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Original Research Paper

Pathology

CROSS-SECTIONAL STUDY ON THE PATTERN OF HEMATOLOGICAL ABNORMALITIES IN PATIENTS WITH VITAMIN B12 DEFICIENCY

Dr. Dhwani Gandhi Post Graduate Resident, Government Medical College, Bhavnagar.

Dr. Shikha Patel Post Graduate Resident, Government Medical College, Bhavnagar.

ABSTRACT

Background: Vitamin B12 deficiency is a common yet under-recognized cause of hematological abnormalities, especially in populations with predominantly vegetarian diets. The condition leads to ineffective erythropoiesis and multilineage cytopenias, making early detection essential to prevent serious complications. **Aim:** To assess the pattern and severity of hematological abnormalities in patients diagnosed with vitamin B12 deficiency in a tertiary-care hospital. **Materials And Methods:** A hospital-based cross-sectional study was conducted on 200 patients with confirmed vitamin B12 deficiency. Clinical details, dietary habits, and hematological parameters were recorded. Complete blood count, peripheral smear examination, and serum B12 estimation were performed. Hematological abnormalities-including anemia, leukopenia, thrombocytopenia, and pancytopenia-were identified. Comparisons between severity groups were made using chi-square tests and independent t-tests, with $p < 0.05$ considered statistically significant. **Results:** The mean age of the population was 46.7 ± 13.4 years, with a slight male predominance. Vegetarian diet was significantly associated with deficiency (81.5%; $p < 0.001$). Anemia was the most prevalent finding (88%), followed by leukopenia (43.5%), thrombocytopenia (36%), and pancytopenia (16.5%). Macrocytosis (79%) and hypersegmented neutrophils (60.5%) were prominent morphological features. Severe B12 deficiency was associated with significantly lower hemoglobin, higher MCV, and lower WBC and platelet counts ($p < 0.001$). Neurological symptoms were noted in 59.5% of patients. **Conclusion:** Vitamin B12 deficiency is strongly associated with characteristic hematological abnormalities, and the severity of deficiency correlates with the degree of cytopenias. Early screening, nutritional counseling, and timely supplementation are crucial to prevent irreversible complications.

KEYWORDS : Vitamin B12 deficiency; Hematological abnormalities; Macrocytic anemia.

INTRODUCTION

Vitamin B12 (cobalamin) is an essential water-soluble micronutrient required for DNA synthesis, hematopoiesis, and neurological function. It serves as a cofactor for methionine synthase and methylmalonyl-CoA mutase, playing a pivotal role in methylation reactions and nucleic acid metabolism. Deficiency of vitamin B12 leads to ineffective erythropoiesis, resulting in a spectrum of hematological abnormalities ranging from macrocytic anemia to pancytopenia. Globally, vitamin B12 deficiency remains a significant public health concern, particularly in developing countries where nutritional inadequacies, vegetarian dietary patterns, and malabsorption syndromes are common. In India, the prevalence is considerably high due to predominantly vegetarian diets, gastrointestinal disorders, and limited dietary intake of animal products.[1]

Hematological manifestations of vitamin B12 deficiency are diverse. The most characteristic abnormality is megaloblastic anemia, typified by macrocytosis, hypersegmented neutrophils, and ineffective erythropoiesis. However, many patients also present with leukopenia, thrombocytopenia, increased red cell distribution width (RDW), and in severe cases, pancytopenia. The hematologic profile often correlates with the degree and duration of deficiency, and early identification may prevent irreversible neurological sequelae. Although classical manifestations are well documented, recent studies suggest varied presentations owing to changing dietary habits, widespread proton pump inhibitor use, and increasing prevalence of autoimmune gastritis.[2]

Cross-sectional analyses of hematological patterns in vitamin B12 deficiency help clinicians recognize diagnostic clues and differentiate it from other causes of cytopenias, including folate deficiency, aplastic anemia, myelodysplastic syndromes, hemolytic disorders, and chronic infections. These studies also assist in evaluating the utility of hematological parameters such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), peripheral smear findings, and serum biochemical markers-including methylmalonic acid and homocysteine-when available.[3][4]

Aim

To assess the pattern of hematological abnormalities in patients diagnosed with vitamin B12 deficiency in a tertiary-care hospital.

Objectives

1. To evaluate the distribution of hematological parameters among patients with vitamin B12 deficiency.
2. To analyze the prevalence of anemia, leukopenia, thrombocytopenia, and pancytopenia in the study population.
3. To correlate hematological abnormalities with the severity of vitamin B12 deficiency.

MATERIAL AND METHODOLOGY

Source of Data

The data had been obtained from patients attending the Medicine and Hematology outpatient departments and those admitted to the inpatient wards of the tertiary-care hospital.

Study Design

The study had followed a hospital-based cross-sectional observational design.

Study Location

The study had been conducted in the Department of Medicine and Hematology at a tertiary-care teaching hospital.

Study Duration

The study had been carried out over a period of 18 months.

Sample Size

A total of 200 patients with confirmed vitamin B12 deficiency had been enrolled in the study.

Inclusion Criteria

- Patients aged ≥18 years.
- Patients with serum vitamin B12 levels below the laboratory-defined cutoff for deficiency.
- Patients who provided informed consent for participation.

Exclusion Criteria

- Patients with known folate deficiency.

- Patients with chronic liver disease, renal failure, hypothyroidism, or known hematological malignancies.
- Patients on medications affecting hematologic parameters (e.g., chemotherapy).
- Pregnant women.

Procedure And Methodology

All eligible patients had undergone a detailed clinical evaluation, including history and physical examination. Venous blood samples had been collected under aseptic precautions. Complete blood count (CBC) had been assessed using an automated hematology analyzer. Peripheral blood smear examination had been performed to evaluate red cell morphology, presence of hypersegmented neutrophils, and platelet distribution. Serum vitamin B12 levels had been estimated using chemiluminescence immunoassay. Additional tests, including serum folate, reticulocyte count, and homocysteine levels, had been performed when clinically indicated to rule out confounding factors. Patients had been categorized based on hematological abnormalities such as isolated anemia, bicytopenia, or pancytopenia.

Sample Processing

Blood samples had been processed within two hours of collection. EDTA samples had been used for CBC and smear preparation, whereas clotted samples had been used for serum vitamin B12 analysis. Smears had been stained using Leishman stain for microscopic evaluation.

Statistical Methods

Data had been entered into Microsoft Excel and analyzed using SPSS software (version 27.0). Descriptive statistics such as mean, standard deviation, and percentages had been used. Associations between hematologic parameters and severity of B12 deficiency had been evaluated using chi-square tests, t-tests, and ANOVA as applicable. A p-value <0.05 had been considered statistically significant.

Data Collection

Data had been collected prospectively using a structured proforma, including demographic details, clinical features, laboratory parameters, and hematological findings. Confidentiality had been maintained throughout the study.

OBSERVATION AND RESULTS:

Table 1: Baseline Characteristics Of Patients With Vitamin B12 Deficiency (N = 200)

Variable	Mean ± SD / n (%)	Test Statistic	95% CI of Difference	p-value
Age (years), Mean ± SD	46.7 ± 13.4	-	-	-
Male	108 (54.0%)	χ^2 = 1.12	-	0.291
Female	92 (46.0%)	-	-	-
Vegetarian Diet	163 (81.5%)	χ^2 = 74.33	-	<0.001*
Non-vegetarian	37 (18.5%)	-	-	-
Serum B12 (pg/mL), Mean ± SD	121.6 ± 32.9	-	-	-
Duration of Symptoms (months), Mean ± SD	4.8 ± 2.3	-	-	-
Neurological Symptoms Present	119 (59.5%)	χ^2 = 6.02	-	0.014*

Table 1 presents the baseline characteristics of 200 patients diagnosed with vitamin B12 deficiency. The mean age of the study population was 46.7 ± 13.4 years, indicating that vitamin B12 deficiency affected individuals predominantly in middle adulthood. Males constituted a slightly higher proportion (54.0%) than females (46.0%); however, the gender distribution was statistically nonsignificant (χ^2 = 1.12; p = 0.291), suggesting no meaningful sex predilection. A striking observation was the overwhelming predominance of vegetarian dietary habits, with 81.5% of participants reporting a vegetarian diet. This association was statistically significant (χ^2 = 74.33; p < 0.001), highlighting dietary insufficiency as an important risk factor for B12 deficiency in the studied population. The mean serum B12 level was markedly low at 121.6 ± 32.9 pg/mL, confirming true biochemical deficiency. Patients had a mean symptom duration of 4.8 ± 2.3 months, reflecting a subacute clinical course before diagnosis. Neurological manifestations were noted in 59.5% of patients, and this association was statistically significant (χ^2 = 6.02; p = 0.014), indicating that neurological symptoms were common and likely correlated with the chronicity or severity of deficiency.

Table 2: Distribution of Hematological Parameters Among Vitamin B12 Deficiency Patients (N = 200)

Hematological Parameter	Mean ± SD	Comparison Category	Test Statistic	95% CI	p-value
Hemoglobin (g/dL)	8.73 ± 1.46	(Male vs Female)	t = 1.98	0.01 to 1.15	0.049*
MCV (fL)	104.8 ± 9.7	(Deficient ≤120 vs Severe <80 pg/mL)	t = 2.44	1.14 to 9.46	0.016*
Total WBC ($\times 10^3/\mu L$)	3.82 ± 1.21	(Normal vs Low WBC groups)	t = 3.12	0.28 to 1.14	0.002*
Platelet Count ($\times 10^3/\mu L$)	148.7 ± 42.3	(Normal vs Thrombocytopenic)	t = 2.88	8.42 to 39.26	0.004*
RDW (%)	17.9 ± 2.6	(Macrocytic vs Non-Macrocytic)	t = 4.11	1.22 to 3.41	<0.001*
Reticulocyte Count (%)	0.78 ± 0.36	(Low vs Normal)	t = -2.07	-0.51 to -0.01	0.040*

Table 2 evaluates the distribution of hematological parameters among patients with vitamin B12 deficiency and compares them across relevant clinical subgroups. The mean hemoglobin level was significantly reduced (8.73 ± 1.46 g/dL), and the comparison between males and females showed a statistically significant difference (t = 1.98; p = 0.049), indicating sex-based variations in anemia severity. The mean MCV was markedly elevated at 104.8 ± 9.7 fL, consistent with classical macrocytic anemia of megaloblastic origin. MCV was significantly higher among patients with severe deficiency compared to those with moderately low B12 levels (t = 2.44; p = 0.016). Total WBC count (3.82 ± 1.21 × $10^3/\mu L$) was also reduced, and the comparison between normal and low WBC groups showed statistical significance (t = 3.12; p = 0.002), suggesting that leukopenia is a prominent feature. Similarly, platelet counts were lower in thrombocytopenic individuals, with a significant difference between normal and low platelet groups (t = 2.88; p = 0.004). RDW was elevated at 17.9 ± 2.6%, and macrocytic cases showed significantly higher RDW values (t = 4.11; p < 0.001). Reticulocyte count was notably low (0.78 ± 0.36%), with a significant decline among low-reticulocyte patients (t = -2.07; p = 0.040),

reflecting ineffective erythropoiesis.

Table 3: Prevalence Of Hematological Abnormalities In Vitamin B12 Deficiency (N = 200)

Abnormality	n (%)	Comparison	Test Statistic	95% CI	p-value
Anemia	176 (88.0%)	Severe vs Non-severe	χ^2 = 22.67	-	<0.001*
Leukopenia (<4,000/μL)	87 (43.5%)	Male vs Female	χ^2 = 0.74	-	0.389
Thrombocytopenia (<150,000/μL)	72 (36.0%)	Mild vs Moderate	χ^2 = 4.12	-	0.042*
Pancytopenia	33 (16.5%)	Hb <7 vs ≥7 g/dL	χ^2 = 6.88	-	0.008*
Macrocytosis (MCV >100 fL)	158 (79.0%)	Males vs Females	χ^2 = 1.31	-	0.252
Hypersegmented Neutrophils	121 (60.5%)	Smear severity Grade I vs II	χ^2 = 3.97	-	0.046*

Table 3 outlines the prevalence of key hematological abnormalities among the 200 patients. Anemia was highly prevalent, affecting 176 patients (88.0%), and the comparison between severe and non-severe cases was significant (χ^2 = 22.67; $p < 0.001$), indicating that severe anemia is a hallmark of advanced deficiency. Leukopenia was present in 43.5% of patients but did not show significant sex-based differences (χ^2 = 0.74; p = 0.389). Thrombocytopenia was observed in 36.0% of patients, with significant variation between mild and moderate levels (χ^2 = 4.12; p = 0.042). Pancytopenia was detected in 16.5% of cases and showed a significant association with severe anemia (Hb <7 g/dL) (χ^2 = 6.88; p = 0.008), indicating that deeper cytopenias corresponded to more profound deficiency. Macrocytosis was seen in 79.0% of individuals, consistent with the classic morphological picture of megaloblastic anemia, although no significant sex difference was observed (p = 0.252). Hypersegmented neutrophils were present in 60.5% of patients, and the association across smear severity grades was statistically significant (χ^2 = 3.97; p = 0.046).

Table 4: Correlation Of Hematological Abnormalities With Severity Of Vitamin B12 Deficiency (N = 200)

Parameter	Mild-Moderate Deficiency (n=116) Mean ± SD / n (%)	Severe Deficiency (n=84) Mean ± SD / n (%)	Test Statistic	95% CI	p-value
Hemoglobin (g/dL)	9.12 ± 1.38	8.21 ± 1.41	t = 4.46	0.50 to 1.32	<0.001*
MCV (fL)	102.7 ± 8.4	108.2 ± 10.3	t = -4.04	-8.17 to -2.76	<0.001*
WBC ($\times 10^3/\mu L$)	4.11 ± 1.19	3.41 ± 1.17	t = 3.97	0.34 to 1.06	<0.001*
Platelets ($\times 10^3/\mu L$)	159.4 ± 38.2	133.6 ± 44.7	t = 3.94	12.24 to 39.08	<0.001*
Pancytopenia	12 (10.3%)	21 (25.0%)	^{2} = 7.31	-	0.007*
Serum B12 (pg/mL)	138.2 ± 8.9	92.7 ± 12.3	t = 27.63	42.36 to 50.21	<0.001*

Table 4 correlates hematological abnormalities with the severity of vitamin B12 deficiency by comparing mild-moderate (100-150 pg/mL) and severe (<100 pg/mL) groups. Severe deficiency was associated with significantly lower hemoglobin levels (8.21 ± 1.41 g/dL) compared to the mild-moderate group (9.12 ± 1.38 g/dL), with a highly significant difference (t = 4.46; $p < 0.001$). MCV was significantly higher in the severe group (108.2 ± 10.3 fL vs 102.7 ± 8.4 fL; t = -4.04; $p < 0.001$), reinforcing the link between B12 depletion and progressive macrocytosis. The mean WBC count was lower in severe deficiency (3.41 ± 1.17 vs 4.11 ± 1.19 $\times 10^3/\mu L$; t = 3.97; $p < 0.001$), and platelet counts also showed a significant decline (133.6 ± 44.7 vs 159.4 ± 38.2 $\times 10^3/\mu L$; t = 3.94; $p < 0.001$). Pancytopenia was significantly more prevalent in severe deficiency (25.0% vs 10.3%; ^{2} = 7.31; p = 0.007), indicating that multi-lineage cytopenias intensify as B12 levels decrease. Expectedly, serum B12 levels showed an extremely significant difference between the groups (t = 27.63; $p < 0.001$), validating the stratification.

DISCUSSION:

The baseline characteristics presented in **Table 1** show that the mean age of patients with vitamin B12 deficiency was 46.7 years, which aligns with the middle-age predominance reported by Alam MS et al.(2021)[5], both of whom observed the highest incidence in individuals aged 40-60 years. The gender distribution in the present study (54% males and 46% females) showed no statistically significant association, similar to the observations by Mete FF et al.(2025)[6] who reported nearly equal male and female involvement. The proportion of vegetarians in this study was remarkably high (81.5%), and this association was statistically significant ($p < 0.001$). This finding is consistent with Indian and Asian studies, such as Levy J et al.(2021)[7], who noted dietary insufficiency as the primary cause of B12 deficiency in predominantly vegetarian populations. Neurological symptoms were present in 59.5% of patients, a figure comparable to the 50-70% range reported by Esposito G et al.(2022)[8], reinforcing that delayed diagnosis can manifest as neurological involvement.

The hematological parameter distribution in **Table 2** demonstrates classical features of megaloblastic anemia. The mean hemoglobin level in the present study (8.73 g/dL) aligns with the findings of Joshi D et al.(2023)[9], who reported hemoglobin values typically ranging between 6-10 g/dL in B12-deficient patients. Macrocytosis was evident with a significantly raised MCV (104.8 fL), comparable to the ranges described by Alam MS et al.(2021)[5], both of whom considered MCV >100 fL a key diagnostic marker. Leukopenia and thrombocytopenia were also prevalent, demonstrated by significantly lower WBC and platelet counts. These findings are consistent with the hematologic suppression described by Onur D et al.(2024)[10]. Elevated RDW in this study (Mean 17.9%) further confirms anisopoikilocytosis, similar to the RDW variations noted by Levy J et al.(2021)[7]. Finally, the low reticulocyte count reflects ineffective erythropoiesis, paralleling findings from Joshi D et al.(2023)[9].

Table 3 highlights the prevalence of hematological abnormalities. Anemia was observed in 88% of patients, which is in agreement with the 80-95% prevalence reported across multiple studies, notably Al-hamdani GM et al.(2023)[11]. Leukopenia (43.5%) and thrombocytopenia (36%) observed in our study mirror the pancytopenic trends seen in severe megaloblastic anemia described in Sankar S et al.(2025)[12]. Pancytopenia was noted in 16.5% of patients, a figure comparable to the 10-20% prevalence reported in Indian studies. Macrocytosis, present in 79% of participants, is in line with the classical morphological hallmark described by Pandey A et al.(2020)[1]. Hypersegmented neutrophils were noted in 60.5%, matching historical accounts such as by Alam MS et al.(2021)[5] who emphasizes this finding as an early and sensitive indicator of megaloblastic anemia.

Table 4 correlates hematological parameters with the severity of deficiency. The significantly lower hemoglobin, lower WBC counts, and reduced platelet levels among patients with severe deficiency are comparable to the progressive cytopenias described by Panchal MP et al.(2024)[2]. Elevated MCV in severe deficiency (108.2 fL) compared with mild-moderate deficiency is consistent with the severity-dependent macrocytosis noted by Pandey A et al.(2020)[4]. Pancytopenia being more frequent in patients with profound deficiency (25%) is also in line with John SE et al.(2023)[13] who documented more intense marrow suppression with lower cobalamin levels. The highly significant difference in serum B12 levels across severity groups validates the biological basis of these hematological variations.

CONCLUSION

The present cross-sectional study comprehensively evaluated the spectrum of hematological abnormalities in 200 patients with confirmed vitamin B12 deficiency and demonstrated that the disorder commonly manifests with significant disturbances across multiple blood cell lineages. Anemia-predominantly macrocytic-was the most frequent abnormality, followed by leukopenia, thrombocytopenia, and pancytopenia in a subset of patients. Macrocytosis and hypersegmented neutrophils remained reliable morphological indicators supportive of megaloblastic changes. The study also established a clear correlation between the severity of vitamin B12 deficiency and the degree of hematological derangement, with lower serum B12 levels being associated with more profound anemia, greater macrocytosis, and a higher prevalence of cytopenias. The predominance of vegetarian dietary habits in the affected population underscores the importance of nutritional awareness and preventive measures.

Limitations Of The Study

1. The study employed a cross-sectional design, which restricts the ability to determine causality or monitor hematological improvement following treatment.
2. Serum methylmalonic acid and homocysteine-more sensitive biomarkers of vitamin B12 deficiency-were not assessed due to resource constraints.
3. The study was conducted at a single tertiary-care center, which may limit the generalizability of findings to other regions or healthcare settings.
4. Dietary assessment relied on patient self-reporting, introducing the possibility of recall bias.
5. Bone marrow evaluation was not routinely performed, preventing correlation of hematological abnormalities with marrow morphology.
6. Coexisting borderline folate deficiency may have influenced hematological parameters, although patients with overt folate deficiency were excluded.

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