

Cross sectional study on the pattern of hemotological abnormalities in patients with vitamin B12 deficiency.

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Abstract

Background: Vitamin B12 deficiency may be associated with anemia, macrocytosis and abnormalities affecting other blood-cell lines. The pattern of hematological and peripheral smear findings can vary between patients. Aim: To study the pattern of hematological abnormalities in patients with vitamin B12 deficiency. **Methods:** A hospital-based cross-sectional study of 200 adults was specified for a one-year period. The proposed assessment included demographic and clinical details, serum vitamin B12, complete blood count and peripheral blood smear examination. **Results:** Anemia was present in 128/200 participants (64.0%), macrocytosis in 83 (41.5%), leukopenia in 31 (15.5%) and thrombocytopenia in 24 (12.0%). Fatigue and paraesthesia were reported in 70.0% and 32.5%, respectively. Macrocytosis was more frequent among participants with anemia than among those without anemia (57.0% versus 15.3%; $p < 0.001$). Smear comparisons showed more frequent hypersegmented neutrophils, macro-ovalocytes, anisopoikilocytosis and megaloblastic-appearing changes in the macrocytosis group. **Conclusion:** Range of hematological and smear abnormalities in vitamin B12 deficiency, with anemia and macrocytosis prominent. These are simulated values; the abstract's results and conclusion must be replaced with findings calculated from the actual study dataset.

Keywords: Vitamin B12 deficiency; hematological abnormalities; peripheral blood smear.



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INTRODUCTION

Vitamin B12, or cobalamin, is essential for DNA synthesis, red blood cell formation and normal neurological function. Deficiency may arise from inadequate dietary intake, impaired absorption, autoimmune gastritis, gastrointestinal disease or certain medications. Its clinical presentation varies: some patients have no obvious symptoms, while others develop fatigue, pallor, glossitis, paraesthesia, gait disturbance or cognitive changes. The hematological effects range from isolated anemia or macrocytosis to leukopenia, thrombocytopenia and, in severe cases, pancytopenia. The classical picture of megaloblastic anemia may include macro-ovalocytes and hypersegmented neutrophils on peripheral smear, but these findings are not present in every patient. A normal mean corpuscular volume also does not exclude deficiency, particularly when other conditions coexist.

Serum vitamin B12 is commonly used to assess vitamin B12 status, although the cutoff for deficiency varies across laboratories and assays. Values below approximately 200-250 pg/mL are generally considered subnormal; borderline results may require interpretation alongside clinical findings and, where available, additional biochemical tests. Complete blood counts and peripheral blood smear examination help identify and classify hematological abnormalities, including changes affecting more than one blood-cell lineage. Recognizing the range of these findings is clinically useful because reliance on macrocytosis alone may fail to identify some affected patients. Systematic assessment of blood counts and

smear morphology in patients with vitamin B12 deficiency can describe the frequency and pattern of hematological involvement and help guide further evaluation. This study assessed the hematological abnormalities observed among patients diagnosed with vitamin B12 deficiency at a tertiary-care hospital.

AIM

To study the pattern of hematological abnormalities in patients with vitamin B12 deficiency.

OBJECTIVES

1. To describe the demographic and clinical profile of patients diagnosed with vitamin B12 deficiency.
2. To determine the frequency and pattern of anemia, macrocytosis and other complete blood count abnormalities in these patients.
3. To describe peripheral blood smear findings and assess their relationship with the hematological profile.

MATERIALS AND METHODS

Source of data

Data were obtained from eligible patients diagnosed with vitamin B12 deficiency who attended or were admitted to Seth GS Medical College and KEM Hospital, Mumbai, during the study period. Clinical details and laboratory findings were recorded from patient interviews, examination and hospital laboratory reports.

Study design

A hospital-based cross-sectional observational study was conducted.

Study location

The study was conducted at Seth GS Medical College and King Edward Memorial (KEM) Hospital, Parel, Mumbai, Maharashtra, India. Patient recruitment and clinical assessment were undertaken through the relevant clinical services, and laboratory investigations were processed by the hospital laboratories.

Study duration

The study was conducted over one year.

Sample size

The calculated sample size was rounded to 200 participants.

Inclusion criteria

- Patients aged 18 years or older, of either sex.
- Patients with serum vitamin B12 deficiency based on the hospital laboratory's validated reference range; a value below 200 pg/mL was used where this was the laboratory cutoff.
- Patients who provided informed consent to participate.

Exclusion criteria

- Patients who had received vitamin B12 supplementation or treatment before the baseline blood sample was collected.
- Patients with a known hematological malignancy or a previously diagnosed primary bone marrow disorder.
- Patients receiving chemotherapy or radiotherapy at the time of assessment.
- Patients whose essential clinical or laboratory records were unavailable or whose blood sample was inadequate for analysis.
- Patients who declined consent.

Procedure and methodology

Eligible patients were identified consecutively during the study period. After obtaining written informed consent, demographic details, relevant symptoms, medical history, dietary history, medication use and examination findings were recorded using a structured data collection form. Relevant comorbidities and history of gastrointestinal surgery or disorders affecting absorption were also documented.

A blood sample was collected for serum vitamin B12 estimation and complete blood count. Hematological variables included hemoglobin, red blood cell count, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total leukocyte count, differential leukocyte count and platelet count. Peripheral blood smears were examined for red-cell morphology, macro-ovalocytes, anisopoikilocytosis, hypersegmented neutrophils and other relevant abnormalities. The study findings were classified using the hospital laboratory reference ranges and

standard adult hematological criteria. Hematological abnormalities were recorded as present or absent and categorized by cell line and pattern.

Sample processing

Blood samples for complete blood count were collected in EDTA tubes, mixed gently and analyzed using the hospital laboratory's automated hematology analyzer according to the manufacturer's instructions and the laboratory's quality-control procedures. Peripheral blood smears were prepared, stained using the laboratory's routine Romanowsky-type staining method and examined microscopically by trained laboratory personnel. Samples for serum vitamin B12 estimation were collected and processed according to the laboratory's routine procedure and assay protocol. Results were interpreted using the laboratory's validated reference range.

Statistical methods

Data were entered into a spreadsheet and analyzed using appropriate statistical software. Continuous variables were summarized as mean and standard deviation or median and interquartile range, depending on their distribution. Categorical variables were summarized as frequencies and percentages. The frequency of each hematological abnormality was reported with 95% confidence intervals where appropriate. Associations between categorical variables were assessed using the chi-square test or Fisher's exact test, as applicable. Continuous variables were compared using the independent-samples *t* test or Mann-Whitney U test, depending on distribution. A two-sided *p* value below 0.05 was considered statistically significant.

Data collection

Data were recorded on a pretested, structured case record form. The form captured participant identification code, age, sex, clinical features, relevant medical and medication history, serum vitamin B12 result, complete blood count parameters and peripheral smear findings. Each record was checked for completeness before entry, and patient names and other direct identifiers were excluded from the analysis dataset.

RESULTS

Table 1: Pattern of hematological abnormalities by sex

Hematological finding*	Total, n (%)	95% CI for total prevalence	Male (n=98), n (%)	Female (n=102), n (%)	χ^2	Difference in prevalence, male - female (95% CI)	p value
Anemia	128 (64.0)	57.1-70.3	62 (63.3)	66 (64.7)	0.05	-1.4% (-14.7 to 11.9)	0.832
Macrocytosis	83 (41.5)	34.9-48.4	45 (45.9)	38 (37.3)	1.55	8.7% (-5.0 to 22.3)	0.214
Leukopenia	31 (15.5)	11.1-21.2	17 (17.3)	14 (13.7)	0.50	3.6% (-6.4 to 13.7)	0.479
Thrombocytopenia	24 (12.0)	8.2-17.2	12 (12.2)	12 (11.8)	0.01	0.5% (-8.5 to 9.5)	0.917
Any listed abnormality	151 (75.5)	69.1-80.9					

151 of 200 patients (75.5%) had at least one listed hematological abnormality. Anemia was the most frequent finding (64.0%), followed by macrocytosis (41.5%). The sex-based comparisons were not statistically significant for anemia, macrocytosis, leukopenia or thrombocytopenia.

Table 2: Demographic and clinical profile by age group

Characteristic	Total, n (%)	<60 years (n=129), n (%)	≥60 years (n=71), n (%)	χ^2	Difference in prevalence, <60 - ≥60 (95% CI)	p value
Female sex	98 (49.0)	62 (48.1)	36 (50.7)	0.13	-2.6% (-17.1 to 11.8)	0.721
Vegetarian diet	116 (58.0)	73 (56.6)	43 (60.6)	0.30	-4.0% (-18.2 to 10.3)	0.586
Fatigue	140 (70.0)	88 (68.2)	52 (73.2)	0.55	-5.0% (-18.1 to 8.0)	0.458
Paraesthesia	65 (32.5)	39 (30.2)	26 (36.6)	0.85	-6.4% (-20.1 to 7.3)	0.356

129 participants (64.5%) were younger than 60 years and 71 (35.5%) were aged 60 years or older. Fatigue was reported by 70.0% and paraesthesia by 32.5%.

Table 3: Complete blood count abnormalities by anemia status

Abnormality	Anemia present (n=128), n (%)	Anemia absent (n=72), n (%)	χ^2	Difference in prevalence, anemia present - absent (95% CI)	p value
Macrocytosis	73 (57.0)	11 (15.3)	32.98	41.8% (29.8 to 53.7)	<0.001
Leukopenia	23 (18.0)	8 (11.1)	1.65	6.9% (-3.0 to 16.7)	0.198
Thrombocytopenia	18 (14.1)	6 (8.3)	1.43	5.7% (-3.0 to 14.5)	0.231
Bicytopenia	15 (11.7)	3 (4.2)	3.21	7.6% (0.3 to 14.8)	0.073
Pancytopenia, overall	7 (3.5)	Not applicable		95% CI for overall prevalence: 1.7-7.0	

Macrocytosis was more frequent among patients with anemia than among those without anemia (57.0% versus 15.3%; $p<0.001$). Differences in leukopenia, thrombocytopenia and bicytopenia were not statistically significant. Pancytopenia was present in 7 patients (3.5%).

Table 4: Peripheral smear findings by macrocytosis status

Peripheral finding*	Macrocytosis present (n=83), n (%)	Macrocytosis absent (n=117), n (%)	χ^2	Difference in prevalence, macrocytosis present - absent (95% CI)	p value
Hypersegmented neutrophils	61 (73.5)	29 (24.8)	46.54	48.7% (36.4 to 61.0)	<0.001
Macro-ovalocytes	56 (67.5)	26 (22.2)	41.10	45.2% (32.7 to 57.8)	<0.001
Anisopoikilocytosis	64 (77.1)	39 (33.3)	37.25	43.8% (31.3 to 56.2)	<0.001
Smear pattern suggestive of megaloblastic change	67 (80.7)	34 (29.1)	51.84	51.7% (39.8 to 63.5)	<0.001

Hypersegmented neutrophils, macro-ovalocytes, anisopoikilocytosis and a smear pattern suggestive of megaloblastic change were each more frequent in the macrocytosis group ($p<0.001$ for each comparison).

DISCUSSION

Table 1 Anemia in 64.0% of participants, macrocytosis in 41.5%, leukopenia in 15.5% and thrombocytopenia in 12.0%. These findings are compatible with the recognized range of blood-count abnormalities in vitamin B12 deficiency, which can include anemia, macrocytosis and one or more cytopenias.

The frequency of each abnormality varies across clinical settings and case definitions. Singh et al. (2022) reported anemia in 87.73% and macrocytosis in 88.64% of their 220 cases, while Shilpy et al. (2025) found anemia in 75% but reported that normocytic blood pictures were more common than macrocytic anemia. Differences in referral patterns, deficiency thresholds, coexisting conditions and participant selection may contribute to this variation. In the table, comparisons between male and female participants were not statistically significant for the listed abnormalities.

This should be interpreted as a feature of the example data, not as evidence of no sex difference in the study population. Published hospital studies report differing sex distributions and clinical profiles; for example, Singh et al. (2022) reported a slight male majority, while Bandyopadhyay et al. (2021) described deficiency among both male and female symptomatic patients. The estimates should not be directly compared without accounting for differences in study populations and diagnostic criteria.

Table 2 Fatigue in 70.0% of participants and paraesthesia in 32.5%. These are clinically plausible symptoms: fatigue may accompany anemia, while paraesthesia may occur with neurological involvement. However, symptom frequencies vary substantially among studies. Agrawal et al. (2024) reported fatigue in 66.7% and tingling or numbness in 54.4% of their patients; Singh et al. (2022) reported paraesthesia in a much higher proportion of their case series. The vegetarian-diet proportion in the table is also not directly comparable with studies that recruited patients based on dietary risk or documented diet in greater detail.

Table 3 shows association between anemia and macrocytosis (57.0% versus 15.3%; $p<0.001$). The association is consistent with the expected relationship between impaired DNA synthesis and enlarged red cells, but macrocytosis is not present in every patient with vitamin B12 deficiency. Studies have described normocytic presentations and variation in red-cell indices, particularly when deficiency is identified before advanced hematological changes or when other causes of anemia coexist. The example table also shows no statistically significant associations between anemia status and leukopenia or thrombocytopenia; those comparisons would need to be recalculated from the actual participant-level data.

Table 4 More frequent hypersegmented neutrophils, macro-ovalocytes, anisopoikilocytosis and a megaloblastic-appearing smear in participants classified as macrocytic. These findings fit the conventional blood-film description of megaloblastic change. Nevertheless, a peripheral smear and mean corpuscular volume should be interpreted alongside the clinical picture and biochemical results. Vitamin B12 deficiency can present without the full classical smear pattern, and serum B12 thresholds and confirmatory testing vary. Current NICE guidance recommends interpreting test results in their clinical context and considering further testing when results are indeterminate.

CONCLUSION

Anemia was the most frequent hematological abnormality, and macrocytosis was associated with anemia. Common smear changes were more frequent in the macrocytosis group. These results are simulated and cannot support conclusions about the actual study population; the conclusion must be rewritten after analysis of the collected data.

LIMITATIONS

The values currently shown are rather than observed, so they cannot be used to estimate prevalence, test hypotheses or support a study conclusion. For the proposed cross-sectional study, relevant limitations may include its single-centre, hospital-based setting, which may limit generalizability; its cross-sectional design, which cannot establish temporal or causal relationships; and possible variation in vitamin B12 assay thresholds and laboratory reference ranges. Coexisting iron or folate deficiency, chronic disease and medication use may influence blood counts and smear morphology. If methylmalonic acid or homocysteine testing was not performed, some borderline biochemical results may also be difficult to interpret. These limitations should be retained only where they accurately describe the completed study.

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