THE DIAGNOSTIC VALUE OF SERUM FERRITIN IN ASSESSING IRON STATUS IN SYRIAN HEMODIALYSIS PATIENTS

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In this research, we aimed to study the diagnostic value of serum ferritin in assessing iron status in hemodialysis patients (HD). While ferritin is one of the most important laboratory parameters used to assess iron status, but the presence of confounding factors such as chronic inflammation in HD patients may confuse the interpretation of laboratory results. The study was conducted on 73 adult patients who were on maintenance HD at Al-Basel Hospital in Homs, Syria, with the presence of 25 age – matched healthy subjects. Blood samples were collected before the HD session, serum CRP was used as an inflammatory marker. The results showed a significant increase in serum ferritin values in the patients group compared to healthy subjects, with an average value of (539.64 ng/ml), and this increase coincided with a decrease in Hb and TSAT values, but with an increase in CRP. Also, we found a significant positive correlation between ferritin and CRP (r2= 0.132, P= 0.002) in the patients group. Thus, the presence of chronic inflammation in HD patients may raise serum ferritin unrelated to iron stores, and reduce its diagnostic value.

Keywords: Anemia, Inflammation, Hemodialysis, C-Reactive Protein, Ferritin

INTRODUCTION

Anemia is one of the most common complications of renal failure, and associated with an increased risk of cardiovascular disease and mortality in patients. The main cause of renal anemia is due to decreased production of erythropoietin hormone (EPO) by the kidneys as a result of loss of renal function[1]. The availability of recombinant human erythropoietin (rHuEPO) since the late 1980s, constituted a new insight and understanding for the treatment of anemia in patients with renal failure, and proved effectiveness in reaching the targeted hemoglobin and reducing the risks of frequent blood transfusions such as iron overload, the transmission of viral hepatitis and other risks[2]. However, anemia can be due to other reasons, such as (iron deficiency, inflammation, resistance to erythropoietin caused by uremic toxins, and the short lifespan of red blood cells in the circulation[3]. In addition to the essential role of iron administration, management of renal anemia relies mainly on the administration of recombinant human erythropoietin. It should be noted that CKD patients on hemodialysis require different target values of iron parameters, different than those of normal individuals, to ensure that the targeted hemoglobin values are achieved. Serum ferritin, Transferrin Saturation Ratio (TSAT), and Total Iron Binding Capacity (TIBC), are the most common parameters worldwide used in the laboratory assessment of iron status[4].

Serum ferritin is a frequently used marker of iron status in dialysis patients. However, The best evaluation of iron status can be obtained by combining serum ferritin and TSAT values[5].

Low TSAT and low ferritin are indicative of iron deficiency, but high serum ferritin in hemodialysis patients may be confusing in the presence of inflammation[6]. The correct evaluation of the iron status in hemodialysis patients is very important because it has a significant impact on making the appropriate decision for treatment, whether by giving iron

to correct iron deficiency anemia, or not giving it to avoid iron overload[7]. Anemia of inflammation AI (or what is known as anemia of chronic disease ACD), has been recognized as a mild to moderately severe anemia (Hemoglobin levels between 7 - 12 g/dL), that develops in the presence of chronic systemic inflammation. Thus, chronic inflammation was considered as one of the most important causes of developing anemia in patients with chronic kidney disease[8].

MATERIALS AND METHODS

This prospective cross-sectional study was conducted at the HD unit of AL-Bassel hospital in Homs, Syria, from May 2020 to September 2020, and informed written consent was taken from each participant in the study.

Patients

Seventy-three (73) adult patients (43 Males and 30 females), diagnosed with End Stage Renal Disease (ESRD), who were on maintenance HD, were enrolled in this study, with the presence of 25 age-matched healthy subjects as a control group.

Inclusion criteria for HD patients were males and females aged > 18 years and inception of maintenance HD ≥ 6 months. The maintenance HD schedule was two sessions per week, each of 4 h duration.

47% of Patients received EPO Alpha (50-100 IU/Kg) once a week, and 70% of Patients received oral iron supplements. All patients received folate 5mg/day.

Exclusion criteria were previous treatment with immunosuppressive
drugs, active inflammatory disease, clinical signs of acute infection, presence of liver disease, presence of malignancy, evidence of blood loss or gastrointestinal bleeding.

Exclusion criteria also included patients who were undergoing blood transfusion and patients receiving parenteral iron.

Methods

Venous blood samples were collected from maintenance hemodialysis patients after an overnight fasting and immediately before the session of hemodialysis. For healthy control subjects, blood was also drawn from a peripheral vein after an overnight fasting.

Two types of tubes were used to collect blood, EDTA anticoagulant tubes (for Complete Blood Count CBC) and plain tubes. To obtain serum, whole blood samples were collected into plain tubes, then the tubes were left in an upright position in the water bath at 37 °C for 30 minutes. After clot formation, the tubes were centrifuged at 3500 rpm for 15 minutes. The serum was separated, and biochemical tests were conducted directly.

CBC was measured by Mindray BC 5000 Auto Hematology analyzer/China, which included: Hemoglobin (Hb), Hematocrit (Hct), Red blood cell count and RBC indices (MCV, MCH, MCHC), White blood cells count (WBC), and the differential counts (Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophiles).

Serum ferritin was assayed by immunofluorescence using I-chroma Boditech/Korea. Serum iron and TIBC were performed by Mindray BS-240 clinical chemistry analyzer/China.
TSAT was calculated as (serum iron×100/TIBC).

Serum CRP was measured by a turbidometric immunoassay in which a serum sample is mixed with latex beads coated with anti-human CRP antibodies forming an insoluble aggregate (normal value < 5 mg/L).

Statistical Analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS) version 24.0 for Windows. Data are expressed as mean ± standard deviation (SD). We used Student's t-test to compare means. For binary correlation, Pearson correlation test was used. Results were expressed in the form of correlation coefficient (r) and P- value, and $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

(Table 1) shows the demographic characteristics of the study subjects. The mean age of the patients group was (50.85 ± 7.57) years, and the mean age for the control group was (50.20 ± 7.23) years, in order to compare the two groups. We found that (60.3%) of the

patients had diabetes mellitus, and (36.7%) had hypertension, while (3%) had polycystic kidney disease. The mean duration of dialysis in patients group was (6.41 ± 2.81) years.
Table 2 shows the comparison of the studied parameter values between patients group and control group.

We found significant differences in favor of the healthy group for the values of Hb, Hct, serum iron, TIBC and transferrin saturation (TSAT).
While the differences were significant for ferritin and CRP in favor of the patients group. The average C-reactive protein in the patients group was (9.43 ± 4.30 mg/dl), and the mean value of serum ferritin was (539.64 ± 152.64 ng/mL).

There was no significant difference in WBC and MCV between the two groups.
the mean values of hemoglobin in patients group were (8.13 ± 0.85 g/dL) and ranged between (6.3 - 10.3 g/dl), meaning that all patients of the study were suffering from anemia (according to the World Health Organization WHO criteria, anemia is

diagnosed When the hemoglobin concentration is less than 13 g/dl in males and less than 12 g/dl in females)[9].

By observing the CRP, neutrophil%, lymphocyte%, and ferritin values, we found that there is a state of a chronic inflammation in patients group.
Chronic elevation of CRP levels is common in (30-60%) of renal failure and HD patients, and it is an important indicator of inflammatory status, where CRP is an acute phase protein that is produced in the liver by stimulation of inflammatory cytokines, particularly IL-1b and TNF-a[10].

Chronic inflammation in HD patients maybe due to reasons related to the dialysis process itself, which involves blood contact with parts of the dialysis system outside the body, which carries the risk of exposure to impurities and pyrogens, as well as the membrane used in the dialysis, which may be responsible for causing chronic inflammation as a result of lack of membrane biocompatibility[11].

Table 1: Demographic data of subjects included in the study.

Demographic characteristics	Values	
	Patients group	**Control group**
Number of subjects	73	25
Male	43	15
Female	30	**10**
Age (years; mean ± SD)	50.85 ± 7.57	50.20 ± 7.23
Diabetes mellitus (%)	60.3 %	-
Hypertension (%)	36.7%	-
Polycystic kidney disease (%)	3%	-
Duration of Dialysis (years; mean ± SD)	6.41 ± 2.81	-

Table 2: Comparison of the studied laboratory parameters between the patient group and the healthy group.

Parameters	Patients group	Control group	P-value
Hb (g/dL)	8.13 ± 0.85	14.31 ± 1.16	0.000
Hct (%)	26.12 ± 2.79	44.47 ± 3.84	0.000
MCV (fl)	81.77 ± 4.99	83.67 ± 7.45	0.154
MCHC (%)	31.08 ± 0.77	32.72 ± 0.50	0.000
WBC (*10^3 / μL)	7.49 ± 0.64	7.26 ± 0.43	0.103
Neutrophil (%)	69.05 ± 1.71	58.68 ± 1.39	0.000
Lymphocyte (%)	24.92 ± 1.56	36.76 ± 1.48	0.000
Serum Iron (μg/dL)	57.10 ± 7.12	84.16 ± 9.94	0.000
TIBC (μg/dL)	238.64 ± 19.28	318.36 ± 17.9	0.000
TSAT (%)	22.78 ± 2.93	26.52 ± 3.47	0.000
Ferritin (ng/mL)	539.64 ± 152.64	115.10 ± 29.74	0.000
CRP (mg/l)	9.43 ± 4.30	1.57 ± 0.32	0.000

*This table shows mean values ± SD of the parameters in patients group (73) and control group (25). *Hb: Hemoglobin, Hct: Hematocrit, MCV: Mean corpuscular Volume, MCHC: mean corpuscular hemoglobin concentration, WBC: White Blood Cells, TIBC: Total iron binding capacity, TSAT: Transferrin saturation, CRP: C-reactive protein.

Depending on the ferritin values, we divided the patients group into two subgroups (according to the recommendations of KDIGO, we considered a cut off value of serum ferritin as 500 ng/ml), and we noticed that there were significant differences between the two subgroups for many of the studied parameters as shown in Table **3**.

We found that Hb and Hct levels in group 1 were significantly higher compared to group 2, while CRP levels were significantly higher in group 2 (which coincided with the levels of ferritin higher than 500 ng/ml).

Also, in group 2, we observed that serum iron, TIBC, and TSAT tend to be from normal to low, but ferritin was high. This pattern of iron indices is similar to anemia of chronic disease (Anemia of inflammation).

As a result of Pearson's test, We found a significant inverse correlation between CRP and Hb (fig.1), and a positive significant correlation between CRP and ferritin in HD patients (fig. 2).

Table 3: Comparison of two patients groups based on ferritin values.

Parameters	Patients group		P-value
	Group 1 Ferritin <500 ng/mL	Group 2 Ferritin ≥500 ng/mL	
Hb (g/dL)	8.5 ± 0.71	7.91 ± 0.86	0.004
Hct (%)	27.32 ± 2.74	25.55 ± 2.81	0.041
WBC (*10^3/µL)	7.42 ± 0.68	7.52 ± 0.61	0.515
Neutrophil (%)	68.52 ± 1.49	69.36 ± 1.77	0.042
Lymphocyte (%)	25.47 ± 1.50	24.59 ± 1.51	0.019
Serum Iron (µg/dL)	52.40 ± 5.38	59.86 ± 6.58	0.000
TIBC (µg/dL)	245.63 ± 17.90	234.64 ± 19.05	0.017
TSAT (%)	21.48 ± 2.72	23.54 ± 2.79	0.003
Ferritin (ng/mL)	413.14 ± 114.4	613.89 ± 120.3	0.000
CRP (mg/l)	7.80 ± 3.81	10.39 ± 4.32	0.012

*This table shows mean values ± SD of the parameters in the two groups of patient.
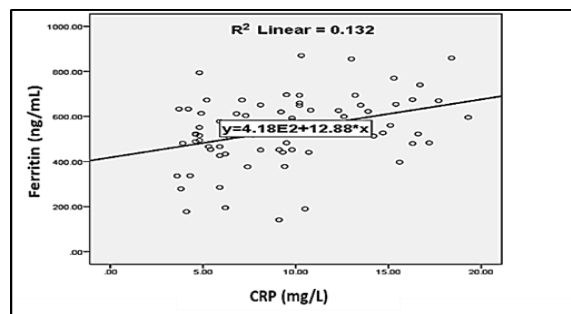
Fig. 1: Correlation between serum ferritin and CRP ($r^2 = 0.132$, P = 0.002).
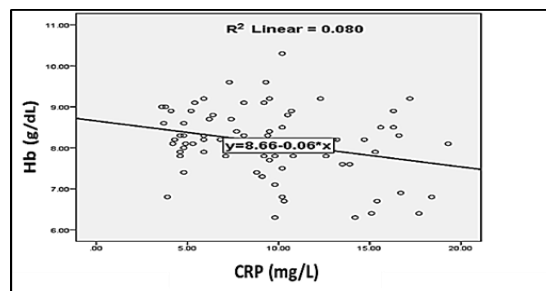
Fig. 2: Correlation between Hb and CRP ($r^2 = 0.080$, P = 0.015).

Since the increase in the inflammatory marker (CRP) coincides with low values of Hb and high values of serum ferritin, here we can say that this type of anemia is similar to anemia of chronic disease ACD (or what is known as anemia of inflammation).

Ferritin is the main storage molecule for iron with an average molecular weight of 450 kDa, while on the other hand, serum ferritin is also known as a positive acute phase reactant, and its serum levels may rise 2-4 times even in mild inflammation. This may be due to the fact that cytokines increase the synthesis of light and heavy subunits ferritin molecule by increasing the translation of preform ferritin mRNA[2&12].

Like ferritin, hepatic synthesis of CRP and Hepcidin (the main iron-state regulating protein in the body) is increased by pro-inflammatory cytokines, such as IL-1b and TNF-a. In contrast, these cytokines inhibit the hepatic synthesis of transferrin. This leads to what is known as "functional iron deficiency", This leads to what is known as 'functional iron deficiency', which is characterized by restriction of iron movement, unresponsiveness to treatment and resistance to erythropoietin, thus in this case of "chronic" inflammation in the study patients, an overexpression of pro-inflammatory cytokines in the bone marrow such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-alpha), could modulate the erythropoiesis process, and It leads to treatment-resistant anemia[13&14].

Our results support the findings of several previous studies, whereas chronic inflammation interferes with the correct assessment of iron markers in HD patients, especially ferritin[15-18].

Serum iron, TIBC, TSAT, and serum ferritin are the main used parameters in assessing iron status and diagnosing iron-deficiency anemia in HD patients. But in addition to the effect of iron stores, there are other factors that affect these laboratory parameters, and one of the most important of these factors is inflammation. In our current study, We found that ferritin levels between (500 ng/ml and 800 ng/ml) may be associated with functional iron deficiency and anemia in maintenance HD patients. Therefore, serum ferritin becomes inexpressive and unreliable for determining iron deficiency and iron overload. Thus, the combination of serum ferritin and TSAT values gives the optimal diagnosis of iron status in hemodialysis patients, and reveals functional iron deficiency.

Conclusion

Anemia of Chronic Disease (or Anemia of Inflammation) is one of the most important types of anemia associated with kidney failure around the world. Since the start of recombinant erythropoietin therapy in the early 1990s, interest has increased in achieving target hemoglobin values and in studying the factors responsible for poor response to treatment, especially iron deficiency and inflammation. Over the past two decades, interest has increased in the study of inflammation in patients with renal failure, and low grade-chronic inflammation has become one of the most important risk factors for morbidity and mortality in patients with renal failure, as it has been linked with both cardiovascular diseases, malnutrition and protein loss. In our study, we found that chronic inflammation in hemodialysis patients interferes the correct laboratory evaluation of iron status, as serum ferritin, which is one of the most important laboratory parameters used, is at the same time a marker for iron evaluation and one of the acute phase proteins. Therefore, the importance of excluding non-iron related factors that cause elevated serum ferritin in hemodialysis patients should be highlighted.

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دراسة القيمة التشخيصية لفيريتين المصل في تقييم حالة الحديد عند مرضى الديال الدموي في سوريا

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يهدف هذا البحث إلى دراسة القيمة التشخيصية لفيريتين المصل في تقييم حالة الحديد عند مرضى الديال الدموي. حيث يعد فيريتين المصل من أهم البارمترات المخبرية المستخدمة لتقييم حالة الحديد، ولكن إن وجود عوامل مشوشة مثل الالتهاب المزمن عند مرضى الديال الدموي قد يؤثر على تفسير النتائج المخبرية. أجريت هذه الدراسة على ٧٣ مريضًا بالغًا يخضعون للعلاج بالديال الدموي المزمن في مشفى الباسل التخصصي في مدينة حمص، سوريا، وبوجود عينة شاهدة من الأصحاء في نفس الفئة العمرية. تم جمع عينات الدم قبل البدء بجلسة الديال، حيث تم استخدام البروتين المتفاعل C (CRP) المصلي كواسم التهابي. أظهرت النتائج وجود ارتفاع معنوي في قيم فيريتين المصل عند عينة المرضى مقارنة بالأصحاء، وبمتوسط بلغ(٥٣٩.٦٤ نانوجرام/مل، وتزامن هذا الارتفاع مع انخفاض في قيم الخضاب ونسبة إشباع الترانسفيرين TSAT، ولكن مع ارتفاع في مستويات الــ CRP. أيضًا وجدنا ارتباط إيجابي معنوي بين الفيريتين والــ CRP (r2 = 0.132, P = 0.002) عند عينة المرضى. بالتالي، إن وجود حالة من الالتهاب المزمن عند مرضى الديال الدموي قد يرفع فيريتين المصل بشكل غير معبّر عن مخازن الحديد، ويقلل من قيمته التشخيصية عند الاعتماد عليه وحده في تقييم حالة الحديد.