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Potential Association of Serum Hepcidin, hs-CRP and Iron Status Levels in Hemodialytic Patients

Shahad F.Obeid[1,*], Zainab A. Salman[2] and Israa Abass Rashed[3]
[1]University of Baghdad, Department of Chemistry, College of Sciences, Baghdad, Iraq.
[2]University of Baghdad, Department of Chemistry, College of Dentistry, Baghdad, Iraq.
[3]Mustansiriyah University, College of Basic Education, Baghdad, Iraq.

Corresponding author: Shahad F.Obeid J(e-mail: shahad.f@sc.uobagdad.edu.iq).

Abstract: Patients on hemodialysis (HD) are usually anemic because of defective erythropoiesis. Hepcidin is a polypeptide that regulates iron homeostasis and could indicate functional iron deficiency in patients with end-stage renal disease (ESRD); this may also aid in assessing a patient's response to erythropoietin (EPO). The present study was directed to investigate serum levels of hepcidin, iron status, and inflammation markers such as C-reactive protein (CRP) in patients with ESRD on maintenance HD and to observe the correlation of serum hepcidin with conventional iron and inflammatory markers. Forty-two patients of both sexes on maintenance HD and EPO therapy were enrolled; 42 age and sex-matched healthy subjects were included as controls. Laboratory tests were performed, including complete blood count, serum hepcidin, total iron binding capacity (TIBC), serum ferritin, serum iron, and CRP. Serum hepcidin levels were significantly higher in patients with ESRD than in the control group (18.2 $\pm$ 2.8 ng/mL and 8.5 $\pm$ 2.3 ng/mL, respectively P = 0.000). The hemoglobin, hematocrit, serum iron, TIBC, and transferrin saturation levels in the patient group were significantly lower than in the control group. Higher hepcidin levels were found in EPO non-responders (19.6 $\pm$ 2.4 ng/mL), while lower levels (16.9 $\pm$ 2.5 ng/mL) were seen in responders (P = 0.001). A positive and significant correlation was observed between the values of serum hepcidin and CRP. Our study indicates that higher hepcidin levels are found in ESRD patients on HD and in those not responding to EPO. Our findings suggest that hepcidin might play a role in the pathophysiology of anemia associated with chronic diseases and EPO resistance.

Key Words: hemodialysis; erythropoiesis; end-stage renal disease; erythropoietin; Hepcidin; total iron binding capacity; C-reactive protein; serum ferritin; hemoglobin

I. INTRODUCTION

In the United States, nearly 26 million people have chronic renal disease [1]. This number is higher in nations where the population is getting older, and diabetes and hypertension are becoming more common. Renal illness significantly shortens life expectancy in addition to its consequences. One of the main consequences is anemia [2], which was estimated to be present in 10% of people with chronic renal disease in stages 1 and 2, increasing to 20% to 40% in stages 3 and 4, and to more than 70% in stages 4 and 5. Less than 25% of patients who begin dialysis in the United States do so with epoetin alfa, and the majority have lower hemoglobin levels [3]. Renal replacement therapy (RRT), or dialysis, is advised for patients who have reached CKD stage 4 (GFR, 30 mL/min/1.73 m2) and have been evaluated for signs and symptoms of renal failure (pruritus, acid-base or electrolyte abnormalities, serositis), volume or BP dysregulation, progressive deterioration in nutritional status despite dietary intervention, or impairment in cognition. American National Kidney Foundation Renal replacement therapy (RRT), or dialysis, is advised for patients who have reached CKD stage 4 (GFR, 30 mL/min/1.73 m2) and have been evaluated for signs and symptoms of renal failure (pruritus, acid-base or electrolyte abnormalities, serositis), volume or BP dysregulation, a progressive deterioration in nutritional status despite dietary intervention, or impairment in cognition [4]. Dialysis is the diffusion of molecules in solution along an electrochemical concentration gradient over a semipermeable membrane. Hemodialysis's main objective is to recreate the fluid environment within and outside cells, which is essential for healthy kidney function. This is done by moving solutes like urea out of the blood and into the dialysate, as well as moving solutes like bicarbonate out of the dialysate and back into the blood. The main factors affecting diffusion rates are solute concentration and molecular weight [5].

Dialysis aims to alleviate the uremic syndrome symptom

complex by replacing kidney excretory function [6]. Acute illnesses such as acute renal damage, uremic encephalopathy, pericarditis, life-threatening hyperkalemia, and refractory acidosis require the start of hemodialysis [7] through vasodilation, cardiac depression, and immunosuppression brought on by these disorders' dysregulation and poor clearance of cytokines (immune response modulators), end-organ damage, hemodynamic instability, or a delay in renal recovery result. In high-cytokine situations like sepsis, RRT accelerates cytokine elimination. Electrolyte imbalances, intradialytic hypotension, and catheter problems all carry a risk of damage [8].

Normocytic normochromic anemia is a significant CKD consequence [9]. Anemia is more common and nearly ubiquitous in patients with end-stage renal illness as kidney function declines [10]. Due to mechanical damage brought on by the dialysis membrane and extracellular circulation, hemodialysis (HD) by itself may also be a factor in the decline in RBC survival. It has been noted that HD causes a modest increase in RBC breakdown that occurs intermittently but does not directly affect hemoglobin levels [11].

According to several research findings, maintenance HD therapy patients' RBC survival is dramatically reduced by 20% [12] to 70% [13] compared to those whose renal function is within the reference range. Hepcidin has recently gained attention as a hormone in that regard. The systemic iron-regulatory hormone has been identified as the hepatic peptide hepcidin. Hepcidin causes the degradation of its receptor in the efferent arc, which controls intestinal iron absorption, plasma iron concentrations, and tissue iron distribution [14].

Hepatocytes create more hepcidin when iron is abundant, which reduces its absorption. The generation of hepcidin is also controlled by the need for iron for erythropoiesis. Hepcidin production decreases during vigorous erythropoiesis, increasing the amount of iron available for hemoglobin formation. In uremia, as in other inflammatory situations, the production of hepcidin is increased. According to several research, hemodialysis patients had higher levels of hepcidin than normal, healthy controls, and higher levels than people who did not receive dialysis but had the same amount of renal impairment. This finding is likely the result of low-grade inflammation [15]. Studies that looked at CKD patients who were not on hemodialysis often reported results between normal and hemodialysis [16]. Others have noted a drop in hepcidin levels following dialysis, although the underlying cause has been postulated to be eliminating this hormone during the dialysis procedure [17].

This study is aimed to investigate the hepcidin level alongside iron status and other parameters in patients on hemodialysis.

II. MATERIALS AND METHODS

This cross-sectional study was conducted at Baghdad Teaching Hospital, Iraq, spanning from January to July 2023.

A total of ninety subjects, aged between 35 and 69 years, participated in the study, categorized as follows:

1) **Control Group:** Comprising 25 females and 25 males, totaling 50 individuals who were deemed healthy with ages ranging from 35 to 69 years.
2) **Patients Group:** Consisting of 40 individuals on hemodialysis, evenly divided between genders.

INCLUSION CRITERIA

Subjects aged between 35 and 69 years undergoing hemodialysis were included in the study.

EXCLUSION CRITERIA

Individuals with diabetes mellitus, those receiving thyroxin drugs, and hemodialysis patients with fever or acute infection were excluded. Additionally, individuals below 35 or above 70 years were not considered.

A venous blood sample (5ml) was collected in gel tubes, subjected to centrifugation at 3000 RPM for 10 minutes, and then frozen at (-20 °C) for subsequent storage until analysis.

The study involved the determination of age and BMI for each group.

- Laboratory tests included assessments of Hb, Ht%, fibrinogen, serum hepcidin, serum transferrin receptor (sTFR), total iron binding capacity (TIBC), serum ferritin, serum iron, TfR-index, urea, creatinine, total protein, albumin, and high sensitive C-reactive protein (hs-CRP).
- Serum hepcidin, hs-CRP, serum ferritin, and sTFR were quantified using the Enzyme-Linked Immunosorbent Assay (ELISA) method.
- Measurements of Hb, Ht%, fibrinogen, TIBC, and TfR-index were performed manually with specialized devices.
- Serum iron, urea, creatinine, total protein, and albumin levels were determined using colorimetric methods.

STATISTICAL ANALYSIS

Statistical analysis was conducted using SAS (Statistical Analysis System - version 9.1). An independent t-test assessed significant differences among means, with a significance threshold set at $P < 0.05$. Receiver Operating Characteristic (ROC) curve analysis was employed to evaluate the diagnostic accuracy of markers.

III. RESULTS

This study included 40 patients on hemodialysis (patients group), 20 of which were females and 20 were males. Healthy individuals (control group) were 25 females and 25 males making a total of 50 individual with ages ranging between 27 to 69 years old and the mean was 46.81 ± 7.56 as shown in Table 1.

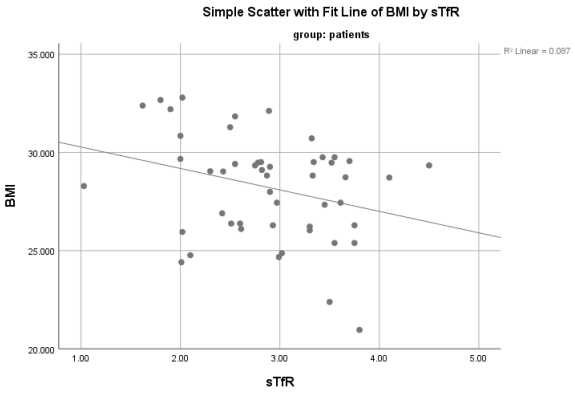
Table 2 shows the comparison between the means of the studied parameters among the two groups, it shows that no significant difference was found between the means of age,

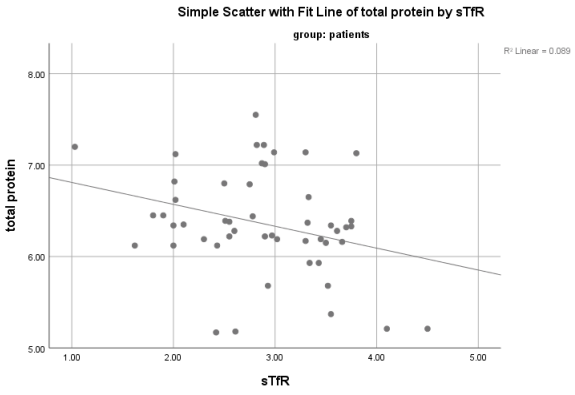
		Group				
		control		Patients		Total
		Count	Row N %	Count	Row N %	
gender	male	20	44.4%	25	55.6%	45
	female	20	44.4%	25	55.6%	45
age	<40 Y.O.	9	33.3%	18	66.7%	27
	40-50 Y.O.	16	35.6%	29	64.4%	45
	>50 Y.O.	15	83.3%	3	16.7%	18
BMI	overweight	33	42.9%	44	57.1%	77
	normal	7	53.8%	6	46.2%	13
	underweight	0	0.0%	0	0.0%	0

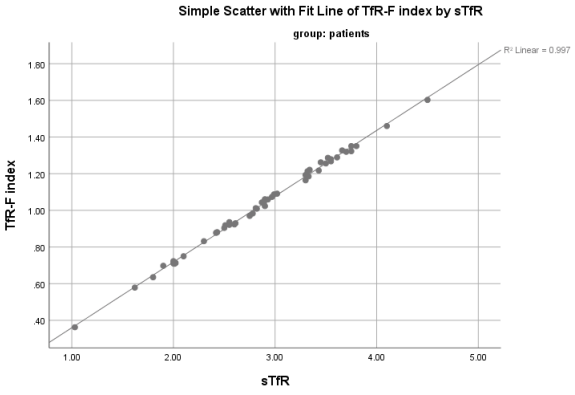
TABLE 1: Demographic Statistics of the Studied Sample

	group				sig
	control		patients		
	Mean	std	Mean	std	
age	45	8	48	7	.528
BMI	26.921	2.200	28.221	2.625	.173
Urea	22.5	5.1	144.9	13.6	.000
Creatinine	.76	.30	8.15	1.31	.000
total protein	7.28	.56	6.36	.57	.835
albumin	4.28	.41	3.22	1.97	.134
Fibrinogen	230.67	7.23	314.83	23.64	.000
h-CRP	1.69	.86	7.23	2.65	.000
GFR	102.74	8.22	8.46	.88	.000
Hb	13.73	.87	9.58	.82	.482
Ht%	44.66	4.55	29.79	1.15	.000
Iron	95.49	4.96	81.30	.78	.000
TIBC	362.55	15.69	228.83	14.76	.421
sTfR	3.69	.93	2.88	.71	.022
Ferritin	57.74	3.29	613.10	50.14	.000
TfR-F index	2.09	.53	1.03	.25	.000
Hepcidine	93.27	4.64	235.55	10.54	.000

TABLE 2: Comparison of the Means of the Studied Parameters between the Groups







BMI, total protein, albumin, Hb, TIBC or sTfR. However highly significant differences were found between the means or urea, creatinine, fibrinogen, h-CRP, ferritin and hepcidin (patients means were higher) while GFR, Ht%, iron and TfR-F index (patients means were lower) all with p values <0.001.

No significant correlation was found in the patients group between ferritin and hepcidin with the other parameters (Table 3), however, significant negative correlation between sTFR and BMI (P value=0.038) was found suggesting that the level of receptors in the blood decrease with the increasing weight.

Total protein was also found to be negatively correlated (P value 0.035) with sTFR levels, and a linear correlation between sTFR and TfR-F index was found as expected due to the formula used to find the index.

Correlations (patients)

			age	BMI	urea	creatinine	total protein	albumin	fibrinogen	h-CRP	GFR	Hb	Ht%	Iron	TIBC	sTfR	Ferritin	TfR-F index
	sTfR	R	.170	-.295*	.099	.234	-.299*	.000	-.080	-.016	-.001	.123	-.011	.192	-.029			.999**
		Sig.	.238	.038	.494	.102	.035	.999	.582	.914	.996	.395	.938	.182	.843			.000
	Ferritin	R	.110	-.095	-.245	-.228	.126	.093	.131	.013	.156	-.210	.009	.081	-.030	-.124		-.173
		Sig.	.448	.514	.086	.111	.382	.522	.364	.929	.279	.143	.951	.578	.837	.390		.230
	Hepcidine	R	.082	.067	.039	.034	.052	-.041	-.143	.007	-.131	.142	-.212	-.037	.007	-.042	-.188	-.036
		Sig.	.570	.644	.785	.815	.721	.777	.322	.961	.364	.324	.140	.798	.959	.771	.191	.804

TABLE 3: Correlation of the Level of sTFR, Ferritin and Hepcidine of the Patients Group with the Studied Parameters

IV. DISCUSSION

While GFR means were lower in the patient group in our study, there were significant differences between the means of urea and creatinine, which were higher in the patient group compared to the control group. This was consistent with previous research because chronic renal failure causes a steady decline in renal clearance, or GFR, which causes the accumulation of urea, creatinine, and other chemicals in the blood [18], [19].

However, when comparing patients on hemodialysis with other renal failure patients, a study found that hemodialyzed individuals with residual renal function have a considerably lower serum creatinine [20].

Both predialysis and dialysis patients have been reported to have higher plasma fibrinogen levels; the causes of the hyperfibrinogenemia are not entirely known. Fibrinogen is a protein found in the acute phase, and end-stage renal disease (ESRD) is linked to substantial increases in the acute phase., one explanation is that fibrinogen production is boosted as part of the acute-phase response [21]. This agrees with the findings of this study, which showed higher fibrinogen levels in the patient group.

This study, in which h-CRP was significantly higher in the patient group, is by other studies by Wang et al. [22]. Kuragano et al. [23] stated that h-CRP was higher in the low filtration group with renal failure, attributing to the fact that it is a well-recognized marker of the chronic inflammatory process.

In our investigation, ferritin levels were much higher in hemodialysis patients; Malyszko et al. likewise found this to be the case. This increase is mostly due to iron overload and inflammation [24]. In line with findings from Kulaksiz et al. [25], who reported elevated hepcidin levels in hemodialyzed patients, our study compared healthy individuals to those on hemodialysis and found hepcidin levels to be higher in the patient group. However, when comparing patients with renal failure, a study by Malyszko et al. [24] found significantly lower serum hepcidin levels in patients on hemodialysis than those with renal failure who are not.

The patients' group's hematocrit levels were found to be lower than those of the control group, which was previously a typical observation in the literature and shows that patients with mild to moderate renal insufficiency may also experience a decline in hematocrit [26] nonetheless, it is more than the levels in patients with renal failure who are not receiving hemodialysis [27].

Due to the large blood losses associated with this treatment, iron insufficiency is a common problem in chronic hemodialysis patients, [28] consistent with our study's findings that demonstrate lower serum iron levels in patients receiving hemodialysis than in healthy individuals.The serum transferrin receptor-ferritin index also decreases as a result of the decline in these parameters.

A significant negative association between sTFR and BMI was discovered, indicating that the number of receptors in the blood decreases as weight increases. Another study found no associations between sTfR levels and obesity, BMI, or any other variables [29]. The study's population could explain this finding because the second study looked at the receptor levels in healthy individuals.

The formula employed to calculate the index led to the expected linear association between sTFR and TfR-F index [30]. Although this was not the case in our investigation, a study by Nemeth et al. [30], and Malyszko et al. [24] demonstrated a significant association between serum ferritin and hepcidin in individuals with anemia of inflammation. Although it significantly correlated with albumin, creatinine, urea, and hsCRP in the latter study, this was not observed in our trial.

Since our study only included dialysis patients, there were likely discrepancies in the study group and the lab methods utilized to measure the amount of this hormone because there currently needs to be recognized standards in this area. By our analysis, there were no connections between hepcidin, iron status, ferritin, and transferrin saturation in the Kulaksiz et al. [25] study.

V. CONCLUSION

A positive and significant correlation was observed between the values of serum hepcidin and CRP. Our study indicates that higher hepcidin levels are found in ESRD patients on HD and in those not responding to EPO. Our findings suggest that hepcidin might play a role in the pathophysiology of chronic diseases and EPO resistance-related anemia.

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This research paper received no external funding.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to this paper. They have all read and approved the final version.

CONSENT

Informed consent was obtained from all participates in the study as needed.

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