THE EFFECT OF CONCOMITANT VITAMIN B12 AND FERRITIN DEFICIENCY ON CERTAIN RED BLOOD CELL PARAMETERS IN END-STAGE RENAL DISEASE PATIENTS UNDERGOING REGULAR HEMODIALYSIS

Nguyen Nhu Nghia[1], Pham Thi Lan Phuong[1,2], Tran Thai Thanh Tam[1], Tran Cong Dang[1], Nguyen Thi Diem Thuy[1], Nguyen The Bao[1]

ABSTRACT

Introduction: Identifying and treating comorbid causes of anemia in patients with end-stage renal disease (ESRD) undergoing regular dialysis plays a crucial role. However, the overlap of vitamin B12 deficiency and ferritin deficiency in this population makes the evaluation of red blood cell parameters complex. ***Objects:*** Evaluate the characteristics of parameters related to red blood cell size, blood ferritin levels, and vitamin B12 levels, as well as the correlation between these parameters. ***Materials and methods:*** A cross-sectional descriptive study with analysis was conducted on 135 patients diagnosed with end-stage renal disease undergoing regular dialysis at Bac Lieu General Hospital from May 2023 to May 2024. ***Results:*** The average age of the study subjects was 49.6 ± 14.8 years, with a male/female ratio of approximately 1/1. The anemia rate was 97.0%, with the majority being moderate anemia (60.0%). Microcytic red blood cells predominated (89.6%), and the hypochromic rate was 20.7%. The median blood ferritin level (Q1-Q3) was 499.3 (310.0-774.9) ng/mL, with a ferritin deficiency rate of 23.2%. The median blood vitamin B12 level (Q1-Q3) was 566.0 (290.0-818.0) pg/mL, with a vitamin B12 deficiency rate of 30.4%. There was a positive correlation between ferritin and MCV in the ferritin-deficient group (r=0.359, p=0.044). In the normal vitamin B12 group, red blood cells in the ferritin-deficient group were smaller (MCV: 87.27 ± 5.28 vs. 90.52 ± 6.1; p=0.017) and more hypochromic (median MCH: 27.9 vs. 29.45; p=0.003) compared to the normal ferritin group. Conversely, there was no difference in MCV and MCH in the vitamin B12 deficient group regardless of ferritin levels (p > 0.05). ***Conclusion:*** In ESRD patients undergoing regular dialysis, those with isolated ferritin deficiency had smaller, more hypochromic red blood cells compared to those without ferritin deficiency. However, if there is concurrent vitamin B12 deficiency, red blood cells may become normochromic and normocytic regardless of ferritin status.

Keywords: *Ferritin deficiency, vitamin B12 deficiency, MCV, MCH, MCHC, end-stage renal disease (ESRD), regular hemodialysis.*

I. INTRODUCTION

Anemia is a common consequence of chronic kidney disease (CKD), not only increasing treatment costs and severely affecting the quality of life but also elevating the risk of mortality. Notably, the severity of anemia tends to escalate with each stage of the disease and is particularly critical in patients with end-stage renal disease (ESRD) [7]. Managing anemia is not merely about supplementing erythropoietin according to the pathogenesis, but also providing essential nutrients that support erythropoiesis, such as iron and vitamin B12, which are often causes of anemia that is unresponsive to treatment in this patient group. However, these substances are not routinely supplemented and should only be prescribed when there is evidence of

[1] *Can Tho University of Medicine and Pharmacy*
[2] *Bac Lieu General Hospital*
Responsible person: Nguyen Nhu Nghia
Email: nnnghia@ctump.edu.vn
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deficiency. Moreover, measuring ferritin and vitamin B12 levels should be conducted when clinically indicated, with clinicians often relying on the characteristics of the patient's anemia — specifically the morphology and chromatic properties of the red blood cells. Traditionally, iron deficiency-induced ferritin deficiency often causes microcytic hypochromic anemia, while vitamin B12 deficiency usually leads to anemia with abnormally large hyperchromic red blood cells [9]. Importantly, in ESRD patients, especially those undergoing dialysis, red blood cell characteristics vary widely due to the overlap of these conditions, not only because of poor dietary intake but also due to nutritional losses through regular dialysis [2]. Thus, in such clinical scenarios, is it still accurate to rely on red blood cell parameters as indicators of nutritional deficiencies in erythropoiesis? Currently, there are no studies focused on examining red blood cell parameters and deeply analyzing the impact of concurrent ferritin and vitamin B12 deficiencies on red blood cell parameters in ESRD patients undergoing regular dialysis. Therefore, we conducted this study.

II. MATERIALS AND METHOD

2.1. Study population

All patients diagnosed with end-stage renal disease undergoing regular hemodialysis at the Artificial Kidney Department, Bac Lieu General Hospital from May 2023 to May 2024.

Inclusion Criteria

Patients diagnosed with end-stage renal disease aged ≥ 18 years undergoing regular hemodialysis (3 times a week, 9-12 hours per week) for at least 3 months.

Patients who agreed to participate in the study.

Exclusion Criteria

Patients with acute conditions such as severe infections, acute coronary syndrome, acute heart failure, cerebral infarction, cerebral hemorrhage, gastrointestinal bleeding.

Patients who died during treatment or were transferred to another healthcare facility.

Patients on a strict vegetarian diet.

Patients with vitamin B12 deficiency due to causes such as ileal resection, Crohn's disease, pernicious anemia, or post-gastrectomy.

2.2. Study method

Study design

A cross-sectional descriptive study with analysis.

Sample size

Convenient sampling was employed during the study for patients meeting the inclusion criteria and not falling under the exclusion criteria. In practice, we recruited 135 patients to participate in the study.

Study contents

General characteristics of study subjects: Age (mean, SD) and gender (male/female).

Parameters related to red blood cells study subjects:

-Red blood cell [RBC] count (mean, SD) (M/µl).

-Hemoglobin [Hb] (mean, SD) (g/dL): normal (12-16 g/dL for women and 14-18g/dL for men), mild anemia (10g/dL-normal limits), moderate anemia (8-10g/dL), severe anemia (6.5-7.9g/dL) and very severe anemia (<6.5 g/dL).

-Mean corpuscular volume [MCV] (mean, SD) (fL): normocytic (80-100fL), microcytic (<80fL), macrocytic (>100fL).

-Mean corpuscular hemoglobin [MCH] (mean, SD) (pg): normochromic (≥28pg), hypochromic (<28pg).

-Mean corpuscular hemoglobin concentration [MCHC] (median, Q1-Q3) (g/dL).

-Red cell distribution with [RDW] (%).

Ferritin serum concentration (median, Q1-Q3) (ng/mL): deficiency (<300 ng/mL), non-deficiency (≥ 300mL).

Vitamin B12 serum concentration (median, Q1-Q3) (pg/mL): deficiency (≤ 300pg/mL), non-deficiency (>300pg/mL)

Statistical analysis

Data were analyzed using R Statistical Environment.

2.3. Ethics in research

The board of directors of Bac Lieu General Hospital and the ethics committee for biomedical research at Can Tho University of Medicine and Pharmacy approved this study (No. 23.262.HV/PCT-HĐĐĐ).

III. RESULTS

Our study was conducted on 135 patients diagnosed with end-stage renal disease undergoing regular hemodialysis, with a male-to-female ratio of approximately 1:1. The average age of the study subjects was 49.6 ± 14.8 years.

Table 1. ***Parameters related to red blood cells, ferritin, and vitamin B12 levels (n = 135)***

Parameters		Value
RBC count (Mean ± SD) (M/µl)		3.11 ± 0.59
Hemoglobin (g/dL)	Normal (n, %)	4 (3.0)
	Mild anemia (n, %)	35 (25.9)
	Moderate anemia (n, %)	81 (60.0)
	Severe anemia (n, %)	13 (9.6)
	Very severe anemia (n, %)	2 (1.5)
	Mean ± SD	8.96 ± 1.67
HCt (Mean ± SD) (%)		27.9 ± 5.05
MCV (fL)	Normocytic (n, %)	10 (7.4)
	Microcytic (n, %)	121 (89.6)
	Macrocytic (n, %)	4 (3.0)
	Mean ± SD	90.0 ± 2.41
MCH (pg)	Normochromic (n, %)	107 (79.3)
	Hypochromic (n, %)	28 (20.7)
	Mean ± SD	29.2 (27.5-30.8)
MCHC (Median, Q1-Q3) (g/dL)		32.2 (31.3-32.9)
RDW (Median, Q1-Q3) (%)		14.9 (14.0-15.9)
Ferritin (ng/mL)	Deficiency (n, %)	32 (23.2)
	Non-deficiency (n, %)	106 (76.8)
	Median, Q1-Q3	499.3 (310.0-774.9)
Vitamin B12 (pg/mL)	Deficiency (n, %)	42 (30.4)
	Non-deficiency (n, %)	96 (69.6)
	Median, Q1-Q3	566.0 (290.0-818.0)

RBC, red blood cell; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution with.

Evaluating the characteristics related to red blood cell parameters, ferritin, and vitamin B12, we noted that 3 out of 5 patients had moderate anemia, and 1 out of 10 had severe anemia. Notably, most patients had microcytic red blood cells (89.6%). Hypochromic red blood cells accounted for about 1/5. Additionally, 23.2% of patients were deficient in ferritin, and 30.4% were deficient in vitamin B12.

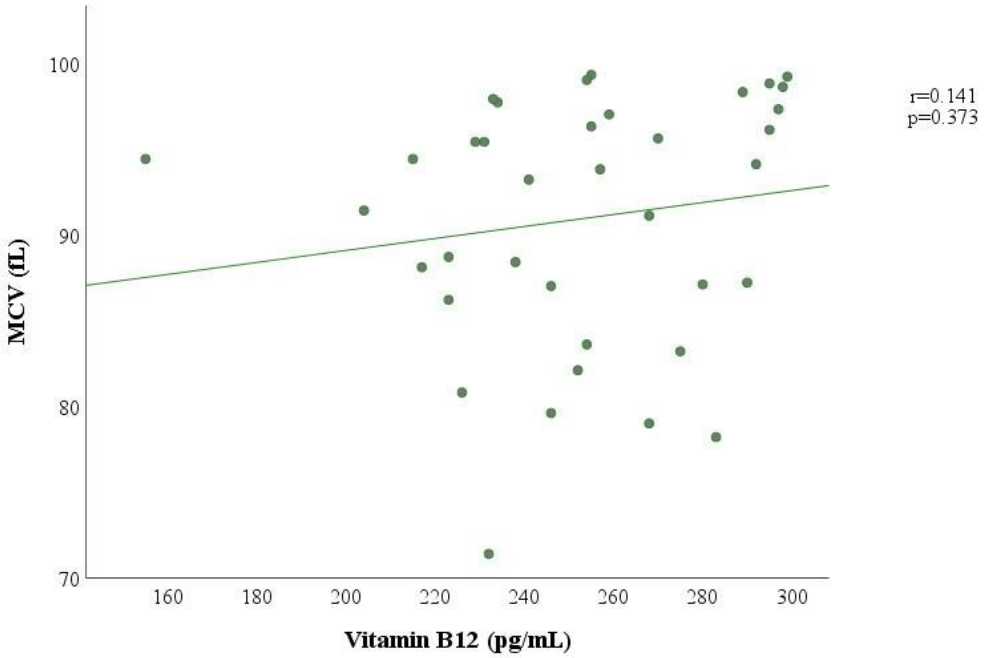

Figure 1. ***Correlation between vitamin B12 level and MCV in the vitamin B12-deficient group (n = 42)***

There was no statistically significant correlation between vitamin B12 levels and MCV (p =0.373).

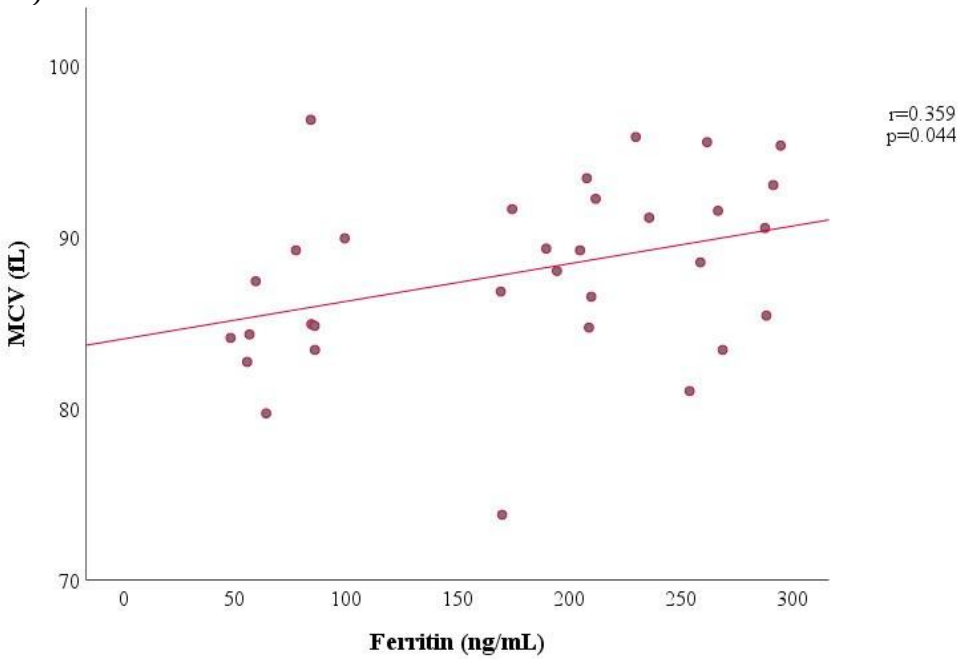

Figure 2. ***Correlation between ferritin level and MCV in the ferritin-deficient group (n= 32)***

We noted a positive correlation between serum ferritin levels and MCV, with a moderate correlation coefficient (r=0.359). The observed difference was statistically significant (p = 0.044).

Table 2. ***Values of parameters as per ferritin deficiency in end-stage renal disease patients undergoing regular hemodialysis (n = 135)***

Para-meters	Deficient vitamin B12 (n = 42)			Non-deficient vitamin B12 (n = 96)		
	Deficient ferritin (n = 5)	Non-deficient ferritin (n = 37)	*p*-value	Deficient ferritin (n = 27)	Non-deficient ferritin (n = 66)	*p*-value
MCV (fL)	91.48 ± 2.68	90.94 ± 7.28	0.756	87.27 ± 5.28	90.52 ± 6.10	**0.017**
MCH (pg)	29.10 (28.10-29.70)	29.90 (27.85-31.05)	0.294	27.90 (26.00-29.10)	29.45 (28.00-31.00)	**0.003**
MCHC (g/dL)	31.20 (30.25-33.05)	32.40 (31.45-32.95)	0.425	31.70 (31.00-32.40)	32.45 (31.78-33.10)	**0.014**
RDW (%)	14.40 (13.00-15.05)	14.30 (13.90-15.70)	0.627	15.10 (14.10-15.90)	14.95 (13.98-16.10)	0.956

MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution with. p-value < 0.05 was considered as statistically significant (bold).

In evaluating the red blood cell indices, we noted that in the group without vitamin B12 deficiency, the red blood cells in the ferritin-deficient group were smaller and more hypochromic compared to the non-ferritin-deficient group, with MCV and MCH values of 87.27 ± 5.28 vs. 90.52 ± 6.10 and 27.90 vs. 29.45, respectively. However, in the group with vitamin B12 deficiency, there were no differences in MCV, MCH, and MCHC values between those with and without ferritin deficiency (p > 0.05). Additionally, no statistically significant differences in RDW were observed.

IV. DISCUSSION

Our study shows that most patients with end-stage renal disease undergoing regular dialysis have anemia, with the majority being of moderate severity (60%) and only about 10% being severe to very severe. Notably, most cases involved microcytic anemia. We observed a positive correlation between serum ferritin levels and MCV values in patients with ferritin deficiency. It is noteworthy that when patients did not have vitamin B12 deficiency, those with reduced ferritin levels had smaller and more hypochromic red blood cells. However, when patients had concurrent vitamin B12 and ferritin deficiencies, there was no significant difference in MCV and MCH characteristics compared to those with normal ferritin levels.

Theoretically, ESRD typically results in normochromic normocytic anemia of severe degree. In our study, the prevalence of anemia, mostly of moderate severity, is likely due to prior anemia interventions, including EPO therapy and frequent blood transfusions. Indeed, our findings align with those of Yin S. (2022), who reported an anemia prevalence of up to 89.29% in ESRD patients undergoing regular dialysis [8]. Another study demonstrated an inverse correlation between dialysis frequency and hemoglobin levels (r=-0.594, p=0.001) [4]. Yin S.'s study also noted that 46.25% of ESRD patients on regular dialysis improved and maintained hemoglobin levels above 11 g/dL post-treatment [8]. This underscores the importance and prevalence of anemia assessment in managing ESRD patients undergoing regular dialysis. In ESRD

patients, anemia is compounded not only by reduced EPO secretion but also by mechanisms such as uremia-induced red blood cell deformities causing hemolysis, chronic inflammation, increased infection susceptibility inhibiting the marrow, and decreased availability of erythropoiesis materials like vitamin B12 and iron [8]. herefore, EPO therapy alone is less effective without evaluating comorbid causes of anemia, including vitamin B12 and iron deficiencies. Notably, a small amount of blood is typically retained in the dialysis apparatus, potentially causing iron deficiency over time in regular dialysis patients [4]. Additionally, literature indicates excessive iron loss in ESRD patients, ranging from 1 to 3 g per year, due to secondary causes like chronic bleeding from uremia-related platelet dysfunction, blood retention in dialysis machines, and frequent venipuncture for tests. Poor nutrition in ESRD patients undergoing regular dialysis is also a primary cause of iron and vitamin B12 deficiencies. Mushtaq's study found that approximately a quarter of ESRD patients on regular dialysis had vitamin B12 deficiency, attributing this to the prolonged dialysis duration associated with high-flux dialysis machines capable of removing medium-sized molecules like vitamin B12 [6]. Thus, our findings of predominantly microcytic anemia (89.6%), hypochromia in about one-fifth, ferritin deficiency in a quarter, and vitamin B12 deficiency in a third of the patients are consistent with the literature.

In ferritin-deficient patients, there was a positive correlation between serum ferritin levels and MCV values. This aligns with the literature as ferritin, a protein that stores and releases iron in a controlled manner, indicates body iron reserves essential for hemoglobin synthesis and normal red blood cell production. Low ferritin levels suggest depleted iron stores, reduced hemoglobin, and smaller red blood cells [3]. However, no significant correlation was found between vitamin B12 levels and MCV values in vitamin B12-deficient patients. This could be due to increased extracellular fluid volume in ESRD patients, primarily in the interstitial space (85%), with reduced intravascular volume compared to healthy individuals or CKD stage 1-3 patients [5]. This decreased plasma volume might artificially elevate blood vitamin B12 levels. Meanwhile, multiple factors may reduce MCV, including overlapping ferritin deficiency causing iron depletion, chronic inflammation from uremia, atherosclerosis, elevated pro-inflammatory cytokines, and immune system activation inhibiting EPO on top of already reduced EPO production. These mechanisms might alter the correlation between vitamin B12 and MCV [2].

When analyzing the impact of iron and vitamin B12 deficiencies on red blood cell indices, we found that in the absence of vitamin B12 deficiency, reduced ferritin levels resulted in smaller, more hypochromic red blood cells. Conversely, concurrent vitamin B12 and ferritin deficiencies did not significantly affect MCV and MCH compared to normal ferritin levels. This may be due to our initial hypothesis that ferritin deficiency causes microcytic hypochromic red blood cells, while vitamin B12 deficiency results in macrocytic hyperchromic red blood cells. The combination of these conditions explains our findings. Indeed, Asif Muhammad’s (2023) study shows that in microcytic anemia with concurrent vitamin B12 deficiency, MCV tends to increase from 64.7 fL in patients with normal vitamin B12 to 72.3 fL in severe vitamin B12 deficiency [1]. Therefore, although normochromic

normocytic anemia is a classic feature of anemia in CKD patients, in ESRD patients on regular dialysis, MCV and MCH alone cannot reliably assess comorbid causes of anemia, specifically overlapping vitamin B12 and iron deficiencies. Hence, clinicians should consider measuring vitamin B12 and ferritin levels, especially in patients with multiple risk factors, when anemia treatment proves ineffective.

In addition to the significant findings, it must be acknowledged that our study has certain limitations. These include a small sample size, a cross-sectional descriptive design, and a lack of in-depth analysis of other confounding factors, which are important drawbacks. Therefore, with these initial contributions, we hope that future studies will involve larger sample sizes, more advanced study designs, and a comprehensive evaluation of multiple factors in analyzing anemia characteristics in end-stage renal disease patients undergoing regular dialysis.

V. CONCLUSION

In end-stage renal disease patients undergoing regular dialysis, those with isolated ferritin deficiency have smaller, more hypochromic red blood cells compared to those without ferritin deficiency. However, if there is concurrent vitamin B12 deficiency, the red blood cells can become normochromic and normocytic regardless of the body's ferritin status.

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