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Research Article

Rediscovering platelet number and the red cell distribution width/mean corpuscular hemoglobin ratio as markers in hemodialysis patients with iron deficiency anemia

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Abstract:

Objectives: Our aim is to evaluate the parameters of complete blood count according to transferrin saturation levels in hemodialysis patients.

Background: Iron deficiency (ID) frequently develops in patients undergoing hemodialysis (HD). It is difficult to assess the need of iron in people with chronic illness such as renal failure. Besides, there is a group of patients with high ferritin and low transferring saturation levels.

Methods: We retrospectively studied 202 subjects (F/M: 101/101) selected among 350 patients and hemoglobin concentration between 8-12 g/dL. Patients were divided into two groups according to their transferrin saturation level, <20% and ≥20% and their results were compared.

Results: The mean age of the patients and median duration of hemodialysis were not different between the groups. Levels of mean corpuscular volume (p=0.007), mean corpuscular hemoglobin (MCH; p=0.023) and lymphocytes (p=0.011) were significantly lower, and red cell distribution width (RDW; p=0.033), the RDW/MCH ratio (p=0.000), neutrophils (p=0.001), the neutrophil/lymphocyte ratio (p=0.006), leucocytes (p=0.002) and platelets (p=0.000) were higher in the transferrin saturation <20% group.

Conclusions: In the case of hemodialysis patients with high to normal serum ferritin concentrations and low transferrin saturation platelet number and the RDW/MCH ratio could assist in the diagnosis of iron deficiency anemia.

Keywords: anemia, complete blood count, hemodialysis, iron deficiency, transferrin saturation,

Introduction

Anemia is a common complication in patients with chronic kidney disease and is primarily due to insufficient production of the glycoprotein hormone erythropoietin (EPO). Iron deficiency (ID) frequently develops in patients undergoing hemodialysis (HD) as a result ofEPO treatment due to the rapid utilization of iron to support erythropoiesis or blood loss. A diagnosis of ID is made using the combination of red blood cell (RBC) indices and serum measurements of transferrin saturation (TSAT) and ferritin levels (1). The serum ferritin concentration and TSAT percentage are the two most widely used tests of iron status for dialysis patients (2). In absolute ID, serum ferritin saturation is reduced, while in functional ID there is an imbalance between the iron needs of the erythroid marrow and the iron supply. Ultimately, iron is not maintained at a rate sufficient to allow normal hemoglobinization of erythrocytes (3). In anemia associated with chronic disease, functional ID may occur even in the presence of large iron stores when iron release is impaired (4). However, ferritin is also an acute phase reactant and its levels can be affected by inflammation. In addition, serum ferritin concentrations are increased in hyperthyroidism, hepatocellular diseases, malignancies and with alcohol consumption and oral contraceptives (5). Kidney disease improving global outcome (KDIGO) guidelines do not recommend routine use of iron supplementation in patients with TSAT >30% or serum ferritin >500 ng/mL (6). Importantly, there is a group of patients who have low TSAT and high ferritin levels. The response to iron therapy to correct anemia in patients on chronic dialysis with serum ferritin values in the 500–1000 ng/mL range has been tested. In the Dialysis Patients' Response to IV Iron with Elevated Ferritin study, intravenous iron treatment was used in patients with serum ferritin levels between 500–1200 ng/mL and TSAT levels <25%, and a significant increase in hemoglobin levels was observed (7). These results demonstrate the challenges in properly assessing body iron stores and identifying and treating ID, and the clinical situation can become even more complicated in settings such as anemia associated with chronic disease and patients undergoing EPO therapy.

Parameters of complete blood count (CBC), such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and red cell distribution width (RDW), are useful in the morphological characterization of anemia, but have not been used for many years in HD patients. Previous studies have shown that MCV and MCH decrease and RDW increases in patients with ID anemia (1). The initial studies that demonstrated these results were performed in small groups,

and in one of these studies, MCV and MCH were both found to be significantly depressed in patients with depleted iron stores (8). In another study neither MCV nor MCH values were effective in detecting iron, B12 or folate deficiencies (9). Increased RDW was the most sensitive feature in those patients but was not indicative of any specific type of deficiency. Recently, different indexes have been used to diagnose ID, such as mean reticulocyte hemoglobin content, percentage of hypochromic RBC and soluble transferrin receptor levels (10-12). However, these indexes have not been widely or routinely used in HD patients. In contrast to the newly developed indexes, many useful parameters can be simply obtained from CBC.

Our aim was to evaluate the CBC parameters MCV, MCH, RDW, neutrophil, lymphocyte, leucocyte and platelet counts according to TSAT level in patients with HD.

Materials and Methods

This is a retrospective and cross-sectional study. We retrospectively studied 202 subjects (F/M: 101/101) selected among 350 patients who were undergoing HD for at least 6 months. Patients with a hemoglobin concentration greater than 12 g/dL or below 8 g/dL, with recent bleeding, malignancy, hepatocellular disease, hemoglobinopathy or in need of blood transfusions were excluded. All of the patients received EPO during the previous month (50–150 U/kg/week according to hemoglobin levels). At the time of the study, each patient was undergoing three bicarbonate HD per week in sessions lasting 4–5 hours using 1.8 m² hemophane membranes. During HD sessions the blood flow rate ranged from 250–350 mL/min (median, 300 mL/min) and mean Kt/V was kept at 1.4±0.2. Blood samples were drawn before mid-week HD session on the same month. Only patients in the morning session were fast at the timing of blood sampling. Laboratory test results were recorded from the patient's cards. CBCs were determined by the spectrophotometer method using a Cell DYN 3700 analyzer (Abbott, Indianapolis, IL, USA), and intact parathyroid hormone (PTH) levels were measured by electrochemiluminescence immunoassays (Roche Diagnostics Corporation, Indianapolis, IN, USA). Serum levels of blood urea nitrogen, creatinine, calcium, phosphorus, albumin, were assessed using standard laboratory methods (Roche Hitachi Analyzer 902, Hitachi High-Technologies Corporation, Tokyo, Japan). Patients were divided into two groups according to TSAT levels being <20% or ≥20%, and results were compared. The institution's ethics committee approved the study.

Statistical analysis

Statistical analyses were performed using the SPSS for Windows version 17.0 software program (SPSS Inc., Chicago, IL, USA). If the continuous variables were normal, they were given as mean ± standard deviation but if they were not normal, they were given as the median. In normally distributed data, comparisons between groups were carried out using Student's *t*-test and for data that were not normally distributed the Mann–Whitney U-test was used. Additionally, the categorical variables between the groups were analyzed with either a chi-square test or Fisher's exact test. A P value of < 0.05 was considered to be statistically significant.

Results

Clinical records of 350 patients were evaluated, and 202 patients were suitable for the study. The etiology of renal failures were as follows: diabetes mellitus (33.2%), hypertension (10.9%), glomerulonephritis (5.9%), kidney stone disease (7.4%), unknown (10.9%), other (31.2%) and amyloid (0.5%). Patients were grouped according to their TSAT levels being <20% or ≥20%. We chose the cutoff level of TSAT as 20% according to the 2006 KDIGO anemia guidelines, which recommended iron therapy to maintain serum TSAT levels at >20% [13]. The clinical characteristics of the enrolled patients are shown in Table I.

Table I: Clinical and laboratory results of the patients according to TSAT levels

	TSAT <20%	TSAT ≥20%	P
N	42	160	
Gender (F/M)	21/21	80/80	
Age (year)	56.42 ± 11.95	54.59 ± 13.58	0.426
Hemodialysis time (month)	47 (8-208)	56 (6-242)	0.857
Glucose (mg/dL)	158 ± 75.46	121.54 ± 58.71	0.001
BUN (mg/dL)	68.5 (28-96)	68.5 (20-114)	0.154
Creatinine (mg/dL)	8.47 ± 2.59	9.37 ± 2.44	0.037
PTH (pg/mL)	318 (32-1378)	361(9-1771)	0.257
Hb (g/dL)	10.22 ± 1.03	10.61 ± 0.95	0.020
Leucocyte (K/mm^3)	8.05 ± 2.20	6.96 ± 1.97	0.002
Platelet (K/mm^3)	247 (126-559)	188 (71-430)	0.000
Ferritin (ng/mL)	462 (102-1732)	742(81-2000)	0.007
TSAT (%)	16 (8-19)	29 (20-85)	0.000
MCV (fL)	88.39 ± 7.63	91.43 ± 6.16	0,007
RDW (%)	17.09 ± 2.01	16.39 ± 1.85	0.033
MCH (pg)	29.01 ± 3.13	30.08 ± 2.55	0.023
RDW/MCH	0.60(0.34-0.83)	0.53(0.32-0.91)	0.000
Neutrophil (%)	68.69 ± 7.85	63.55 ± 9.34	0.001
Lymphocyte (%)	20.5 (7.9-38)	22 (9.60-63)	0.011
Neutrophile/Lymphocyte	3.36(1.39-10.46)	2.88(0.49-8.18)	0.006
CRP (mg/mL)	13 (1-152)	5(1-78)	0.002

Abbreviations: BUN, blood urea nitrogen; CRP, c reactive

protein; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin PTH, parathyroid hormone; RDW, red cell distribution width; TSAT, transferrin saturation.

The mean age of the patients and median duration of HD were not different between the groups. In the TSAT <20% group, levels of MCV (p=0.007), MCH (p=0.023) and lymphocytes (p=0.011) were significantly lower, and RDW (p=0.033), the RDW/MCH ratio (p<0.001; Figure I),

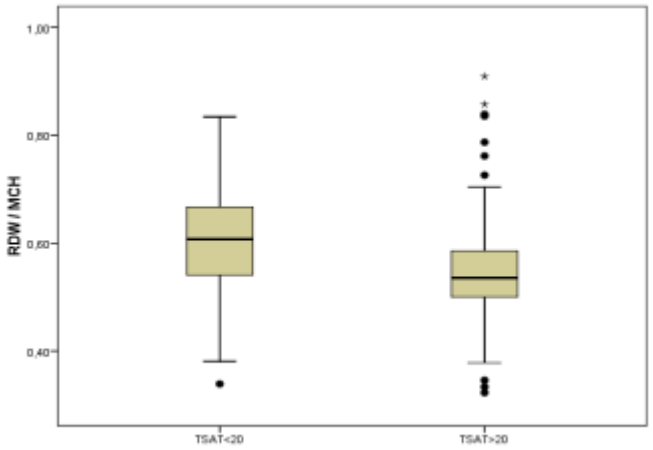

Figure I: RDW/MCH ratio according to TSAT levels.

the neutrophil/lymphocyte (N/L) ratio (p=0.006), and levels of neutrophils (p=0.001), leucocytes (p=0.002) and platelets (p<0.001; Figure II)

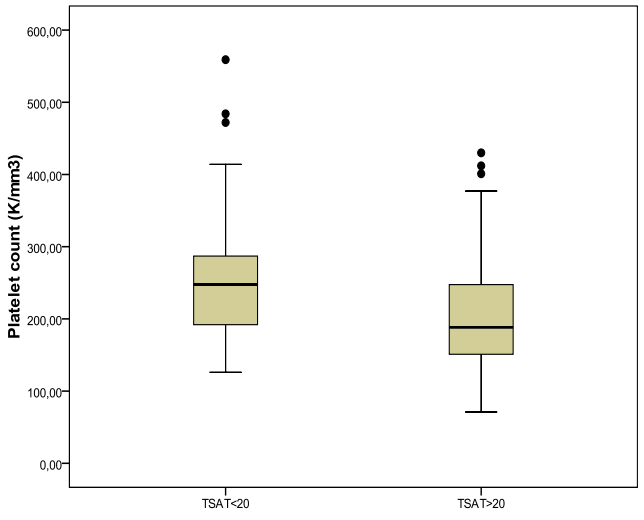

Figure II: Platelet count according to TSAT levels.

were higher, than in the TSAT ≥20% group. The complete list of clinicopathological results are shown in Table I. As these results show, the significance of the RDW/MCH ratio was more prominent than the significance of RDW or MCH alone. The significance of platelet counts and the RDW/MCH ratio were the most prominent characteristics in the patients with low TSAT scores.

Discussion

ID is one of the main causes of anemia in HD patients. There are two ID syndromes; absolute and functional. Absolute ID is defined as serum ferritin <100 ng/mL. HD patients suffer from absolute ID because of increased blood loss from blood left in the dialyzer circuit, frequent blood sampling and/or low grade gastrointestinal bleeding. Functional ID is particular to patients who are being treated with erythropoiesis stimulating agents because their supraphysiologic rate of RBC production outstrips the ability of transferrin bound circulating iron. In functional ID, serum ferritin levels are generally normal or greater than 100 ng/mL [14]. The basic problem in these patients is supply and demand of iron.

Serum ferritin and TSAT are the favored markers for assessing iron status, because of their widespread availability and extensive literature base. It is not uncommon for patients to have high ferritin and low TSAT levels. The frequent paradox of those patients is whether to use intravenous iron therapy. In this study, we evaluated the efficacy of using the simply obtained CBC parameters—MCV, RDW, MCH, leucocytes, neutrophils, lymphocytes and platelets—in HD patients with ID anemia because studies evaluating those parameters in HD patients are lacking.

RDW provides a quantitative measure of heterogeneity of red cells in the peripheral blood. Increased platelet number is evident on the peripheral smear of the iron deficient patients (1). There have been different results in studies evaluating RDW for ID anemia. A study that subjected ID among otherwise healthy 0.5–3-year-old children found that RDW facilitated the identification of ID as the cause of anemia, and that RDW values were not influenced by the presence of infection. Platelet count was found to have no role in diagnosing ID among this group of children (15).

In another study, eight discrimination indexes, including RDW, were used to differentiate ID anemia and beta thalassemia trait. None of the indices were found to be reliable in discriminating between those patient populations (16). Another study showed that RDW was the parameter with the highest sensitivity for ID (94%) and concluded an RDW value within the reference interval can be used to exclude ID, especially in patients in which the serum ferritin concentration does not accurately reflect the iron stores owing to severe tissue damage, as in inflammation or malignancy (17). The role of RDW and RBC indexes was also evaluated in determining ID in a group of patients including 190 pregnant women. This study concluded that ID anemia could be screened-out early by increased RDW when RBC indexes were within the normal range (18). Importantly, all of these studies were performed in patients with normal renal function. To the best of our knowledge there has been only one study evaluating HD patients. Morgan et al. evaluated 40 HD patients to determine the usefulness of RDW in screening these patients for ID and found the specificity of RDW elevation for ID to be 45%, and the positive predictive value to be 32% (19).

Decreased MCV and MCH are two indexes of ID. Nuwayri-Salti et al. showed significantly depressed MCH and MCV levels in HD patients with depleted iron stores. They evaluated

only 32 HD patients but their results are very valuable, because they detected bone marrow iron stores on Prussian-blue stained smears (8). We found that levels of MCV and MCH were significantly depressed in the TSAT <20% group. Additionally, we evaluated the significance of the RDW/MCH ratio. The significance of this ratio was more prominent than the significance of RDW or MCH alone.

Increased platelet number is evident on the peripheral smear of iron deficient patients, and thrombocytosis usually occurs in a mild to moderate degree (1). Park et al. studied 41 women with ID anemia and thrombocytosis and found the platelet count showed an inverse correlation with serum iron and TSAT, while no correlation between serum ferritin was found (20). A study of premenopausal patients showed the platelet count to be significantly higher and the percentage of lymphocytes to be lower in the ID anemia group as compared with the control group. But they did not find any statistically significant difference between the two groups for mean relative counts of white blood cells, or percentage of neutrophils (21). The suspected mechanisms underlying these findings are prolonged platelet survival or extramedullary hematopoiesis, faster maturation time and/or increased polyploidy of megakaryocytes resulting in increased platelet production in ID (22). Experimental ID anemia in rats showed the inhibitory effects of iron on platelet production (23). however these changes may be associated with low tissue levels of iron.

In a study by Mujib et al., 150 children with ID anemia were evaluated and levels of N and L were found to be higher than the control group (24). Higher N and lower L numbers were the results of our study; however, these discrepancies could be due to our patients being adults with renal failure, and uremic milieu might be the cause of the lower lymphocyte values we observed.

Higher N/L ratios have been shown to be associated with progression of chronic kidney disease and to predict the increased risk of cardiovascular disease events in HD patients (25,26). Kocyigit et al. demonstrated that patients whose N/L ratio =3 had higher progression rates compared with patients who had an N/L ratio <3 (25). Abe et al. studied 86 incident Japanese dialysis patients in a prospective cohort study. The median N/L ratio was 3.72, and patients who had higher N/L ratios than the median showed a shorter duration from the start of dialysis therapy to the first cardiovascular disease event (26). In our study the median N/L ratio was 3.36 in the low TSAT level group. We concluded that N/L ratio could be a predictor of ID, and an N/L ratio >3 may be the best clinical cutoff value.

Conclusion

A group of simple tests could be used to evaluate ID in addition to serum ferritin concentration and TSAT. Those markers should be easily measured and always available. In our study platelet number and the RDW/MCH ratio were the most valuable tests from a simple CBC. For patients with high or normal serum ferritin and low TSAT these markers are the most valuable. Monitoring these simple parameters related to hemoglobinization and iron availability could help us in the assessment of the iron status of the patients and thus their therapy requirements.

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