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Hepcidin And C – Reactive Protein Levels Among Pateints With End Stage Renal Disease On Regular Hemodialysis At Abass Ibrahim Dialysis Unit, White Nile State – Sudan

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Abstract

Review: The incidence curve of end stage renal disease is in dramatic exaggeration. On the same side in spite of intensive efforts to improve dialysis care events, anemia remains a life threatening problem among hemodialysis patients. Improper follow up and management of patients on dialysis is the main cause of iron deficiency anemia associated with significant morbidity, and increased risk of mortality.

Objectives: This study aimed to evaluate serum levels of Hemoglobin hepcidin and CRP among end stage renal disease patients.

Methods: This study is a cross- sectional study conducted in Kosti city , White Nile State, Sudan, during a period of Jan to April 2019. 104 subjects were selected of them 52 diagnosed as ESRD patients while the other closely matched were represent ted the reference group. The gender among the study groups were 40 males and 12 are female all of them older than 17 years. Hemoglobin, reactive protein and hepcidin were estimated.

Result: The estimated levels of the parameters under investigation when compared with reference value revealed significance increase. There is no correlation between parameters on side and weight, age and sex on other side. Hepcidin and C- reactive protein were significant increased in patients compared to control group.

Conclusion: Serum hepcidin and C- reactive protein were increased reflecting active inflammatory process and iron status abnormality among patients with end stage renal disease on regular hemodialysis this may be attributed to weak points in follow up program regarding nutrition and periodical screening of inflammation screening among those patients.

Key word: ESRD: Hepcidin, C – reactive protein, Dialysis

Introduction

Iron deficiency anemia is commonly associated with all stages of renal disease but is much more seen in patients with stage 5 (end-stage renal disease - ESRD) [1]. Anemia due to iron deficiency in chronic kidney disease (CKD) patients are at increased needs of monitoring such as of management and hospitalization, Unfortunately increased length of hospital stay, reduced quality of life and higher mortality.[2-1] As approved the main causes of anemia in patients with CKD are decreased erythropoietin (EPO) production, chronic inflammation, shortened half-life of erythrocytes and iron deficiency.[3-2]

Alteration in status of biological iron in our body coupled with various abnormalities and diseases. Renal diseases approved by multiple studies attributed to the disturbance that involve the metabolism of iron in those patient. Consequently, iron deficiency anaemia with normal red blood cell size and hemoglobin content (normocytic & normochromic) occurs throughout the late

stages of chronic renal disease and is almost the main cardinal feature in stage 5 disease. Regarding this stage (stage 5) lack of erythropoietin, the hormone that responsible for the synthesis of red blood cells through the process known as erythropoisis is the stone of corner in the pathophysiology of anaemia. That why anemia is one of the common complication that seen to be associated with chronic kidney diseases and this is mainly due to progressive loss and damage of kidney tissue. [1-3]

Defect in the distribution of biological iron is the main player in the occurrences of iron deficiency anemia in chronic kidney disease patients. The level of iron in circulation is depends depend on the that amount fraction taken via the intestinal mucosa in addition to that fraction released from the of storage iron. Hence any interruption at these two levels affect the amount of the total iron on the circulation. [4,5,6] Multiples proteins sharing in monitoring iron status in human body one of them an intracellular iron storage protein known as ferritin. Estimation of ferritin reflects the level of stored iron in the body. [7, 8] Ferritin is one of acute phase protein that associated with chronic inflammation as well as iron overloading, liver disease, haemoglobinopathies and in malignancies.

The low level of serum ferritin indicate iron deficiency anaemia. [9] Another protein synthesized and secreted by liver called transferrin deal with transportation of dietary iron the fraction that absorbed by mainly at duodenum and upper jejunum of the gastrointestinal tract. With the occernce of anemia in chronic inflammation associated with end stage renal disease, hepcidin is an important biomarker reflecting the impaired in iron metabolism in those pasteints. [11 -12] Unfortunately few repots and studies touching the implications of hepcidin and its association iron deficiency anemia in end stage renal disease. [13]

The biological effect of hepcidin reflected by inhibiting the release of iron from macrophages and the absorption of dietary iron from the intestine. The mechanism involve internalization and degradation of the cellular iron exporter ferroportin, which is highly expressed in macrophages and duodenal enterocytes. [13] The net effect of hepcidin is to increase intracellular iron stores, decrease dietary iron absorption and decrease circulating iron concentrations. Dysregulation of iron homeostasis represents another key-player in ACD; levels of iron in the circulation

are decreased as intestinal iron-absorption is reduced and the release of storage iron is inhibited .[14] The hormone hepcidin, with its active isoform hepcidin-25, seems to be the main regulator of iron homeostasis in this setting [15] and is itself regulated by inflammatory processes[16] . While anemia and chronic inflammation are frequently detected in CKD, hepcidin is an important biomarker, determining impaired iron metabolism in ACD [17]. However, evidence on the prognostic implications of hepcidin is sparse, as only few reports described its association with clinical outcome [18], and importantly, to our knowledge, not by considering endogenous EPO levels simultaneously.

Various physiologic and pathologic processes regulate the synthesis of hepcidin. An augmented demand for circulating iron due to iron deficiency, hypoxia[19], anaemia [19], conditions characterized by ineffective erythropoiesis or the use of erythropoietin leads to a decrease in hepcidin synthesis. Inflammation and infection are the corner stone that increasing the synthesis of hepcidin [19]. This is believed lead to the anaemia of chronic disease that is characterized by a decrease in circulating iron available for erythropoiesis, despite apparently normal iron stores. These latter features are similar to those observed in patients with impaired renal function. The diagnosis of iron deficiency using tradional parmeters such as hemoglobin, ferritin transferrin total iron binding capacity is unproductive, as it can be affected by many confounding factors such as age, sex, inflammation and nutritional factors. Several pervious studies concluded that determining hepcidin concentrations together with other protein associated with iron metabolism improved the identification of patients with iron deficiency by 26.1%. [20]

Materials and Methods

Prior to go on through the study, permission was obtained from the Ethical research Committee, University of Elimmam Elmahdi, Sudan. It was a case control, comparative study and was conducted at the Abass Ibrahim dialysis center in Kosti city, White Nile, Sudan between Jun and March 2019. The study involved 104 subjects, both male and female. The selected subjects were divided into two groups. Cases group included 52 patients subdivided farther into group I represented the by 40 males and group II include 12 females both of them were

well known as end stage renal disease (ESRD) patients on maintenance hemodialysis . Group III comprised of 52 healthy subjects selected from the community they were age- and sex that closely matched with the patients groups. The age range among the study groups was 17 - 60 for group I (males), 19 – 55 for group II (females) and that for the control group III was 18–62

A consecutive sampling technique was used. Five milliliters of venous blood was drawn under aseptic conditions. Three milliliters was transferred to gel vaccutainers for the measurement of serum hepcidin and CRP levels. The remaining 2 mL was transferred to the EDTA vaccutainer for the determination of Hb . The CRP levels were measured quantitatively by immunoturbidimetric test on an Olympus (Germany) analyzer. The hepcidin level was measured by a Biorad-680 Microplate reader, Germany using a Human Hepcidin ELISA kit from Creative Diagnostics Company. India.

Statistical Analysis

The data were entered and analyzed using PASW 18.0. Mean ± SD were calculated for quantitative variables (Hb, serum hepcidin, CRP). Two independent sample T tests were applied to observe mean difference between the two groups (controls and ESRD patients). Pearson correlation correlation was applied to observe correlations between hepcidin and conventional markers of iron status and inflammation. A P-value of ≤0.05 was considered to be statistically significant.

Results

One hundred and four subjects were recruited in this study. Fifty two of them were patients with ESRD on regular hemodialysis at Abass Ibrahim center for renal dialysis and the other fifty two were healthy subjects selected from kosti community included as the control group 76.9% were males and 23.1% were females in both the patient and the control groups. The mean age in group I was 48.3 ± 7.2 while that of group II and III were 43.8 ± 8.4 and 42± 6.2 respectively. . Demographic details of the participants are summarized in Table 1. Hemoglobin, serum hepocidin and C- reactive protein were evaluated for the clinical assessment of anemia and inflammation. The results of estimated parameters were expressed in mean± standard deviation shown in Table 2.

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

Parameters	GroupI	Group II	Group II
Number of subjects	40	12	52
Age (years; mean ± SD)	48.3 ± 7.2	43.8 ± 8.4	42± 6.2
Range of age (years)	17–60	19 - 55	18–62

Table 2. Level of hemoglobin, Serum hepcidin and markers of inflammation in both groups.

Parameters	Group I mean±SD	Group II mean±SD	Group II mean±SD	P-value
Hb (g/dL)	9.8 ± 1.5 4	9.2 ± 1.5 4	13.7 ± 1.7	<0.001**
CRP (mg/L)	13.8 ± 19.7	14.3 ± 23.2	2.1 ± 3.1	<0.001**
Hepcidin (ng/mL)	16.7 ± 4.3	17.2 ± 2.7	9.4 ± 3.6	<0.001**
Range of age (years)	48.3 ± 7.2	43.8 ± 8.4	42± 6.2	>0.05^

Mean, standard deviation (SD) & P value

P >0.05 (Non Significant) = ^ P <0.05 (Significant) = * P <0.01 (Highly Significant) = **

Group I : Male patients with chronic renal failure .

Group II : Female patients with chronic renal failure. Group III: Control group.

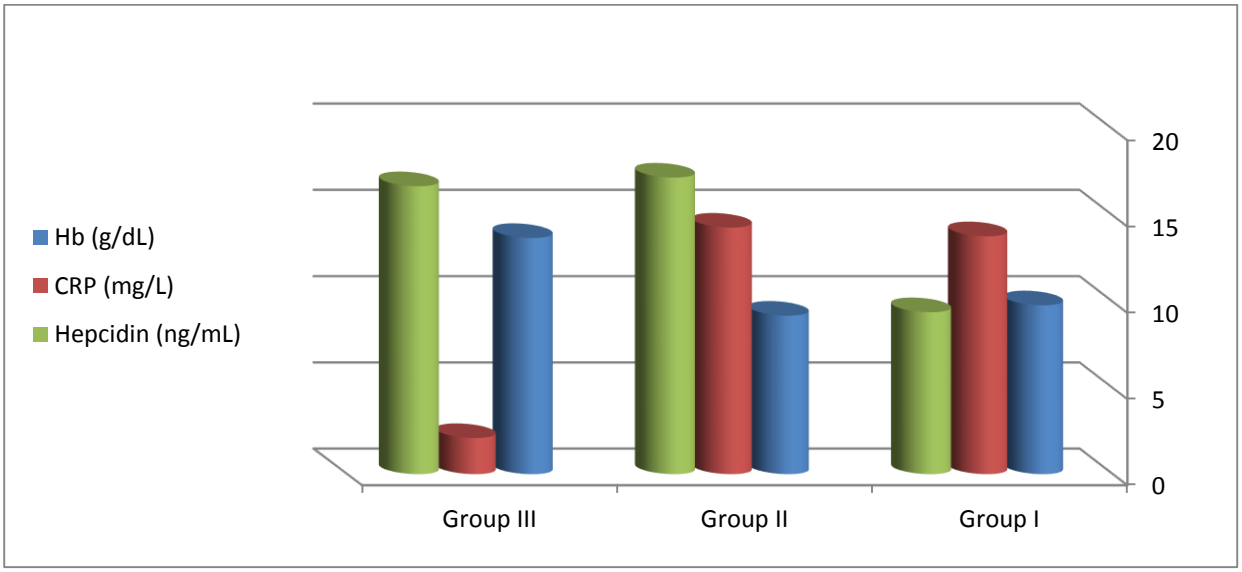

Figure 1. Relationship between serum hepcidin and CRP among study groups

Mean, standard deviation (SD) & P value

Group I : Male patients with chronic renal failure .

Group II : Female patients with chronic renal failure.

Group III: Control group.

Discussion

Hepcidin levels are affected by iron homeostasis and rate of red blood cells production.14 Many studies approved that the level of hepcidin seemed to be reduced by anemia and shortage of oxygen supply while the level increased by inflammation. 15 Renal anemia is considered a special form of anemia of inflammation. [16] The current study focused on estimation of the serum hepcidin level among patients with ESRD on maintenance Hemodialysis (HD and their estimated levels were then compared with controls.

The level of serum hepcidin was determined by using enzyme-linked immunosorbent assay (ELISA) method and found that the levels were significantly increased among end stage renal disease patients on maintenance hemodialysis (HD) as compared with healthy controls. Comparable results were also approved by other researcher dealing with the same parameters in patients with chronic kidney disease. [12] Moreover our finding revealed that hepcidin levels were approximately twice time higher in patients with end stage renal disease than in the control healthy group.13 the elevation in Hepcidin levels among those patients are attributed mostly to inflammation caused by tissue iron overload as aconsquence of defect in the process involve hepcidin excretion in urine. [17]

Moreover the estimated level of C – reactive protein (CRP), one of the famous a cut phase protein that taken in current study as conventional marker of inflammation reflected higher levels in patients with end stage renal disease than controls. This point may explain the elevation of hepicidin in diseased groups beside that and the findings showed significantly correlated between CRP and serum hepcidin. This correlation between hepcidin and C- reactive protein is explained by the fact that hepcidin synthesis is trigger by inflammation, a process that is mediated by IL-6. Since chronic kidney disease is considered an inflammatory state, this positive correlation was expected to be found . [8] findings in our results are comparable to that observation in other studies on patients with renal failure, which showed strong relation between hepcidin levels and C – reactive protein (CRP).10 However, there are other studies in chronic kidney disease (CKD) patients where no correlation was seen between the level of serum

hepcidin and acute phase C –reactive protein (CRP levels). [8,17] The absence of this correlation between the two proteins may be attributed to the basis of differences in the half-lives of CRP and hepcidin. [17]

Conclusion

The levels Serum hepcidin found to be increased in patients with end stage renal disease ESRD patients on regular hemodialysis (HD) and, hence, may be used in the evaluation of anemia in such patients. The level of the protein gives clear information about the level and availability and distribution of iron during inflammation as compared with traditional markers of iron status.

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