Role of Hepcidin as a biomarker for iron status in patients on regular hemodialysis

A Thesis

Submitted for partial fulfillment of Master degree
in Internal Medicine

By

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2020

Acknowledgments

First and foremost, I feel always indebted to **Allah**, *the* ***Most Beneficent*** *and* ***Merciful*** *who gave me the strength to accomplish this work.*

My deepest gratitude to my supervisor, **Prof. Dr. Magdy Mohamed El Sharkawy,** *Professor of Internal Medicine and Nephrology, for his valuable guidance and expert supervision, in addition to his great deal of support and encouragement. I really have the honor to complete this work under his supervision.*

I would like to express my great and deep appreciation and thanks to **Prof. Dr. Mohamed Saeed Hassan,** *Lecturer of Internal Medicine and Nephrology, Faculty of Medicine, Ain Shams University, for his meticulous supervision, and his patience in reviewing and correcting this work.*

I must express my deepest thanks to my **Dr. Lina Essam Khedr,** *Lecturer of Internal Medicine and Nephrology, Faculty of Medicine, Ain Shams University for guiding me throughout this work and for granting me much of her time. I greatly appreciate her efforts.*

Special thanks to my **Parents,** *my* **Wife** *and all my* **Family** *members for their continuous encouragement, enduring me and standing by me.*

Marco Monir Hanna

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List of Abbreviations

Abbr.	Full-term
AI	**:** Anemia of Inflammation
CKD	**:** Chronic kidney disease
EPO	**:** Erythropoietin
ESA	**:** Erythropoietin stimulating agent
GFR	**:** Glomerular filtration rate
Hb	**:** Hemoglobin
Hct	**:** Hematocrite value
IDA	**:** Iron deficiency anemia
IL	**:** Interleukin
LEAP-1	**:** Liver-expressed antimicrobial peptide
LPS	**:** Lipopolysaccharide
MCV	**:** Mean corpuscular volume
NKG	**:** National kidney foundation
rhEPO	**:** Recombinant human erythropoietin
ROSs	**:** Reactive oxygen species
SD	**:** Standard deviation
SPSS	**:** Statistical package for social science
Tf	**:** Transferrin
TSAT	**:** Transferrin percentage saturation

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Abstract

Background: The etiology of anemia in End Stage Renal Disease is multifactorial. In addition to relative erythropoietin deficiency, shortened erythrocyte survival and the erythropoiesis inhibitory effects of accumulating uremic toxins also contribute to the anemia of CKD. Importantly, ESRD patients also have several abnormalities in systemic homeostasis of iron, an essential component in the production of red blood cells. **Aim of the Work:** to assess hepcidin level in regular hemodialysis patients with negative virology & its relation to iron status. **Patients and Methods:** This cross sectional study was conducted on 45 prevalent End Stage Kidney Stage patients. The patients on regular hemodialysis sessions three times weekly with low-flux synthetic membrane, bicarbonate dialysate & heparin as anticoagulant. In all patients the length of the dialysis session was set at 240 minutes, in Nasser Institute Dialysis Unit. **Results:** *******. **Conclusion:** Hepcidin plays 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. Hepcidin levels do not appear to help predict who will or will not have an improved Hemoglobin response to intravenous iron, since it doesn't perform better than the traditional markers of iron status (ferritin and TSAT). Increased hepcidin across the spectrum of CKD may contribute to abnormal iron regulation and erythropoiesis and may be a novel biomarker of iron status and erythropoietin resistance. Further studies including a large number of cases may be needed.

Key words: Hepcidin, biomarker, iron status, regular hemodialysis

Introduction

Anemia is an important and common problem associated with chronic kidney disease (CKD), which is caused by erythropoietin deficiency, iron-restricted erythropoiesis ***(Ueda & Takasawa, 2017).***

The gold standard for diagnosing iron deficiency anemia is assessment of iron stores in the bone marrow, an invasive and impractical method for routine testing The conventional indices used in current practice include serum concentrations of iron and ferritin and the transferrin percentage saturation (TSAT) ***(Bahrainwala & Berns, 2016).***

Adequate iron stores are essential for achieving maximum benefit from erythropoietic agents, such as recombinant human erythropoietin (rhEPO). Decreased iron stores or decreased availability of iron are the most common reasons for resistance to the effect of these agents ***(Cançado and Muñoz, 2011).***

Ferritin is a marker of tissue iron stores, whereas TSAT is a marker of iron available for erythropoiesis in the circulation. TSAT is calculated as follows: (serum ferritin/total iron binding capacity) × 100. Absolute or true IDA is present when the body iron stores are low and usually is represented by ferritin levels less than 100 ng/mL in predialysis and peritoneal

dialysis patients and by ferritin levels less than 200 ng/mL in hemodialysis patients and a TSAT less than 20% ***(Rocha et al., 2009).***

Hepcidin, an acute phase reactant protein produced in the liver, plays a major role in the anemia of inflammation and rhEPO resistance. Because of its renal elimination and regulation by inflammation, it is possible that progressive renal insufficiency leads to altered hepcidin metabolism, subsequently affecting enteric absorption of iron and the availability of iron stores ***(Ueda & Takasawa, 2017).***

Hepcidin level is significantly increased in patients with chronic renal failure on maintenance hemodialysis and that increased hepcidin seriously affects the prognosis of chronic renal failure ***(Zhang et al, 2014).***

Hepcidin levels are correlated to the glycemic status in CKD and HD patients and hepcidin levels in hemodialysed patients were significantly correlated with eGFR but it is not considered as an independent predictor for hepcidin level in these patients ***(Ali et al, 2014).***

Treatment with agents that lower serum hepcidin levels or inhibit its actions may be an effective strategy for restoring normal iron homeostasis and improving anemia in CKD patients ***(Tsuchiya & Nitta, 2013).***

The interaction of pro-inflammatory cytokines with hepcidin in the genesis of functional iron deficiency in CKD patients is an area of intense research. Several studies have shown elevated hepcidin levels in CKD, and it is now considered to be the critical link between inflammation and anemia in CKD patients ***(Jairam et al., 2010).***

Hepcidin is a polypeptide that regulates iron homeostasis and could serve as an indicator of functional iron deficiency in patients with end-stage renal disease (ESRD); this may also aid in the assessment of patient's response to erythropoietin (EPO) ***(Rubab et al., 2015).***

Aim of the Work

To assess hepcidin level in regular hemodialysis patients with negative virology & its relation to iron status.

Chapter (1)
Anemia of chronic kidney disease

Anemia of Chronic Kidney Disease

Anemia had been defined by the World Health Organization (WHO) as an Hemoglobin (Hb) concentration below 13.0 g/dl for adult males and post-menopausal women, and a Hb below 12.0 g/dl for premenopausal women ***(El-Achkar et al., 2005).*** Anemia generally appears in patients with Chronic Kidney Disease (CKD) once the glomerular filtration rate (GFR) declines to less than 60 ml/min ***(Schwartz et al., 2012).*** The prevalence of anemia was increased at less depressed levels of GFR in persons with diabetes compared to persons without diabetes. Both men and women with diabetes had increased prevalence of anemia; however, diabetes conferred greater odds of anemia in men than in women ***(Cappellini and Motta, 2015).***

Based upon these criteria, nearly 90 percent of patients with a GFR of less than 25 to 30 ml/min have anemia, many with Hb levels below 10g/dl ***(Kazmi et al., 2001).*** While decreased Hb often accompanies CKD, there is no quantitative definition of anemia in CKD ***(Gejyo et al., 2004)***; instead, anemia is defined according to physiological norms. All patients with CKD who have Hb levels lower than physiologic norms are considered anemia ***(Mikhail et al., 2017).***

Anemia is the most common hematological abnormality in CKD patients. In the past, blood transfusion was the essential method in the treatment of renal anemia, whereas the transfusion requirement has recently lessened by the use of Erythropoietin (EPO) ***(Goodnough and Schrier, 2014).*** Iron deficiency is frequent in patients with CKD and iron need is increased by EPO therapy; therefore, iron replacement is very important in the treatment of renal anemia. The relationship between CKD, EPO deficiency and anemia has been well established ***(Sonkar et al., 2018).***

(1) Etiology of anemia of CKD:

a- Factors that decrease erythropoiesis
b- Blood loss
c- Factors that decrease red cell survival
d- Hematological problems
e- Bleeding disorders

Erythropoietin deficiency

EPO is a hormone that is endogenously synthesized by the kidney. It is an essential stimulus for erythroid precursors in the bone marrow to sustain normal erythropoiesis ***(Geiszt et al., 2001).*** Development of anemia is mainly due to decrement in the levels of EPO reflecting a reduced number of EPO producing cells. With any degree of anemia, EPO

levels are low compared to a patient with anemia and normal GFR ***(Kaplan et al., 2018).***

Iron deficiency in dialysis patients

There are three important mechanisms that have been proposed to explain the high frequency of iron deficiency in dialysis patients; include abnormal iron absorption, external blood loss, and functional iron deficiency ***(Motonishi et al., 2018).***

a- Iron absorption in dialysis patient:

Uremic patients are anorexic and malnourished; the ability of the gut to absorb iron may be decreased due to uremic enteritis or increased gastric PH with frequent use of H_2 blockers ***(Zhang et al., 2014).*** High ferritin level, which can occur in dialysis patients despite low iron stores, may also impair the normal feedback that would increase absorption during deficiency states ***(Dignass et al., 2018).***

b-External blood loss:

There are several factors that contribute to ongoing blood loss in dialysis patients, including blood retained in the dialyzer and blood tubing at the end of each dialysis treatment, frequent blood testing and occult gastrointestinal tract (GIT) blood loss ***(Saha and Allon, 2017).***

C-Functional iron deficiency:

Functional iron deficiency is present when the usual tests for iron deficiency in dialysis patients do not indicated absolute iron deficiency (Ferritin of more than 100 ng/ ml, transferrin saturation (TSAT) more than 25 %), but patients respond to additional iron administration with rise in hematocrit (Hct) at a stable EPO dose or maintain a stable Hct with a lower EPO dose ***(Aoun et al., 2018).***

Hyperparathyroidism:

Indeed, hyperparathyroidism is usually listed among the possible reasons for impaired response to recombinant human erythropoietin (r-HuEPO) in patient with renal disease ***(Working Party for European Best Practices, 2004; Alves et al., 2015).***

In the presence of excess amounts of PTH as in primary hyperparathyroidism, PTH decreases the number of erythropoietin receptors on the erythroid progenitors, making these cells insensitive to EPO. This anemic effect of PTH is especially pronounced if this state is combined with inadequate amounts of erythropoietin as in secondary hyperparathyroidism ***(Chutia et al., 2013).***

It has also been shown that PTH increases the osmotic fragility of the peripheral red blood cells by increasing the influx of calcium into the cells. This hemolytic effect of PTH