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Original research

COMPARISON OF PERIPHERAL BLOOD CELL CHARACTERISTICS IN PATIENTS WITH VITAMIN B12 DEFICIENCY ANEMIA WITH AND WITHOUT IRON DEFICIENCY

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ABSTRACT

Objective: *To compare hematological characteristics in patients with vitamin B12 deficiency anemia with and without concomitant iron deficiency at the National Institute of Hematology and Blood Transfusion during the period 2024 - 2025.*

Methods: *A retrospective cross-sectional study was conducted on 281 patients diagnosed with vitamin B12 deficiency anemia from January 2024 to December 2025. Patients were divided into two groups: vitamin B12 deficiency alone and vitamin B12 deficiency with concomitant iron deficiency. Hematological parameters were compared between the two groups.*

Results: *The mean age was 51.4 ± 19.9 years, with a male-to-female ratio of 1:1.98. Mild and moderate anemia accounted for 93.6% of cases. Among 281 patients, 156 had isolated vitamin B12 deficiency, and 125 had concomitant iron deficiency. The isolated vitamin B12 deficiency group had significantly higher Hb, MCV, MCH, and MCHC compared to the combined deficiency group (90.5 g/L vs. 79.0 g/L; 104.6 fL vs. 69.1 fL; 34.8 pg vs. 20.8 pg; 332 g/L vs. 306 g/L, respectively; p < 0.001). Thrombocytopenia was more frequent in the isolated vitamin B12 deficiency group than in the combined deficiency group (39.1% vs. 5.6%; p < 0.05). The proportion of hypersegmented neutrophils was high in both groups (66.0% and 70.4%, respectively), with no statistically significant difference.*

Conclusion: *Concomitant iron deficiency was associated with differences in red cell indices and some white blood cell and platelet features, potentially masking the typical hematological manifestations of vitamin B12 deficiency.*

Keywords: *Anemia, vitamin B12 deficiency, iron deficiency, peripheral blood cells.*

I. INTRODUCTION

Anemia is a common health problem, among which nutritional deficiency anemia accounts for a significant proportion, including iron, folate, and vitamin B12 deficiencies [1, 2]. Vitamin B12 deficiency is an important cause of anemia; however, its hematological manifestations are not always typical. In some cases, particularly when concomitant iron deficiency is present, the characteristic macrocytic features may be masked, leading to alterations in hematological parameters and posing diagnostic challenges.

Iron deficiency is one of the most common micronutrient deficiencies worldwide and may coexist with vitamin B12 deficiency [3-5]. This coexistence may alter the characteristics of red blood cells, white blood cells, and platelets, thereby complicating the identification of the underlying cause of anemia [6, 7]. In patients with combined nutritional deficiency, the hematological presentation may be atypical, making diagnosis more challenging when based only on conventional red cell indices. Although several studies worldwide have addressed this

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combined deficiency [4, 8], data from Vietnam remain limited. Therefore, we conducted this study to compare hematological characteristics in patients with vitamin B12 deficiency anemia with and without concomitant iron deficiency at the National Institute of Hematology and Blood Transfusion during the period 2024 - 2025.

II. SUBJECTS AND METHODS

2.1. Study Subjects.

A total of 281 patients diagnosed with vitamin B12 deficiency anemia at the National Institute of Hematology and Blood Transfusion (NIHBT) were included. All patients were aged ≥18 years, met the WHO 2011 criteria for anemia [9], and had serum vitamin B12 levels < 200 pg/mL [10]. In this study, vitamin B12 deficiency was identified based on serum vitamin B12 concentration recorded in the medical charts. Additional functional biomarkers, such as methylmalonic acid or homocysteine, were not routinely available in all cases [11].

Exclusion criteria: Patients receiving medications or chemotherapy, or those with other hematological disorders, including autoimmune hemolytic anemia, hereditary hemolytic anemia, acute leukemia, and primary myelodysplastic syndromes, were excluded. However, due to the retrospective design, some potential confounding conditions that may affect MCV, platelet count, and white blood cell parameters, such as folate deficiency, chronic inflammatory disease, liver disease, and other comorbid conditions, could not be fully assessed or excluded in all cases.

2.2. Methods.

Study design: A retrospective cross-sectional study based on medical records.

Study setting and duration: The study was conducted from January 2024 to December 2025 at NIHBT.

Data collection: Demographic data (age, sex) and peripheral blood cell parameters were collected using a standardized data collection form.

Peripheral blood smear morphology was assessed by physicians at the Department of Cytology and Histology, National Institute of Hematology and Blood Transfusion, all of whom hold valid professional certificates in hematology laboratory practice. Hypersegmented neutrophils were defined as neutrophils with five or more nuclear lobes [12].

Definition of iron deficiency: Serum ferritin < 30 ng/mL and/or transferrin saturation < 30% [13]. Because ferritin may be elevated in inflammatory states, reliance on ferritin may have led to underrecognition of iron deficiency in some patients.

2.3. Statistical analysis.

Data were analyzed using SPSS version 27.0. Continuous variables were expressed as mean ± SD or median and interquartile range, while categorical variables were presented as frequencies and percentages (%). Comparisons of categorical variables were performed using the chi-square (χ^2) test, and continuous variables were compared using the Student's T-test or Mann - Whitney test, as appropriate. A p-value < 0.05 was considered statistically significant.

2.4. Ethical approval.

This study was approved by the Scientific Committee of the National Institute of Hematology and Blood Transfusion and Hanoi Medical University. All procedures were conducted in accordance with ethical standards, and patient confidentiality was strictly maintained.

III. RESULTS

3.1. General characteristics

Among the 281 patients included in the study, the male-to-female ratio was 1:1.98. The mean age was 51.4 ± 19.9 years, with the ≥ 60 - year age group accounting for the highest proportion (43.1%).

3.2. Characteristics of Peripheral Blood Cells

Mild and moderate anemia accounted for a very high proportion (93.6%), while only 6.4% of patients had severe anemia (Figure 1).

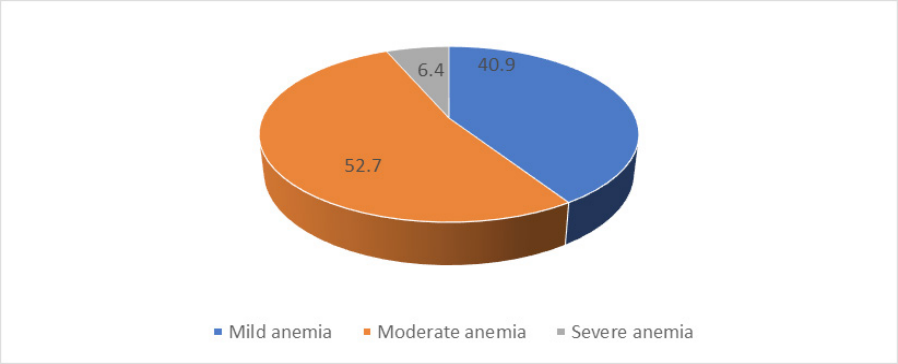

Figure 1: Severity of anemia in the study population

The median MCV, MCH, and MCHC in the isolated vitamin B12 deficiency group were 104.6 fL, 34.8 pg, and 332 g/L, respectively, which were significantly higher than those in the group with iron deficiency (69.1 fL, 20.8 pg, and 306 g/L, respectively) ($p < 0.001$). The RBC count in the group with concomitant iron deficiency (3.76 T/L) was higher than that in the isolated vitamin B12 deficiency group (2.55 T/L) ($p < 0.001$). Hb levels in the isolated vitamin B12 deficiency group (90.5 g/L) were higher than in the group with concomitant iron deficiency (79.0 g/L) ($p < 0.001$). RDW and RET (%) showed no statistically significant differences between the two groups (Table 1).

Table 1: Distribution of patients by selected red blood cell parameters

Parameters	Median (Q1-Q3)			p-value
	Overall (n=281)	Isolated vitamin B12 deficiency (n=156)	Vitamin B12 deficiency with iron deficiency (n=125)	
RBC (T/L)	3.16 (2.38 - 3.81)	2.55 (2.06 - 3.15)	3.76 (3.33 - 4.18)	< 0.001
Hb (g/L)	84.0 (74.0 - 97.0)	90.5 (76.3 - 100.7)	79.0 (70.5 - 89.5)	< 0.001
Hct (%)	26.6 (23.6 - 29.5)	27.4 (23.1 - 30.4)	26.1 (24.1 - 28.7)	> 0.05
MCV (fL)	89.2 (69.7 - 110.4)	104.6 (91.5 - 120.4)	69.1 (60.9 - 77.3)	< 0.001
MCH (pg)	29.0 (20.9 - 36.6)	34.8 (30.5 - 40.4)	20.8 (17.8 - 24.9)	< 0.001
MCHC (g/L)	323 (306 - 336)	332 (323 - 342)	306 (292 - 320)	< 0.001
RDW (%)	18.9 (16.0 - 22.4)	18.6 (15.7 - 24.9)	19.0 (16.9 - 20.9)	> 0.05
RET (%)	2.08 (1.31 - 2.80)	1.86 (1.05 - 3.27)	2.18 (1.54 - 2.54)	> 0.05

**Abbreviation: RBC: Red blood cells; Hb: Hemoglobin; Hct: Hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; RDW: Red Cell Distribution Width; RET; Reticulocyte.*

The proportion of thrombocytopenia (<150 G/L) in the isolated vitamin B12 deficiency group was 39.1%, which was significantly higher than in the group with iron deficiency (5.6%) ($p < 0.05$) (Table 2).

Table 2: Distribution of patients according to platelet count

Parameters	Overall n (%)	Isolated vitamin B12 deficiency n (%)	Vitamin B12 deficiency with iron deficiency n (%)	p-value
< 150	68 (24.2)	61 (39.1)	7 (5.6)	< 0.05
150 - 450	189 (67.3)	86 (55.1)	103 (82.4)	
> 450	24 (8.5)	9 (5.8)	15 (12.0)	

The median WBC count was higher in the group with concomitant iron deficiency than in the isolated vitamin B12 deficiency group (8.09 vs. 5.01 G/L, $p < 0.001$). The proportions of neutrophils and monocytes were lower in the isolated vitamin B12 deficiency group, while the lymphocyte proportion was higher ($p < 0.001$) (Table 3).

Table 3: Distribution of patients according to white blood cell (WBC) count

Parameters	Median (Q1-Q3)			p-value
	Overall (n=281)	Isolated vitamin B12 deficiency (n=156)	Vitamin B12 deficiency with iron deficiency (n=125)	
WBC (G/L)	6.44 (3.88 - 8.91)	5.01 (3.07 - 7.65)	8.09 (5.87 - 9.48)	< 0.001
Neutrophil (%)	65.0 (53.5 - 72.0)	59.5 (48.2 - 70.0)	67.0 (61.0 - 74.0)	< 0.001
Lymphocyte (%)	25.0 (19.0 - 37.0)	31.0 (22.0 - 44.0)	21.0 (18.0 - 25.5)	< 0.001
Monocyte (%)	7.0 (5.0 - 8.0)	5.0 (4.0 - 7.0)	8.0 (7.0 - 9.0)	< 0.001
Neutrophil (G/L)	3.97 (2.09 - 6.18)	2.88 (1.62 - 5.09)	5.34 (3.67 - 6.71)	< 0.001
Lymphocyte (G/L)	1.57 (1.20 - 1.96)	1.49 (1.13 - 1.87)	1.67 (1.29 - 2.00)	0.013
Monocyte (G/L)	0.46 (0.24 - 0.65)	0.27 (0.13 - 0.49)	0.59 (0.46 - 0.79)	< 0.001

The proportion of hypersegmented neutrophils was relatively high in both groups (66.0% and 70.4%, respectively), with no statistically significant difference between them (Table 4).

Table 4: Distribution of patients according to neutrophil morphological features

Morphology	Overall n (%)	Isolated vitamin B12 deficiency n (%)	Vitamin B12 deficiency with iron deficiency n (%)	p-value
Normal	90 (32.1)	53 (34.0)	37 (29.6)	> 0.05
Hypersegmented neutrophils	191 (67.9)	103 (66.0)	88 (70.4)	
Total	281 (100)	156 (100)	125 (100)	

IV. DISCUSSION

The study included 281 patients diagnosed with vitamin B12 deficiency anemia at NIHBT. The mean age was 51.4 years, with most elderly patients. This may be related to reduced vitamin B12 absorption, inadequate nutritional intake, or the presence of chronic gastrointestinal disorders in this age group.

A key finding of this study is the marked difference in hematological characteristics between patients with isolated vitamin B12 deficiency and those with concomitant iron deficiency. In the combined deficiency group, Hb, MCV, MCH, and MCHC were significantly lower than in the isolated vitamin B12 deficiency group ($p < 0.001$),

whereas RBC tended to be higher (Table 1). These findings reflect the combined effects of two distinct pathophysiological mechanisms on erythropoiesis. Vitamin B12 deficiency leads to impaired DNA synthesis, delayed nuclear maturation, and ineffective hematopoiesis, resulting in macrocytic anemia [2, 10]. In contrast, iron deficiency reduces hemoglobin synthesis, leading to microcytic, hypochromic red blood cells [14]. When these two conditions coexist, the resulting hematological profile represents an interaction between these mechanisms, in which the microcytic, hypochromic features of iron deficiency may attenuate or mask the characteristic macrocytosis of vitamin B12 deficiency. Remacha et al. (2013) similarly reported that macrocytosis may not be evident in cases of combined vitamin B12 and iron deficiency, as iron deficiency can mask the macrocytic features of vitamin B12 deficiency [4]. In clinical practice, MCV is commonly used to guide the differential diagnosis of anemia: elevated MCV suggests vitamin B12 or folate deficiency, whereas reduced MCV suggests iron deficiency or thalassemia. However, our findings indicate that in patients with concomitant iron deficiency, MCV may be markedly reduced or remain within the normal range, resulting in an atypical presentation of vitamin B12 deficiency. Therefore, reliance solely on MCV may lead to underdiagnosis of vitamin B12 deficiency in such cases. This is particularly important in patients with persistent anemia, suboptimal response to treatment, or clinical features that are disproportionate to red blood cell morphology. Although ferritin is an acute-phase reactant that may be elevated in inflammatory states, its use in conjunction with transferrin saturation and the observation of significant decreases in MCV, MCH, and MCHC indicate that the classification of iron deficiency in this study is scientifically grounded and reflects clinical reality.

In addition to differences in red cell indices, variation in platelet parameters was also noteworthy (Table 2). In our study, thrombocytopenia was more frequent in patients with isolated vitamin B12 deficiency than in those with concomitant iron deficiency. One possible explanation is that vitamin B12 deficiency and iron deficiency may influence platelet production in different ways: vitamin B12 deficiency may lead to ineffective hematopoiesis and thrombocytopenia, whereas iron deficiency is more often associated with normal platelet counts or reactive thrombocytosis [15]. This suggests that coexisting iron deficiency may attenuate the thrombocytopenic effect of vitamin B12 deficiency in some patients. However, previous studies have reported mixed findings regarding platelet indices in combined nutritional deficiency. Beyan et al. found higher platelet counts in patients with combined deficiency than in those with isolated iron deficiency [8]. Taken together, these findings suggest that concomitant iron deficiency may modify platelet-related parameters in vitamin B12 deficiency, but the magnitude and direction of this effect may vary across different study populations and study designs.

Regarding the white blood cell lineage, a high proportion of patients in both groups exhibited hypersegmented neutrophils, with no statistically significant difference between the groups (Table 4). This suggests that the presence of hypersegmented neutrophils are a common and potentially useful morphological clue in vitamin B12 deficiency, even in the presence of concomitant iron deficiency; however, because this feature was absent in a considerable proportion of patients, it should not be considered a standalone diagnostic marker. This is a classic morphological feature of vitamin B12 deficiency related to impaired DNA synthesis [12]. Thompson et al. (1989) reported that neutrophil hypersegmentation has higher sensitivity than MCV in detecting vitamin B12 deficiency; however, its specificity is limited, and it cannot be used as a standalone diagnostic criterion [16]. In our study, this feature was absent in 32.1% of patients. Moreover, hypersegmented neutrophils may also be observed in various hematological disorders, particularly myelodysplastic syndromes. Therefore, accurate diagnosis requires integration of peripheral blood morphology, red cell indices, and serum vitamin B12 measurement.

The diagnosis of vitamin B12 deficiency in this study was based on serum vitamin B12 concentration. Although this approach is commonly used in clinical practice, serum vitamin B12 alone may have limited diagnostic accuracy in some cases, particularly in borderline results. Because confirmatory biomarkers such as methylmalonic acid or homocysteine were not

routinely available, some degree of misclassification could not be excluded.

Limitations: This study has several limitations. First, its retrospective cross-sectional design means that the analysis depended on the completeness and quality of medical records and only allowed the assessment of associations, not causal relationships. Second, the study was conducted at a single center, which may limit the generalizability of the findings. In addition, because ferritin is an acute-phase reactant, concomitant acute infection or inflammation may have influenced ferritin levels in some patients, and these conditions could not be fully excluded in this retrospective study. Nevertheless, with a relatively large sample size, this study provides useful data on peripheral blood cell characteristics in vitamin B12 deficiency anemia, particularly in the presence of concomitant iron deficiency.

V. CONCLUSION.

Based on the analysis of 281 patients with vitamin B12 deficiency anemia at the National Institute of Hematology and Blood Transfusion, anemia was predominantly mild to moderate in severity (93.6%). Concomitant iron deficiency significantly decreased MCV, MCH, and MCHC, while increasing red blood cell count compared to isolated vitamin B12 deficiency. Thrombocytopenia was more commonly observed in the isolated vitamin B12 deficiency group. Hypersegmented neutrophils were common in both groups and may provide supportive morphological evidence of vitamin B12 deficiency. However, because this feature was not observed in all patients, it should not be considered a universal or standalone diagnostic marker.

Conflict of Interest

The authors declare no conflicts of interest.

REFERENCES

1. Allen LH. How common is vitamin B-12 deficiency? Am J Clin Nutr. 2009; 89(2): 693S-6S.
2. Andres E, Loukili NH, Noel E, Kaltenbach G, Abdelgheni MB, Perrin AE, et al. Vitamin B12 (cobalamin) deficiency in elderly patients. CMAJ. 2004; 171(3): 251-9.
3. Iolascon A, Andolfo I, Russo R, Sanchez M, Busti F, Swinkels D, et al. Recommendations for diagnosis, treatment, and prevention of iron deficiency and iron deficiency anemia. Hemasphere. 2024; 8(7): e108.
4. Remacha AF, Sarda MP, Canals C, Queralto JM, Zapico E, Remacha J, et al. Combined cobalamin and iron deficiency anemia: a diagnostic approach using a model based on age and homocysteine assessment. Ann Hematol. 2013; 92(4): 527-31.
5. Osmola M, Chapelle N, Vibet MA, Bigot-Corbel E, Masson D, Hemont C, et al. Iron and Vitamin B12 Deficiency in Patients with Autoimmune Gastritis and Helicobacter pylori Gastritis: Results from a Prospective Multicenter Study. Dig Dis. 2024; 42(2): 145-153.
6. Balci YI, Akpinar FO, Polat A, Uzun U, Ergin A. Evaluation of Reticulocyte Parameters in Iron Deficiency, Vitamin B12 Deficiency and Mixed Anemia. Clin Lab. 2016; 62(3): 343-7.
7. Clancy CC, Browne LD, Gilligan R, Blake O, Stack AG. Prevalence of anaemia, iron, and vitamin deficiencies in the health system in the Republic of Ireland: a retrospective cohort study. BJGP Open. 2024; 8(2).
8. Beyan C, Kaptan K, Beyan E, Turan M. The platelet count/mean corpuscular hemoglobin ratio distinguishes combined iron and vitamin B12 deficiency from uncomplicated iron deficiency. Int J Hematol. 2005; 81(4): 301-3.
9. Cappellini MD, Motta I. Anemia in Clinical Practice-Definition and Classification: Does Hemoglobin Change With Aging? Semin Hematol. 2015; 52(4): 261-9.
10. Devalia V, Hamilton MS, Molloy AM, British Committee for Standards in H. Guidelines for the diagnosis and treatment of cobalamin and folate disorders. Br J Haematol. 2014; 166(4): 496-513.
11. Obeid R, Andres E, Ceska R, Hooshmand B, Gueant-Rodriguez RM, Prada GI, et al. Diagnosis, Treatment and Long-Term Management of Vitamin B12 Deficiency in Adults: A Delphi Expert Consensus. J Clin Med. 2024; 13(8).
12. Ankar A, Kumar A, Vitamin B12 Deficiency, in StatPearls. 2026: Treasure Island (FL).
13. Bach Quoc Khanh, Nguyen Truong Son, Huong dan chan doan va dieu tri mot so benh ly Huyet hoc. Vol. 528. 2022.
14. Andrews NC. Iron metabolism: iron deficiency and iron overload. Annu Rev Genomics Hum Genet. 2000; 1: 75-98.
15. Evstatiev R, Bukaty A, Jimenez K, Kulnigg-Dabsch S, Surman L, Schmid W, et al. Iron deficiency alters megakaryopoiesis and platelet phenotype independent of thrombopoietin. Am J Hematol. 2014; 89(5): 524-9.
16. Thompson WG, Cassino C, Babitz L, Meola T, Berman R, Lipkin M, Jr., et al. Hypersegmented neutrophils and vitamin B12 deficiency. Hypersegmentation in B12 deficiency. Acta Haematol. 1989; 81(4): 186-91.