatr 2025;12:602-8

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