



Serum Hepcidin as an Inflammatory Marker in Chronic Kidney Disease

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Hepcidin is known to be the central regulator of iron homeostasis in the body. It is up-regulated by inflammation and downregulated by anemia. CKD is a state of chronic inflammation seen in kidney. Previous work has shown that serum hepcidin levels were increased in patients with CKD. This was surprising as these patients had a chronic inflammatory state and co-existent anemia.

Aim and Objectives: The aim of the study is to estimate the levels of hepcidin in CKD patients and to check the correlation of hepcidin to inflammation in chronic kidney disease.

Methods: This cross-sectional study was conducted at the Department of Biochemistry, Central Laboratory, Sree Balaji Medical College and Hospital, Chromepet, Chennai during January 2017 - June 2018 among 50 patients of chronic kidney disease in the age group of 18-60 years. The blood samples were collected using vacutainer system. Samples for serum hepcidin, ferritin and hsCRP were collected in red topped plain vacuum tube. The samples were centrifuged at 3000 rpm for 15 minutes. The samples were then processed, and values were obtained. The data were analysed using SPSS package.

Results: The mean values of s. Hepcidin, s. ferritin and hsCRP levels were found to be increased

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in the study population. The mean value of s. hepcidin was found to have strong positive correlation with the mean values of s. ferritin and hsCRP with r-value > 0.7.

Conclusion: Hpcidin levels are elevated in CKD and hepcidin is a predictor of inflammation since it correlated well with the inflammatory markers hsCRP and ferritin levels.

Keywords: Chronic Kidney Disease (CKD); hepcidin.

1. INTRODUCTION

Chronic kidney disease (CKD) is associated with an irreversible progressive deterioration in renal function. CKD has become a worldwide, chronic, non-communicable disease epidemic with adverse outcomes of renal failure, cardiovascular disease and premature death. In developed countries, it affects 10-15% of adult general population.

The discovery of hepcidin and its functions has led to a better understanding of iron metabolism disorders in CKD. Hpcidin, a small cysteine rich liver-derived peptide hormone, is a key regulator of systemic iron homeostasis. Hpcidin has evolved as a probable mediator of anemia of inflammation.

Increased serum hepcidin levels may contribute to the development and severity of anemia and the resistance to erythropoiesis-stimulating agents. The metabolism of hepcidin is profoundly altered in chronic kidney disease (CKD). What little data are available in this area are inconclusive. Markers of inflammation and iron status were positively associated with serum hepcidin level, regardless of CKD stage. However, glomerular filtration rate was inversely associated with serum hepcidin level, particularly in patients with CKD stages 3b–5 but not in those with CKD stages 1–3a. Hence in the present study, the serum level of hepcidin was estimated in patients with CKD and the relationship between serum hepcidin and inflammation and were analysed.

1.1 Aim

The aim of the present study was to estimate the levels of serum hepcidin in patients with chronic kidney disease and to check the correlation of hepcidin to inflammation in chronic kidney disease.

The objectives of the present study were to:

- Estimate serum hepcidin levels in patients with chronic kidney disease.

- Determine whether hepcidin levels correlated to inflammatory marker in chronic kidney disease.

2. MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Biochemistry, Sree Balaji Medical College and Hospital, Chromepet, Chennai during the period of January 2017 – June 2018 among 50 patients of chronic kidney disease visiting the Nephrology outpatient services in the age group of 18-60 years. Age, gender, duration of chronic kidney disease, general history and medications and blood pressure were recorded. The study was explained to the participants and informed consent was obtained from them before taking the blood sample. After obtaining informed consent, 5 ml of blood was collected from each participant, from a peripheral vein under aseptic precautions in specific vacutainers. Plain tube for hepcidin, ferritin and hsCRP were used. Blood samples collected were used for the estimation of serum levels of hepcidin, ferritin and hsCRP. Transferrin saturation value was obtained by calculation. Blood collected in the plain tube was allowed to clot, then each tube was centrifuged for 10 minutes at 3500 rpm within 2 hours of blood collection to separate serum. Serum sample was used for the estimation of all parameters. Hpcidin was estimated by competitive ELISA kit (Enzyme Linked Immuno Sorbent Assay). Estimation of serum ferritin and hsCRP were done by chemiluminescence assay in Siemens, ADVIA Centaur CP Immunoassay system. Data was categorised based on demographics (Age and Gender).

All the results obtained were statistically analyzed using SPSS software version 16.0. Shapiro-Wilk test was used to test for normality of the data. Mean and standard deviation were used to represent normally distributed data. Median and interquartile ranges were used to represent data which were not normally distributed. Bivariate correlation analyses were done using Pearson correlation to correlate hepcidin with other parameters. The results of

statistical analysis were arranged in tabular form and were plotted in graphs.

2.1 Inclusion Criteria

Known cases of chronic kidney disease on conservative management in the age group 18-60 years.

2.2 Exclusion Criteria

- Age group less than 18 years and greater than 60 years
- Pregnancy
- Any malignancy
- Pre-existing liver disease
- Active inflammatory disease

3. RESULTS

Kruskal-Wallis Test was used to test the statistical significance of hepcidin and other parameters among the study group. The mean values of s. Hepcidin, s. ferritin and hsCRP levels were found to be increased in the study population.

The mean value of s. hepcidin was found to have strong positive correlation with the mean values of s. ferritin and hsCRP with r-value > 0.7.

4. DISCUSSION

Anemia is a significant co-morbidity in patients with CKD. Iron deficiency and inflammation are the most common causes of anemia in CKD. However, despite the reported high and alarming prevalence of anemia in CKD resulting in significant co-morbidity, anemia is often untreated or treated improperly in clinical practice. The reason for this is mainly in the difficulty involved in determining the etiology of anemia in this condition which is vital in providing the right treatment. Hepcidin is the master regulator of systemic iron homeostasis [1] and plays a key role in anemia associated with inflammation. Physiologically, hepcidin inhibition occurs in cases of anemia, iron deficiency or hypoxia. In inflammatory conditions, levels of pro-inflammatory cytokines are increased [2]. It is plausible that increased hepcidin concentrations

may cause iron-restricted erythropoiesis in CKD-associated anemia.

In the present study, a total of 50 CKD subjects were studied. Hepcidin levels were significantly higher in patients with CKD than in control subjects. Serum ferritin and hsCRP were found to be elevated in CKD cases.

In the present study, serum hepcidin concentrations were found to be significantly increased in patients with CKD [Mean value: 149.484 ± 47.539 ng/mL]. Serum hepcidin levels were found to be increasing with the progression of CKD. This finding is supported further by the highly significant positive correlation observed between serum hepcidin and creatinine in CKD cases (r= 0.8437, p<0.001). These findings are in accordance with the study of Tarek et al. [3], which reported an increase in serum hepcidin levels in all stages of CKD among 54 CKD patients under conservative management and 40 CKD patients under hemodialysis.

Hepcidin is cleared from the body by the kidneys. The increase in hepcidin seen in CKD is due to its reduced renal clearance associated with the deteriorating renal function. Depending on the damage of kidney, the elimination of hepcidin is limited and leads to the increase in its serum level. Another reason could be the chronic inflammatory state of CKD which stimulates hepcidin production. Uremia is a state of heightened inflammatory activation. Hepcidin synthesis is induced in the liver as a response to IL-6 stimulation and it expresses its activity by decreasing the absorption of dietary iron and prevents iron release from macrophages. Elevated serum hepcidin levels mediate iron-restricted erythropoiesis and contribute to inducing anemia in CKD patients. Short-term increases in serum hepcidin levels impair the release of storage iron, and long-term increases in serum hepcidin levels result in iron deficiency. High serum hepcidin levels cause iron blockade and anemia in chronic disease. Chronic kidney disease patients with anemia have been found to have elevated serum hepcidin levels, and the high hepcidin levels are likely to contribute to anemia in CKD and to ESA hypo-responsiveness.

Table 1. Descriptive statistics of the study group

Parameters	N	Mean	STD. deviation	Mean rank
S. Hepcidin (ng/ml)	50	149.484400	47.5399263	75.50
S. Ferritin (mg/dl)	50	187.757400	93.1905705	75.22
hsCRP (ng/ml)	50	5.251400	2.5022708	75.22

Table 2. Pearson’s correlation between hepcidin and other parameters among CKD cases

Variables		S. hepcidin
S. ferritin	r value	0.907
	p value	0.000
S. hsCRP	r value	0.942
	p value	0.000

** Correlation is significant at the 0.01 level (2-tailed)

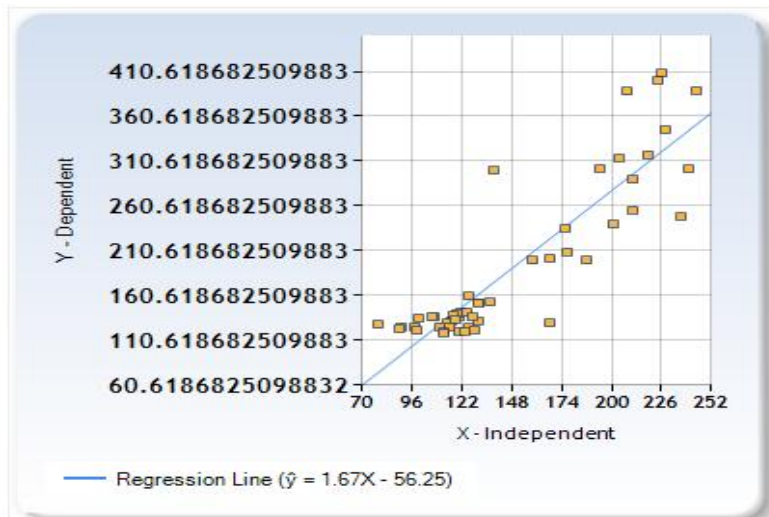


Fig. 1. Scatter plot with linear regression of hepcidin vs ferritin

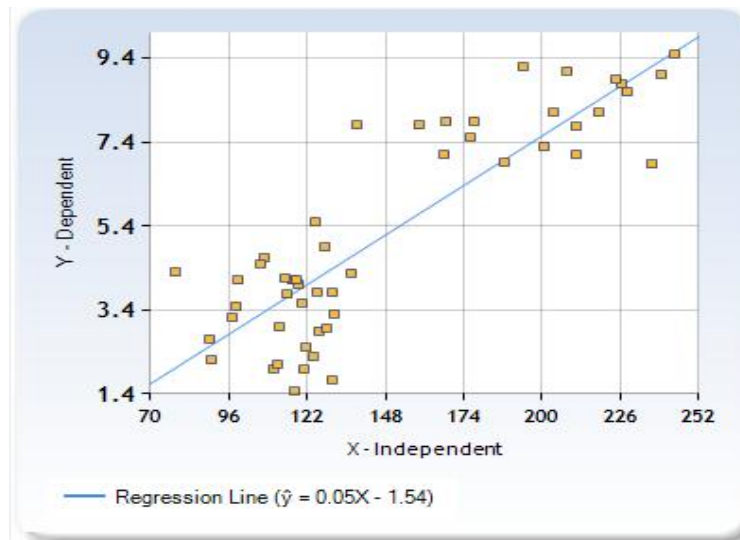


Fig. 2. Scatter plot with linear regression of hepcidin vs hsCRP

Ferritin is considered as marker of iron status. Though ferritin is a marker of body iron stores, it also increases in acute inflammation and therefore becomes less valuable as an indicator of iron status during inflammation seen in CKD. In this study, hepcidin and ferritin were

significantly higher in chronic kidney disease patients compared to control subjects. The mean value of ferritin was 187.757 ± 93.19 mg/dl. Also, Malyszko et al. [4] found that in his study on patients with chronic renal failure and hemodialyzed patients, that serum ferritin and

hepcidin were significantly higher than in the healthy volunteers. There was a strong positive correlation between ferritin and hepcidin among the CKD cases ($r = 0.907$, $p < 0.001$) which is in accordance with the study of Mercadal et al. [5]. He observed a positive correlation between hepcidin and ferritin in his study among 199 non-dialysed non-transplanted patients with CKD stages 1-5.

The chronic inflammatory state of CKD is due to the chronic imbalance between prooxidant and antioxidant factors. Oxidative stress induces insulin resistance by decreasing internalization of insulin [6] and increased ferritin synthesis. Functional iron deficiency is present in some patients with chronic renal failure, on conservative treatment and on dialysis, which is characterized by the presence of adequate iron stores with serum ferritin level either normal or elevated. In addition to this, inflammatory iron block occurs among these patients largely due to an underlying inflammatory state. The inflammatory block along with functional iron deficiency cause elevated ferritin level [4]. Hepcidin level of the two study groups in the present study was related to ferritin that agrees with the results of Peters et al. [7], who revealed that serum ferritin concentration was a significant predictor of hepcidin-25 levels in CKD by means of multiple regression analysis. Similar correlation between ferritin and hepcidin was reported by Dallalio et al. [8], in anemic patients undergoing diagnostic bone marrow examination. However, Malyszko et al. [4] could not find a significant correlation between hepcidin and ferritin in his study population of patients with chronic renal failure on conservative treatment and on hemodialysis. Reduced levels of serum ferritin or transferrin saturation (TSAT) are present in most patients with CKD.

CKD is a state of chronic persistent low-grade inflammation with persistent elevation of pro-inflammatory markers. The prototype marker of inflammation in the clinical setting is hsCRP, a positive acute phase reactant and higher level of this inflammatory biomarker is associated with cardiovascular mortality in patients with renal insufficiency. The elevated levels serum hepcidin in patients with CKD indicates an underlying inflammatory state associated with advanced renal failure, with loss of renal function, and development of anemia of chronic disease, the thing that was obvious in the present study in the form of high hsCRP in chronic kidney disease patients when compared to control [Mean level:

Cases - 5.25 ± 2.50 ng/mL; $p < 0.001$]. This is similar to the finding of Carmen et al. [9]. He observed a positive correlation between hsCRP and hepcidin in anemic CKD patients. However, he could not establish any relation between hepcidin and hsCRP among non-anemic CKD patients. As the renal function declined, there was a progressive increase in the hsCRP levels. Further a strong positive correlation was found between hepcidin and hsCRP levels ($r=0.942$; $p < 0.001$) which shows that hepcidin is a positive acute phase reactant. Similar finding of positive correlation between hsCRP and hepcidin was observed by Karthik et al. [10] and Tarek et al. [3] in their studies as well.

5. CONCLUSION

The present study suggests that hepcidin levels are elevated in CKD and hepcidin is a predictor of inflammation since it correlated well with the inflammatory markers hsCRP and ferritin levels. This increase in hepcidin levels reflects both the renal impairment leading to reduced renal clearance of hepcidin and the state of chronic inflammation. These findings highlight the close relationship between inflammation and hepcidin in CKD.

CONSENT

As per international standard or university standard written participant consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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