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Original Article

Evaluation of serum ferritin level and hepatitis b and hepatitis c viral infection in chronic hemodialysis patients

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Abstract

The most popular treatment for end-stage renal illness is hemodialysis (HD). The study aimed to assess serum ferritin levels and their connection to Epoetin alfa resistance, along with exploring the link between hepatitis C virus, iron overload, and the prevalence of hepatitis C and B infections in chronic HD patients. This was a descriptive-analytical study conducted on 50 Patients with chronic kidney disease (CKD) who were on regular HD in the dialysis unit of Ibin Sina Teaching Hospital in Mosul City, Iraq. Out of 50 patients, 26 (52%) tested positive for Hepatitis C Virus (HCV) Antibody, 10 (20%) for Hepatitis B surface Antigen (HBsAg), and 14 (28%) tested negative for both. Higher serum iron and ferritin levels were found in HCV antibody-positive patients ($p < 0.05$). Despite Epoetin alfa treatment, patients with elevated ferritin levels exhibited lower Hemoglobin (HB) and Packed Cell Volume ($p < 0.05$). Non-diabetics exhibited significantly higher serum ferritin, Hemoglobin, Blood urea, and serum creatinine than diabetics ($p < 0.05$). A noteworthy association was seen between the quantity of blood transfusions and elevated levels of serum ferritin and total serum iron ($p < 0.05$). Most HD patients were anemic, with Hepatitis B and C prevalent. The main CKD causes were diabetes and hypertension. HCV-positive patients often showed mild to moderate iron overload, and high serum ferritin was linked to poor Epoetin alfa response. Dialysis can elevate blood urea, ferritin, and creatinine, worsening anemia. High ferritin levels may hinder response to Epoetin alfa and iron replacement. Excessive blood transfusions can lead to iron overload and inhibit erythropoiesis. Maintaining HB at 110-120 g/l improves quality of life and reduces anemia-related risks.

Keywords: Hemodialysis, Hepatitis B, Hepatitis C, S ferritin, Epoetin alfa.

1. Introduction

The gradual and irreversible kidney function problem known as chronic renal failure (CRF) causes the body to lose its capacity to clear metabolic waste products and maintain the proper balance of fluids and electrolytes, which in turn causes uremia [1]. Hemodialysis (HD) is the most common method used to treat end-stage renal disease (ESRD) in which a volume of the patient's blood, at a time, flows through a special filter that removes excess fluids and waste materials [2]. Although HD techniques have improved compared to the past, the morbidity and mortality of chronic HD patients are still high [3]. Despite adequate dialysis, many dialysis patients still exhibit uncontrolled hypertension, a major risk factor for cardiovascular complications. Other treatment-related medical complications during HD include post-dialysis fatigue syndrome, increased pruritus, headache, pain, nausea and vomiting, and pyrogenic reaction [4]. Machine-related complications are another group of complications of HD caused by accidents or failure of the safety mechanisms of HD treatment and include air embolism, hemolysis, hyperthermia or hypothermia, blood loss, and conduction problems. Hemolysis during HD is caused by oxidizing agent, reducing agent, hyperthermia, mechanical problems and hyperosmolar dialysate [5].

Patients with chronic kidney disease (CKD) frequently have anemia because the kidneys produce the hormone erythropoietin, which encourages the production of red blood cells (RBC) in the bone marrow (BM) [6]. In the United States (US), the prevalence of anemia is twice as high among those with CKD (15.4%) as in the overall population (7.6%) [7]. The prevalence of anemia was 45.0% among non-dialysis CKD patients in Korea [8]. Anemia may begin in the early stages, when 20 to 50% of normal kidney function remains, but worsen as the disease progresses [9]. It is caused by erythropoietin, iron, and vitamin B12 or folate deficiency, blood loss, shortened RBC life span, the uremic milieu, aluminum toxicity, pure red cell aplasia, and inflammation [10, 11]. The direct traumatic effect of the dialysis circuit on the RBCs and the toxic substances in the dialysate (chloramine, nitrate, copper, zinc, fluorine, etc.) can cause anemia to worsen [3]. Diabetic nephropathy, CKD stages, smoking, body mass index, serum albumin, iron markers, leukocyte count, calcium, and phosphorus concentration were identified as independent risk factors for anemia in patients with CKD [8]. In individuals with CKD, anemia is a powerful marker of morbidity and death from cardiovascular consequences

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[12].

Erythropoietin is an endogenous glycoprotein produced by the liver and renal cortex in response to hypoxia. It exerts its action in the BM, where it stimulates the maturation of the progenitor stem cells to erythrocytes. Epoetin alfa is produced by means of recombinant DNA technology and contains the same sequence of 165 amino acids as that found in human erythropoietin [13]. The FDA approved epoetin alfa for the treatment of anemia of various etiologies, including anemia in chronic renal failure [14]. However, epoetin therapy is associated with significant side effects, including hypertension, venous and pulmonary thrombosis, injection site reactions, flu-like symptoms, and seizures. There may also be an association between the development of neutralizing antibodies to epoetin alfa and pure RBC aplasia [15].

Among the issues HD patients deal with are viral infections. Hepatitis B and C indicators are more prevalent in dialysis patients than in everybody as a result of intravenous treatments, blood transfusions, etc [3]. Serum ferritin, which serves as a measure for the body's iron store [16], may be elevated in conditions such as inflammation, infection, malignancy, and liver disease and these conditions are common in CRF patients [17]. Elevations in serum ferritin levels are linked to increased mortality in people with HD and may be impacted by iron consumption and inflammation [16].

The present study aimed to assess the prevalence of anemia in HD patients, the response to Epoetin alfa, the relation between high ferritin with resistance to Epoetin alfa, and the relation between hepatitis C virus and iron overload.

2. Materials and Methods

In the dialysis unit of the Ibn Sina Teaching Hospital in Mosul, Iraq, 50 patients with CKD who were receiving regular HD participated in this descriptive-analytical study.

In this study, we analyzed hemoglobin (HB), packed cell volume (PCV), serum creatinine, blood urea, serum ferritin, serum iron, total iron-binding capacity (TIBC), Transferrin saturation (TSAT) that is the ratio of serum iron to total iron-binding capacity. Blood samples were taken from patients before dialysis. Anemia is defined as HB <12g/dl in males and <11g/dl in females according US National Kidney Foundation -Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) practice guidelines recommendation [18]. HB was measured using HB cyanide method which is the WHO recommended for HB determination; the method is based on dilution of blood in a solution containing potassium cyanide and potassium ferri cyanide. Diluents are based on Drabkins cyanide-ferri cyanide solution & spectrophotometric method is used by machines of HORiBA ABX diagnostic [ABXMicros60, France]; PCV as percentage should be nearly three times the Hb value. Urea is measured using kit [Urease – Berthol reaction]; endpoint colorimetric method (Biomerieux, France); Creatinine is measured using colorimetric method spectrophotometer Jaffes-reaction (Biomerieux, France).

Ferritin is measured using the Enzyme-linked immunofluorescent assay (ELIFA) technique (Minividas), (Biomerieux, France); TIBC is measured using the colorimetric method manually with the aid of magnesium carbonate (RANDOX, UK); the Serum iron is measured using spectrophotometric determination [CECIL7200] (endpoint method). TSAT is calculated from formula s. iron / TIBC x %; Internal quality control was assayed within the sample run (Multiprecision level 1, 2, 3).

2.1. Statistical Analysis

Data management and statistical analyses were conducted using SPSS version 16.0 model for determination of relationship between iron indexes and HB with biochemical markers. In every statistical analysis, p-values of less than 0.05 were deemed as statistically significant.

3. Results

The age of studied patients was between 26-67 years (mean ± SD) (44.66 ± 12.43) years, the total number of studied patients was 50, of which 30 patients (60%) were males and 20 patients (40%) were females. Five patients (10%) were from rural areas and 45 patients (90%) were from urban areas; 16 patients (32%) were unemployed, 16 patients (32%) were housewives, 13 patients (26%) were workers, 3 patients (6%) were retired, 2 patients (4%) were employee. Twenty-six patients (52%) were Hepatitis C Virus Antibody (HCV AB) positive, 10 patients (20%) were Hepatitis B surface Antigen (HBS Ag) positive, 14 patients (28%) were negative for both HCV AB and HBS Ag (Table 1).

According to causes of chronic renal failure, 21 patients (42%) had no known cause for their CKD, 16 patients (32%) were caused by Hypertension, 5 patients (10%)

Table 1. Demographic characters of the studied patients.

Demographic parameters		No. of Patients	Percentage (%)
Sex	Male	30	60%
	Female	20	40 %
Residence	Rural	5	10 %
	Urban	45	90 %
Occupation	Unemployed	16	32 %
	Housewife	16	32 %
	Worker	13	26 %
	Retired	3	6 %
	Employee	2	4 %
Type of hepatitis	HCV Ab positive	26	52 %
	HBS Ag positive	10	20 %
	HBS Ag and HCV Ab negative	14	28 %

Table 2. Descriptive statistic of some hematological and biochemical parameters in patients with chronic renal failure on HD.

Parameters*	Mean ± SD	Range
Hb (g/L)	89.28 ± 15.86	59 - 134
PCV(L/L)	28.50±5.50	19 - 48
Total serum iron (microgram/dl)	82.29±56.19	8 - 209
Serum ferritin (ng/ml)	498.24 ±594.16	8 - 2458
TIBC (Mg/dl)	261.38±117.32	15 - 564
TSAT (%)	60.82±163.45	2.72 - 1160
Blood urea (mmol/L)	28.13±6.45	17 - 42.20
Serum creatinine (mmol/L)	859.66±278.87	227 - 1546

*HD: hemodialysis, Hb: Hemoglobin, PCV: packed cell volume, TIBC: total iron-binding capacity, TSAT: Transferrin saturation

Table 3. Comparison between some hematological and biochemical parameters between patients with HCV AB positive and HCV ab negative.

Parameters	HCV AB positive No. of pts =26 (mean ± SD)	HCV AB negative No. of pts =24 (mean ± SD)	P-value
Total serum Iron (Mgm/dl)	99.10±66.25	64.08±35.94	*0.024
Serum ferritin (ng/ml)	682.40±723.97	298.72±328.18	*0.019
Hb (g/L)	89.65±17.60	88.87±14.11	NS
PCV (L/L)	28.69±6.27	28.29±4.65	NS
Blood urea (mmol/L)	28.93±7.37	27.25±5.29	NS
Serum Creatinine (mmol/L)	907.61±290.14	807.70±262.22	NS
TIBC (MG/dl)	259.34±137.65	263.58±93.37	NS
TSAT (%)	91.18±223.74	27.94±18.29	NS

* = Significant according to unpaired t-test, NS=non-significant according to unpaired t-test. HCV AB: Hepatitis C Virus Antibody, Hb: Hemoglobin, PCV: packed cell volume, TIBC: total iron-binding capacity, TSAT: Transferrin saturation.

Table 4. Comparison between some hematological and biochemical parameters between patients who received Erythropoietin (Epo) and those who did not receive Erythropoietin.

Parameters	No. of patients who received Epo= 16 (mean ± SD)	No. of Patients who not received Epo =34 (mean ± SD)	P-value
Total serum Iron (Mgm/dl)	78.35 ± 60.84	84.14±54.73	NS
Serum ferritin (ng/ml)	708.56 ± 404.50	399.26±850.81	*0.05
Hb (g/L)	86.31±14.82	90.67±16.36	NS
PCV (L/L)	27.25±4.85	29.08±5.75	NS
Blood urea (mmol/L)	29.95±6.07	27.27±6.53	NS
Serum Creatinine (mmol/L)	942.56±231.08	820.64±293.77	NS
TIBC (MG/dl)	273.56±155.28	255.64±96.80	NS
TSAT (%)	44.72±56.81	68.40±194.98	NS

NS = non-significant according to unpaired t-test, * = Significant according to unpaired t-test. Hb: Hemoglobin, PCV: packed cell volume, TIBC: total iron-binding capacity, TSAT: Transferrin saturation.

were caused by diabetes mellitus (DM), 4 patients (8%) were caused by obstructive uropathy (stones); 3 patients (6%) were caused by chronic infections (repeated UTI); 1 patient (2%) were caused by bladder tumor.

Table 2 shows the descriptive statistic of some hematological and biochemical parameters in patients with CRF; HB range was 59-134g/L with a mean of 89.28±15.86; PCV range was 19-48L/L at a mean of 28.50±5.50; total serum iron range was 8-209 microgram/dl at a mean of 82.29±56.19; Serum ferritin range was 8-2458ng/ml at a mean of 498.24±594.16; TIBC range was 15-564Mg/dl at a mean of 261.38±117.32; TSAT range was 2.72-1160 % at a mean of 60.82±163.45; Blood urea were in a range of 17-42.20 mmol/L at a mean of 28.13±6.45; Serum creatinine ranged from 227-1546 mmol/L at a mean of 859.66±278.87.

Table 3 shows the comparison of some hematological and biochemical parameters between patients with HCV AB positive and HCV AB negative; Unpaired t-test revealed that patients with HCV Ab positive had greater levels of total blood iron and serum ferritin than patients with HCV Ab negative. These differences were statistically significant ($p < 0.024$ and $p < 0.019$, respectively); while the differences in HB, PCV, TSAT, TIBC, blood urea and serum creatinine values were statistically not significant ($p > 0.05$).

Table 4 shows the comparison of some hematological and biochemical parameters between patients who received Erythropoietin and those who did not receive Erythropoietin. HB, PCV, TSAT, TIBC, blood urea and serum creatinine values were statistically not significant. Higher ferritin level causes resistance to Erythropoietin replacement and remain anemic ($p < 0.04$).

Table 5 shows the differences between some hematological and biochemical parameters in DM and non-DM patients; serum ferritin, HB, Blood urea and serum creatinine values were significantly higher in non-DM (($p < 0.001$), ($P < 0.05$), ($p < 0.005$), ($p < 0.037$), respectively). The differences between hematological and biochemical parameters in Hypertensive and non-hypertensive patients and according to hours per week on HD were differences in the values but were not significant statistically. There were noticeable improvements in both hematological and biochemical parameters with HD session length but were not statistically significant.

Table 6 shows the differences between some hematological and biochemical parameters according to number of blood transfusion units in their lives; There was a significant relation between number of transfused blood units and increment in the level of total serum iron ($p = 0.05$), the relationship between serum ferritin and amount of transfused blood units were significant according to ANOVA test ($p < 0.01$). The relation between TIBC and TSAT with number of transfused blood units did not reach significant values.

4. Discussion

The mean age of Patients with HD in this study was 44 years which was lower by 20 years than that of Western countries which may be due to better medical facilities in the Western world. The increased incidence of CKD in adults and older adults may be explained by long-term HTN, DM, BPH, and nephrotoxic substances that individuals are exposed to throughout their lives [19]. Most laboratories evaluate kidney function using serum creatinine. In this study mean serum creatinine was significantly higher in males than females which may be due to higher muscle mass in males and more adipose tissue in females [20].

Most HD Patients were HCV Ab positive which accounts for majority of viral hepatitis worldwide [21]. In this study, DM and HTN were the most common causes of CKD in HD. Throughout the whole adult age range, stage 3 or 4 CKD and albuminuria are linked to HTN and DM. Crucially, for younger age groups, this connection was noticeably greater [22, 23]. The large proportion of patients with no known causes of CKD may be due to late referral to nephrologist.

This study revealed that there is strong positive correlation between iron overload and HCV AB-positive patients;

Table 5. Differences between some hematological and biochemical parameters in DM & non-DM patient.

Parameters	DM No. of pts = 5 (mean ± SD)	Non-DM no. of pts =45 (mean ± SD)	P-value
Total serum Iron (Mgm/dl)	61.60±40.57	84.59±57.56	NS
Serum ferritin (ng/ml)	122.30±101.94	540.01±611.85	*0.001
Hb (g/L)	108.44±17.72	87.66±15.30	*0.05
PCV (L/L)	32.80±5.06	28.02±5.39	NS
Blood urea (mmol/L)	23.26±2.67	28.67±6.53	*0.005
Serum Creatinine (mmol/L)	707.40±124.21	876.57±286.84	*0.037
TIBC (MG/dl)	236.80±36.71	264.11±123	NS
TSAT (%)	26.04±18.30	64.69±171.96	NS

NS = non-significant according to unpaired t-test, * = Significant according to unpaired t-test. DM: diabetes mellitus, Hb: Hemoglobin, PCV: packed cell volume, TIBC: total iron-binding capacity, TSAT: Transferrin saturation.

Table 6. Comparison between some hematological and biochemical parameters according to number of blood transfusion units.

Parameters	Units of blood transfusion no. of pts=5 (mean ±SD)	1-5 units of blood transfusion no. of pts=21 (mean ±SD)	6-10 units of blood transfusions no. of pts=8 (mean ±SD)	11-20 units of blood transfusions no. of pts=9 (mean ±SD)	>20 units of blood transfusions no. of pts=7 (mean ±SD)	P. value
Total serum iron (Mgm/dl)	45.92 ±32.54	72.47±52.29	109.00±51.05	69.55±51.26	123.57±69.31	*0.05
Serum ferritin (ng/ml)	129.60±145.48	268.69±227.48	476.00±575.28	707.05±853.78	1207.14±623.23	*0.001
Hb (g/L)	90.00±9.89	88.61±14.79	96.00±18.05	79.66±12.25	95.42±20.87	NS
PCV (L/L)	27.80±3.70	28.23±4.72	30.75±5.89	25.22±4.02	31.42±8.26	NS
Blood urea (mmol/L)	32.46±5.30	26.60±5.71	26.81±4.48	27.84±7.79	31.48±8.28	NS
Serum creatinine (mmol/L)	979.40±125.86	844.71±290.45	925.75±282.30	808.77±371.62	808.85±201.94	NS
TIBC (MG/dl)	351.60±157.72	250.52±88.60	303.12±155.58	270.44±102.56	170.14±89.62	0.069
TSAT (%)	16.83±18.98	33.60±28.40	56.75±72.16	30.21±26.35	217.92±416.93	0.096

NS = non-significant according to unpaired t-test, * = Significant according to unpaired t-test. Hb: Hemoglobin, PCV: packed cell volume, TIBC: total iron-binding capacity, TSAT: Transferrin saturation a result which is similar to Farinati et al. [21]. A unique characteristic of HCV is that it can actively produce free radicals without the need for inflammation. Its core protein has a specific activity that interacts with intracellular proteins like interleukin-1 (IL-1) and tumor necrosis factor (TNF), causing transcriptional activation that is associated with an excess of free radicals [21]. The infection of HCV was independently linked to a nearly fourfold rise in levels of 8-OHdG, a chemical alteration of guanine that leads to a genetic mutation in the offspring DNA strands and thus serves as a dependable indicator of DNA harm. This association persisted even in instances of asymptomatic infection [24]. This evidence supports the data that indicates individuals with normal transaminases may still exhibit varying levels of liver damage [25]. Additionally, it verifies the existence of tissue damage associated with the formation of free radicals. A study conducted using a comparable mouse model, which was transgenic for HCV polyprotein, demonstrated that co-factors can impact the formation of reactive oxygen species (ROS). For example, iron overloading was found to promote liver carcinogenesis in the presence of increased 8-OHdG formation [26]. Patients with chronic HCV-mediated liver damage have higher levels of both blood ferritin and liver tissue iron. Iron buildup is frequently seen in instances of HCV hepatitis [27].

When there are sufficient iron reserves, anemia that is resistant to appropriate erythropoietin dosages may result from a combination of the following: Acute or ongoing inflammation, insufficient dialysis, extreme hyperparathyroidism, persistent hemolysis or blood loss, ongoing infection, or cancer. Hemoglobinopathy patients, such as those with sickle cell disease or thalassemia, typically do not react well to exogenous EPREX; yet, many of these patients still exhibit a rise in Hb concentration [28]. Given the potential for increased susceptibility to hepatitis, iron overload, and transplant sensitization, it is advisable to refrain from administering blood transfusion until the patient exhibits symptoms and the anemia remains unresponsive to EPREX treatment. In the context of CKD, blood transfusion may impede erythropoiesis. Another cause of the failure of response Erythropoietin was the development of neutralizing antibody which may cause pure red cell aplasia and this antibody affects all types of Erythropoietin even Owen erythropoietin [15].

The reasons behind lower HB values in non-diabetic patients than diabetic patients were the higher level of inflammatory markers (serum ferritin) and more uremic toxins levels (blood urea and serum creatinine) in non-diabetic patients because dialysis performance is judged by the level of solutes (blood urea and serum creatinine), ultrafiltration characteristics, volume and HTN control [29]. In addition, most DM patients in this study were HCV Ab negative.

There were no significant improvements in HB values in patients who received both Epoetin alfa and iron because anemia in CKD is an anemia of chronic disease and is due to the anti-proliferative effects of accumulating urinary excretion products from the pathophysiological basis in this setting [30]. Moreover, numerous infection episodes and/or contact stimulation of immune cells by dialysis membranes might cause chronic immunological activation in individuals with end-stage renal disease, leading to pathophysiological alterations characteristic of ACD [30]. Iron therapy's detrimental effects on immunity may further raise an ACD patient's risk of septicemia or other infectious problems [31]. Neutrophil dysfunction has been demonstrated to be induced by iron treatment in patients with chronic HD. As a result, these individuals are unable to phagocytose invasive microorganisms [32]. Because of the potential for negative effects, iron treatment is presently not advised for ACD patients with high/normal ferritin levels (>100 μg/L) [32].

The study shows that total serum iron and ferritin rise with the number of blood transfusions, aggravating secondary iron overload exacerbated by HCV infection (HCV infection is linked to mild to moderate iron accumulation due to HCV itself and the hepatic cell damage associated with it) [33]. Due to the potential for hepatitis, iron overload, and the formation of alloantibodies in individuals undergoing dialysis, blood transfusions can heighten the susceptibility of these patients' human leukocyte antigen (HLA) to the presence of donor kidney antigens, thereby complicating the process of renal transplantation. Additionally, blood transfusions may exert an adverse impact on the production of RBCs in patients with CKD [34].

5. Conclusion

Virtually all HD patients were anemic which increases their mortality rate. Hepatitis B and C infections were prevalent in HD patients. The most common causes of CKD were DM and HTN. HCV antibody-positive patients had mild to moderate iron overload. High serum ferritin was associated with failure to respond to Epoetin alfa. Most HD patients were under dialyzed causing them to retain higher blood urea and serum creatinine which further aggravates anemia and it is management. Due to high ferritin values, HD patients were not responding to combination of Epoetin alfa and iron replacement. Excessive blood transfusion led to secondary iron overload, inhibiting erythropoiesis and causing tissue damage. Increase HB value to reach 110-120g/l to improve quality of life, and reduce morbidity and mortality associated with anemia.

Recommendation

Avoidance of iron replacement in patients with ferritin >100ng/ml; Avoidance of blood transfusion in ESRD to prevent development of alloantibody that could sensitize the patients to donor antigen (HLA) and make renal transplantation more problematic; Use of darbepoetin instead of Epoetin alfa because of longer half-life and less incidence of neutralizing antibody; Increasing dose of Epoetin alfa when using ACE inhibiter drugs for HTN therapy, screening CRF patients for viral screens (HCV, HBV, HIV) before rushing them for urgent HD.

Conflict of interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author reviewed and gave her approval for the published version of the work.

Ethics approval and consent to participate

Ethical permission for the study was granted by the hospital authorities. The Declaration of Helsinki's guiding principles were followed in this investigation.

Informed Consent

Participants provided verbal informed consent before their enrollment.

Availability of data and material

The corresponding author can provide the study's data upon reasonable request.

Authors' contributions

Hemn R. Shawgery did all the steps in the research work.

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