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Evaluation of Iron Metabolism in Chronic Kidney Disease Patients by Serum Hepcidin level

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Abstract

Background: A glomerular filtration rate (eGFR) of less than 60 ml/min/1.73 mt2 indicates chronic kidney disease (CKD). Low levels of haemoglobin (Hb) cause the kidneys to produce insufficient amounts of the hormone erythropoietin, which in turn causes anaemia. Blood iron levels may be controlled by the liver-produced peptide, Hepcidin, which regulates iron absorption in the digestive tract. Serum hepcidin levels were measured in individuals with chronic renal disease to determine their iron status. Researchers at Benha University Hospital and Benha Teaching Hospital recruited 60 patients with chronic renal disease (stages I to V) and 20 healthy volunteers for the research. The connection between HB, Iron, TIBC, and transferrin was stronger in the HD group than in the Pre HD group, whereas ferritin was considerably higher in the HD group than in the Pre HD group. The HD group and the Pre HD group differed significantly. However, when looking at Po4 and PTH, HD group had a considerable advantage over Pre HD and control groups because Ca was much greater in HD than in any of the other groups. It was found that Hepcidine correlated positively with ferritin, SGPT and SGOT, ALP, UREA and creatinine as well as proteinuria, Po4 and PTH while negatively correlating with HB, iron and TIBC and Transferrin. Hepcidine was also found to be positively associated with CRP, ferritin, CRP-SGPT, and ALP-SGOT. The involvement of hepcidin in the control of dietary iron absorption and cellular iron release has been established in this study. Hemodialysis patients' erythropoiesis is regulated by hepcidin, which is predominantly found in iron reserves.

Keywords: Iron Metabolism, Chronic Kidney Disease, Hepcidin

1. Introduction

Chronic renal disease patients on haemodialysis are more likely to suffer from anaemia, which has a negative impact on their quality of life (11).

Dialysis patients' anaemia is a complicated problem, and its clinical treatment is still difficult. Chronic inflammation and low erythropoietin levels combine, making it difficult to isolate the exact cause of anaemia and the best treatment options (31).

Iron deficiency anaemia affects 25% of hemodialysis patients (10).

Patients with chronic renal disease are at risk of iron shortage for a variety of reasons. Iron deficiency may occur in certain people, with both circulating iron levels and total body iron storage falling to dangerously low levels. In some cases, a reduction in circulating iron restricts erythropoiesis even when the body's iron reserves are normal or sufficient, leading to functional iron shortage. It's possible that a mixture of these characteristics exists as well (34).

Hemodialysis patients are at risk for iron insufficiency because of increased blood loss, increased iron usage from Erythropoietin treatment, decreased dietary iron absorption, and impaired iron release from body storage sites. Antacids and phosphate binders may interfere with the absorption of iron from the diet. Hemodialysis patients with high levels of hepcidin have reduced iron absorption from their food and reduced iron release from their body's storage sites because hepcidin inhibits ferroportin expression, preventing iron from entering the bloodstream (33).

Anemia and Erythropoietin resistance in chronic kidney disease (CKD) patients may be caused by iron deficiency, hence iron supplementation is an essential aspect of anaemia care in CKD (27).

Deficiencies in iron metabolism and macrophage iron trapping result in decreased plasma iron levels, which prevents the creation of new haemoglobin (30).

Hepcidin is a key regulator of iron homeostasis in the body and is mostly found in the liver, with smaller levels found in the kidney, heart, and brain. Hepcidin is a liver-specific protein. Hepcidin's primary function is to destroy the iron efflux transporter ferroportin, which is found on all cells that excrete iron (19).

The release of iron by macrophages is inhibited by hepcidin, which reduces iron intake in the intestines. Hepcidin may also have a direct influence on the proliferation and survival of erythroid-progenitor cells (9).

Preprohepcidin (HAMP) is a precursor protein of 84 amino acids encoded by the HAMP gene (22).

Histidine levels are controlled by iron, erythrocyte demand, hypoxic conditions, and inflammatory signals, among others (19).

ESA medication has been shown to lower blood levels of hepcidin, which may account for the rapid iron release (12).

Several possibilities, including as the soluble transferrin receptor (sTfR) (16) and the growth differentiation factor (GDF), have been postulated as possible regulators of erythropoiesis in light of the connection between hepcidin synthesis and erythropoiesis seen (6).

2.Aim of the Work

Assess the iron status in patients with chronic kidney disease by measurement of serum hepcidin as a novel biomarker of iron metabolism.

Compare this novel marker in chronic kidney disease patients with healthy patients.

3.Patients and Methods

1 Ethical Approval: The study protocol was approved by the ethical committee of Faculty of Medicine, Benha University.

The study had started after medical ethical committee approval. All included cases were informed about aim of our study, risk factors, possible complication and risk of failure.

Study design: This is a cross sectional study includes 60 patients with chronic kidney disease (stage I to V) & 20 healthy as control group. These patients will be chosen from Benha University Hospital and Benha teaching Hospital.

Study population:

Inclusion criteria:

- Age above 18 years.
- Stage I to V chronic kidney disease.

Exclusion criteria:

- Anemia due to non renal cause (other than iron deficiency).
- Evidence of active or occult bleeding.
- History of malignancy.
- History of end-stage liver disease
- Recent hospitalization or infection requiring antibiotics within the past 4 weeks.
- Blood transfusion within past 4 months
- Pregnancy.

All patients will be subjected to:

- Full medical history.
- Investigations.

I-Full medical history:

Full history taking including all demographic data (age and sex), co morbidity and drug history.

3.4 Investigations:

- Complete blood picture.
- Blood urea, serum creatinine and uric acid.
- Measurement of Albumin to creatinine ratio
- GFR was calculated using Modification of Diet in Renal Disease equation.
- Liver function tests: - serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase and serum Albumin.
- Serum iron, Total iron binding capacity, serum Ferritin and Transferrin Saturation.
- Serum calcium, serum phosphorous and serum PTH.
- CRP.
- Serum Hepcidin.

4. Statistical Analysis:

Data collected throughout history, basic clinical examination, laboratory investigations and outcome measures coded, entered and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) **(Statistical Package for the Social Sciences)** software for analysis. According to the type of data qualitative represent as number and percentage, quantitative continues group represent by mean ± SD, the following tests were used to test differences for significance; difference and association of qualitative variable by Chi square test (X^2). Differences between quantitative multiple groups by ANOVA followed by LSD, correlation by Pearson's correlation. P value was set at <0.05 for significant results & <0.001 for high significant result.

5. Results

The study group was subdivided into two groups:-

Group 1: including 60 patients with chronic kidney disease with anemia

This group was divided into two subgroups:-

Group 1A (pre hemodialysis patients): including 30 patients not on hemodialysis (pre HD group).

Group 1B (Hemodialysis patients): including 30 patients on regular hemodialysis (HD group).

Group 2: included 20 healthy volunteers as a control group**.**

The mean hemoglobin, serum iron, TIBC and transferrin were statistically lower in pre-hemodialysis (pre HD) and hemodialysis (HD) groups compared to control group (P value= 0.00**). However these parameters were statistically lower in HD group compared to control group and pre HD groups. Meanwhile serum ferritin was significantly higher in HD group compared to preHD and control groups, the serum ferritin is significantly higher in preHD than that of control group (tabl 1).

Table (1): Hemoglobin (HB) and Iron profile distribution among studied groups

	Control Group	Pre HD Group	HD Group	F	P
HB(g/dL)	12.56±0.76	10.28±0.92	8.66±0.95	112.239	0.00**
Iron (ug/dL)	83.45±5.28	52.1±14.03	35.9±3.15	160.837	0.00**
TIBC (ug/dL)	322.8±23.99	246.46±24.3	210.53±16.2	164.579	0.00**
Ferritin(ng/mL)	67.35±18.82	228.13±54.8	289.46±79.4	84.266	0.00**
Transferrin (%)	185.35±16.1	43.0±10.09	32.83±7.57	1317.829	0.00**

All 60 patients (100%) were anemic, mild anemia was (43.3%) in pre HD group and (23.3%) in HD group, moderate anemia was most common in both groups of patients (50.0%) of pre HD group (51.7%) in HD group. Although severe anemia was more common in HD group (6.7% in preHD and 23.3% in HD patients). The peripheral blood smear examinations showed that most frequent morphologic features were normocytic normochromic anemia was most frequent (86.7% in preHD group and 83.3% in HD group). While hypochromic-microcytic represented (13.3%) in both pre HD group and HD group and the macrocytic normochromic type was (3.3% in HD group) (table 2).

The mean serum calcium was significantly lower in pre HD group and HD group than that of the control group; meanwhile it was significantly decreased in HD group compared to preHD group. The mean serum phosphorous (PO4) and PTH were significantly higher in pre HD group and HD group than that of the control group, the highest level of these two parameters was observed in HD group (table 3).

Hepcidin was significantly positive correlated in both preHD group and HD group with Ferritin, CRP, SGPT, SGOT, ALP, UREA, Creatinin, Uric acid, proteinuria, Po4 and PTH but significantly negative correlated with HB, iron, TIBC, Transferrin, eGFR and Ca (tale 4).

Table (2) Degree of the anemia and its percentage among studied group.

Hb (g/dl)			Group		Total
			Pre HD	HD	
Sever	**< 8**	Count	2	7	9
		%	6.7%	23.3%	15.0%
Moderate	**8 - 10**	Count	15	16	31
		%	50.0%	53.3%	51.7%
Mild	**10 - 12.5**	Count	13	7	20
		%	43.3%	23.3%	33.3%
Total		Count	30	30	60
		%	100.0%	100.0%	100.0%

Table (3) Ca, Po4 and PTH distribution among studied groups.

	Control Group	Pre HD Group	HD Group	F	P
Ca (mg/dl)	9.68±0.16	8.68±0.84	7.29±0.66	82.001	0.00**
Po4 (mg/dl)	3.65±0.58	4.07±0.83	5.15±0.93	23.171	0.00**
PTH (pg/ml)	15.2±3.56	70.95±22.8	178.68±46.9	149.546	0.00**

Table (4) Correlation between hepcidin and other parameters.

		HEPCIDINE
Age	**r**	**0.048**
	P	**0.671**
BMI	**r**	**-0.062**
	P	**0.582**
Hb	**r**	**-0.463****
	P	**0.000**
iron	**r**	**-0.446****
	P	**0.000**
TIBC	**r**	**-0.495****
	P	**0.000**
Ferritin	**r**	**0.766****
	P	**0.000**
Transferrin	**r**	**-0.491****
	P	**0.000**
CRP	**r**	**0.478****
	P	**0.000**
SGPT	**r**	**0.295****
	P	**0.008**
SGOT	**r**	**0.274***
	P	**0.014**
ALP	**r**	**0.228***
	P	**0.042**

ALBUMIN	**r**	**-0.209**
	P	**0.063**
UREA	**r**	**0.493****
	P	**0.000**
Creatinin	**r**	**0.346****
	P	**0.002**
Uric acid	**r**	**0.411****
	P	**0.000**
eGFR	**r**	**-0.456****
	P	**0.000**
CKD_STAGES	**r**	**0.153**
	P	**0.244**
Proteinuria	**r**	**0.247***
	P	**0.027**
Ca	**r**	**-0.412****
	P	**0.000**
Po4	**r**	**0.295****
	P	**0.008**
PTH	**r**	**0.418****
	P	**0.000**

6. Discussion

In There was a statistically significant difference between the pre-hemodialysis (pre HD) and hemodialysis (HD) groups in the mean haemoglobin, serum iron, TIBC, and transferrin levels (P value = 0.00**).

However, compared to the control and pre-HD groups, these values were significantly lower in the HD group.

A substantial difference was found between the HD and preHD groups when comparing serum ferritin levels, and a similar difference was seen between preHD and control group serum ferritin levels.

According to Ali et al. (4), CKD patients and HD patients have considerably lower haemoglobin levels than healthy individuals (P less 0.001).

As previously reported (25), anaemic parameters in CKD patients before hemodialysis showed significant decreases in iron, TIBC and TS percent as well as significant increases in serum ferritin; post-hemodialysis, these anaemic parameters increased as well as the percentage of serum ferritin decreased significantly. However, functional iron insufficiency may develop when iron reserves are reduced due to loss or decreased intake, rather than a deficiency that occurs when iron stores are exhausted (8).

All 60 patients in this study were anaemic, with mild anaemia affecting 43.3% of patients in the pre-HD group and 23.3% of patients in the HD group; moderate anaemia affected the majority of patients in both groups, with 50.0% of those in the pre-HD group and 51.7% of those in the HD group being anaemic.

In the HD group, severe anaemia was more prevalent (6.7 percent in preHD and 23.3 percent in HD patients).

As George et al. (13), showed, the prevalence of CKD participants being anaemic increased with increasing severity of CKD.

Our findings are consistent with those of a prior investigation. It has been shown that people with an eGFR of less than 60 mL/min/1.73 m2 had a lower RBC count, haemoglobin concentration, and percentage haematocrit, regardless of their age or gender.

Mean Hb and Hct levels may drop in dialysis patients with a reduced GFR due to decreased erythropoietin and inflammation production, as well as a decreased ability to produce red blood cells (Raji et al., 23).

Normocytic normochromic anaemia was the most common kind of anaemia seen in peripheral blood smears in our investigation (86.7 percent in preHD group and 83.3 percent in HD group).

A macrocytic form of hypochromic-microcytic anaemia was seen in 13.3% of both the pre- and post-HD groups, however (3.3 percent in HD group).

Similar to the work of Suega et al., we found a substantial change between pre- and post-dialysis Hb and Hct levels in our patients (28).

The findings of Afshar et al., (1), who observed that the majority of pre and post HD patients had mild anaemia, severe anaemia was present in 15% of postdialysis patients and 5% of predialysis patients, respectively.

Our findings are consistent with those of previous investigations. The normocytic and normochromic form of anaemia was shown to be the most common morphological characteristic, followed by microcytic and hypochromic anaemia, in studies by Akinsola et al. (3), Afshar et al. (1), and Suega et al. (28)

The most prevalent cause of microcytic hypochromic anaemia is iron deficiency, which may be caused by either a reduction in iron intake or an increase in iron loss (Cappellini et al., 8).

When the GFR falls below 20-30 ml / min, it is common for a patient to develop normochromic normocytic anaemia. Reduced red blood cell lifespan, bleeding, and an insufficient rise in erythropoiesis

compared to Hb decline are all factors in this phenomenon. Reduced susceptibility to mechanical, osmotic, or oxidative stress, as well as extra corpuscular influences, may diminish red blood cell lifespan. Dialysis, diagnostic sampling, and occult gastrointestinal bleeding Banerjee et al. all cause blood loss (5).

The loss of water-soluble B12 and folate during hemodialysis causes macrocytic anaemia in CKD patients, particularly those on hemodialysis. Banerjee and others (5).

There is a negative connection between hepcidin and haemoglobin in both the pre-HD group and the HD group in our research.

Shinzato et al. (26), Rubab et al. (24), Ahmed and El-Maghraby (2) and Petrulien et al. (21) observed considerably greater hepcidin and lower Hb levels in hemodialysis patients. This considerable negative connection between hepcidin and haemoglobin level is constant.

On the other hand, contrary to our findings, those individuals with severe iron shortage (i.e., those with ferritin levels below 1 mg/dL) demonstrated a positive connection between serum hepcidin and haemoglobin levels.

There is a negative connection between serum iron and hepcidin in both the preHD and HD groups in this research.

Iron restriction in erythropoiesis and anaemia are caused by high hepcidin levels, which block intestinal iron absorption and macrophage iron recycling. These findings may help to better understand the importance of a new treatment for anaemia in hemodialysis patients that is currently being tested: hepcidin antagonists.

Hepcidin and serum iron were shown to have a substantial negative connection, in keeping with recent findings by Ahmed and El-Maghraby, (2) and Kadery et al., (15).

There was no link between serum hepcidin levels and iron levels, in contrast to our findings.

Compared to the control group, the mean serum calcium level was lower in the preHD and HD groups than it was in the latter, while it was lower in the former when compared to the latter.

Serum phosphorous (PO4) and PTH levels were considerably greater in the pre-HD and HD groups compared to those in the control group, with the HD group reporting the highest levels.

(7) Boronat et al., reported that pre-HD serum calcium levels were considerably lower than those in the HD group.

EPO synthesis, RBC survival, and myelofibrosis have all been linked to PTH, which has been seen as a uremic toxin that may impede EPO production (Tanaka et al., 29).

Hepcidin and serum calcium were shown to be negatively correlated in the current research in both the preHD and HD groups.

They discovered that there was no significant association between the amount of serum hepcidin and the total calcium in the bloodstream (21).

However, Ibrahim et al. (14) investigation demonstrated a substantial positive association between blood hepcidin level and serum total calcium, in contrast to our findings.

Treatment regimens such as Vitamin D, which raises serum calcium levels, calcimimetics, which lowers calcium levels, and agents that lower phosphorous levels could account for these discrepancies in results, as could the different types of hyperparathyroidism (the patient has secondary or tertiary hyperparathyroidism) (Calcium based or non-calcium based medications). Serum albumin and nutritional status of hemodialysis patients may be to blame for discrepancies between our research and other studies, since total calcium values should be interpreted using this information.

In the current research, hepcidin and serum phosphorous (Po4) had a favourable connection in both the preHD and HD groups.

Hepcidin and phosphorous clearance may be affected by decreasing renal clearance, which might explain this association.

Zaritsky et al. (32) found a connection between hepcidin and phosphorous levels in hemodialysis patients that was statistically significant.

According to Petrulien et al. (21) and Ibrahim et al. (14) research, there is no association between serum hepcidin and serum phosphorus in individuals with chronic kidney disease. This is in contrast to our data.

7. Conclusions

- Hepcidin play a major role in regulation of dietary iron absorption and cellular iron release.
- Hepcidin is primarily associated with iron stores and involved in regulating iron availability for erythropoiesis in Hemodialysis patients.
- If used as a diagnostic tool, it might help improving iron therapy if there is reticuloendothelial blockage of iron transport.
- Increased hepcidin across the spectrum of CKD may contribute to abnormal iron regulation and erythropoiesis and may be a novel biomarker of iron status.

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