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Analysis of Hepcidin, Ferritin, CRP and Iron Levels in ESRD Patients and Their Correlation in CKD-4 & 5 Stages with/without Iron Intake

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Abstract

Inflammation interferes with iron utilization in chronic kidney disease through hepcidin. In our study, iron levels, ferritin, CRP and hepcidin levels were analyzed in newly diagnosed end-stage renal disease (ESRD) patients. A total of 50 ESRD patients and 5 healthy controls were studied. 40 recently detected ESRD patients on hemodialysis and 10 patients with Stage 4 CKD not received HD or parenteral iron, 22 out of 40 ESRD patients had already received prior parenteral iron or blood products. The ESRD patients had a significantly lower estimated albumin; and higher transferrin saturation (TSAT) and raised serum ferritin and Hepcidin levels. Hepcidin levels correlated significantly with Ferritin levels. Whereas ferritin levels correlated significantly with CRP levels. There have been elevated serum hepcidin levels in ESRD patients more in those receiving Iron therapy. High hepcidin levels could explain the functional iron deficiency. Larger randomized multicenter studies could throw more light on the diagnostic and therapeutic potentials of using Hepcidin-25 levels in regular practice.

Keywords: Chronic Kidney Disease; Hepcidin; CRP; Hemodialysis.

Introduction

The burden of chronic kidney disease (CKD) is increasing all over the world including in India. The best-known adverse consequence of CKD is end-stage kidney disease (ESRD). The incidence of ESRD in India has been estimated at 165–225 per million population [1].

The anemia that accompanies chronic renal disease (CKD) is associated with precocious mortality and morbidity rates, as well as with a decrease in life quality of patients. The chief etiology of anemia in CKD is erythropoietin (Epo) deficiency. Despite the widespread Epo use, over 50% of the patients do not reach the target hemoglobin levels[1,2]. The most common reason for poor response to Epo therapy is iron deficiency[1]. Inflammation has been implicated as another important cause of poor response[3]. C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) are acute phase reactants that have been used to reliably assess the degree of inflammatory activation [4]. Hepcidin, a regulator of body iron stores, has been identified to play a critical role in the pathogenesis of anemia of chronic disease.

Ferritin, other than being a marker of body iron stores, also increases in acute inflammation, and becomes less valuable as an indicator of iron status during inflammation [5]. However, studies have suggested that parenteral iron therapy might itself contribute to morbidity and mortality by inducing a pro-inflammatory state, due to increased oxidative

stress [6]. Additionally, assessment of iron status itself may be rendered difficult on account of inflammatory activation. A vast majority of Indians are vegetarians, and anemia due to iron deficiency is very common in the general population [7]. Hence, in our study, we analyze the body iron status, levels of CRP and hepcidin levels in ESRD population.

Materials and Methods

A non randomized cross sectional observational study was conducted at Narayana Medical College in the Department of Nephrology from January 2014 to January 2016. Study approved by Institutional ethics committee, a written informed consent was taken from all patients. All patients fulfilling inclusion criteria were screened and investigation done. Recently diagnosed ESRD patients on dialysis and CKD stage 4 of either sex were included. Healthy adult individuals were recruited as controls. The exclusion criteria were: The exclusion criteria were: age less than 18 years, evidence of acute infection or trauma in the last four weeks, history of parenteral iron injection in the last 14 days, history of blood transfusion in the last one month, hemoglobinopathies, malignancy, recent overt blood loss, and post-transplant status. 50 CKD patients including 10 patients with stage 4 CKD and 40 patients with ESRD who had been recently initiated on dialysis (< 3 months) and 5 healthy volunteers as controls.

All patients underwent a thorough physical examination, nutritional status and anthropometrical data, Skin fold thickness, mid arm muscle circumference (MAMC), Body fat percentage and Body mass index.

For dialysis patients, the modality and schedule of dialysis were also recorded. Hemogram, serum iron, total iron binding capacity (TIBC), serum ferritin, percentage transferrin saturation (TSAT), and quantitative CRP levels were analyzed. Anemia patients were stopped to oral iron for a week before sampling.

Serum Iron: Serum iron was measured as recommended by international committee for standardization. Protein was precipitated and chromogen was added to supernatant followed by measurement of absorbance

TIBC: Excess iron was added to sample as ferric chloride, excess unbound iron was removed with magnesium carbonate. The iron concentration was measured.

Serum Ferritin: Ferritin was estimated by an immunometric enzyme immunoassay.

Serum CRP: It was estimated using quantitative CPR assay kit, principle was based on immune precipitation in a liquid phase.

Serum Hepcidin: hepcidin-25 was estimated using the DRG® Hepcidin 25 bioactive ELISA (EIA-5258) hormone enzyme immune assay kit. The DRG Hepcidin-25 ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.

The micro titer wells are coated with a monoclonal (mouse) antibody directed towards an antigenic site of the Hepcidin-25 molecule. Endogenous Hepcidin-25 of a sample competes with a Hepcidin-25-biotin conjugate for binding to the coated antibody. After incubation, the unbound conjugate is washed off and a streptavidin-peroxidase enzyme complex is added to each well. After incubation, unbound enzyme complex is washed off and substrate solution is added. The blue colour development is stopped after a short incubation time, turning the colour from blue to yellow. The intensity of colour developed is reverse proportional to the concentration of Hepcidin in the sample.

Statistical Analysis

Data were presented as mean ± S.E. ANOVA test was used to test the mean difference between three groups. Pearson correlation test was used to test the correlation between the variables. All the p value of less than 0.05 was considered as statistically significant.

Results

The study includes 55 individuals, 40 recently detected ESRD patients on hemodialysis and 10 patients with Stage 4 CKD not received HD or parenteral iron, 22 out of 40 ESRD patients had already received prior parenteral iron or blood products and 5 healthy control subjects. Males 39 and Females 16 were recorded. The mean age of patients in the study group was 48.76 ± 13.983 years. The minimum age of our individual are 20 years and maximum age was 74 years. The CKD-5 and CKD-4 patients had higher TSAT, CRP, Hepcidin and markedly raised serum ferritin levels (Table 1).

The hepcidin level, Ferritin level, CRP level, Transferrin Saturation levels were observed to be higher in CKD-5 group with Iron than CKD-5, CKD-4 without Iron . Hepcidin levels correlated

significantly with Ferritin levels [rho=0.589, p< 0.0001). Hepcidin levels correlated with Serum CRP [rho = 0.176] with no significant difference p>0.05). Ferritin levles correlated significantly with CRP levels [rho = 0.510, p< 0.0001) (Table 2) (Figure 1, Figure 2 & Figure 3).

Table 1: Characterization of various parameters in CKD patients and control subjects.

Disease	N	Hemoglobin	Transferrin Saturation	Ferritin	CRP	Mean Hepicidin	Mean MAMC	Mean Albumin	Mean TIBC	Mean Iron
CKD-4 without Iron	10	8.7900±1.65089	27.1080± 14.00924	215.00± 40.969	18.34± 16.334	27.6900± 13.04611				
CKD-5 without Iron	22	8.4318±1.92388	20.7186± 8.33124	275.36± 42.639	20.98± 16.059	66.6727± 40.91265				
							19.33 ± 2.99.61 (cm)	3.24± 0.50g/dl	263.91 ± 116.8	66.18 ± 27.4 g/dl
CKD-5 with Iron	18	8.2778±1.90466	35.6783±11.80983	401.94± 68.209	31.82± 30.878	116.3444± 65.86968				
Control	5	14.5400±1.16103	31.7400± 6.41779	43.20± 8.468	0.96±0.329	71.1691± 58.09552				

Table 2: Correlations between CRP, Hepcidin and Ferritin levels

Correlations				
		Serum CRP	Serum Ferritin	Serum Hepcidin
Serum CRP	Pearson Correlation	1	.510**	.176
	Sig. (2-tailed)		.000	.197
	N	55	55	55
Serum Ferritin	Pearson Correlation	.510**	1	.589**
	Sig. (2-tailed)	.000		.000
	N	55	55	55
Serum Hepcidin	Pearson Correlation	.176	.589**	1
	Sig. (2-tailed)	.197	.000	
	N	55	55	55

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

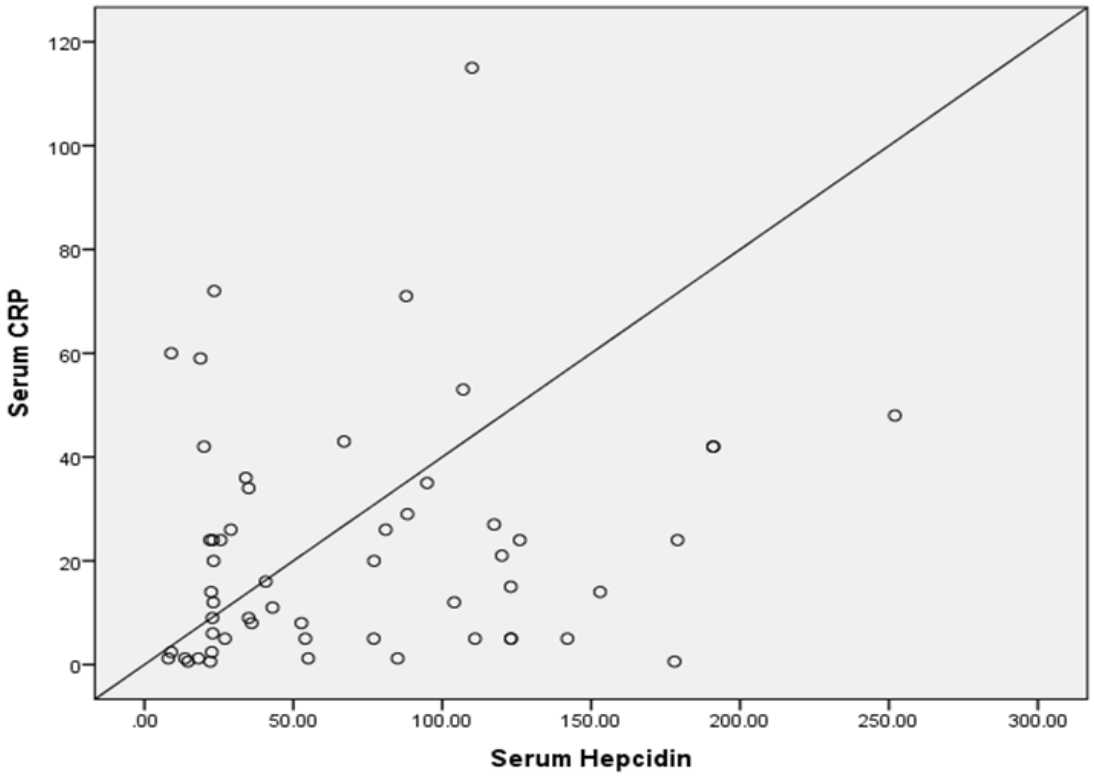

Fig. 1: Correlation between serum hepcidin & serum CRP [Scatter diagram]

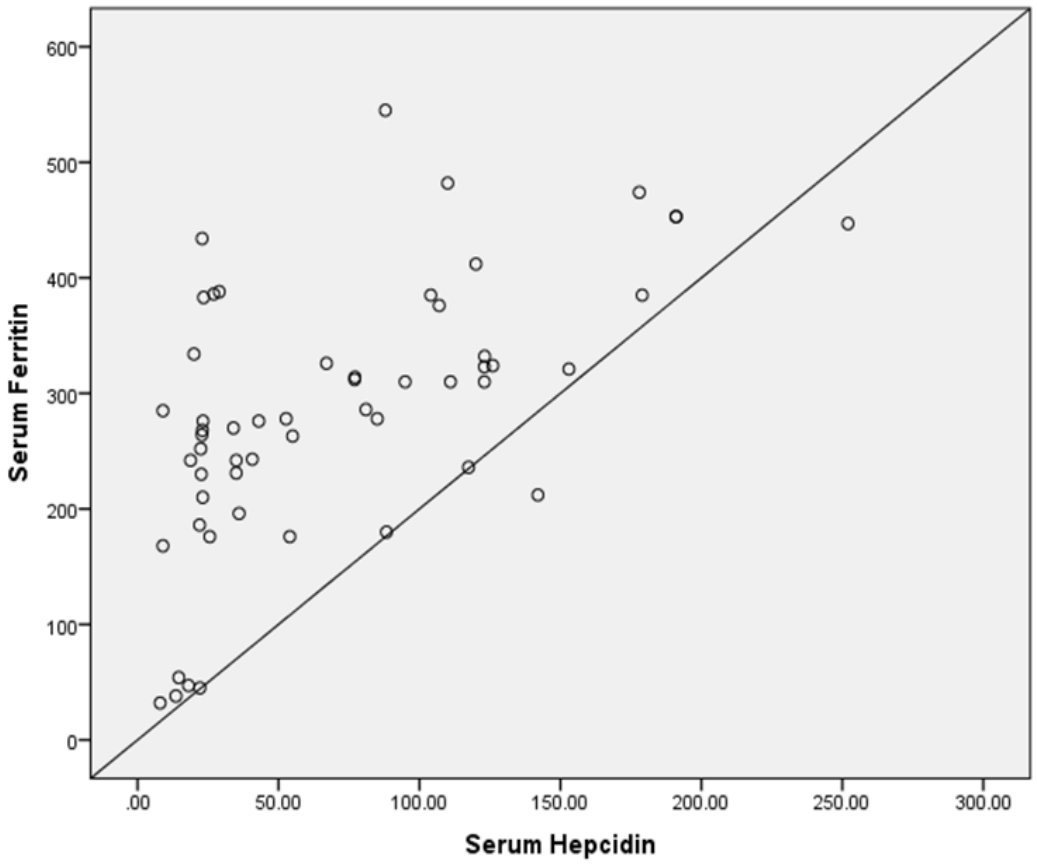

Fig. 2: Correlation between serum hepcidin and serum Ferritin [Scatter diagram]

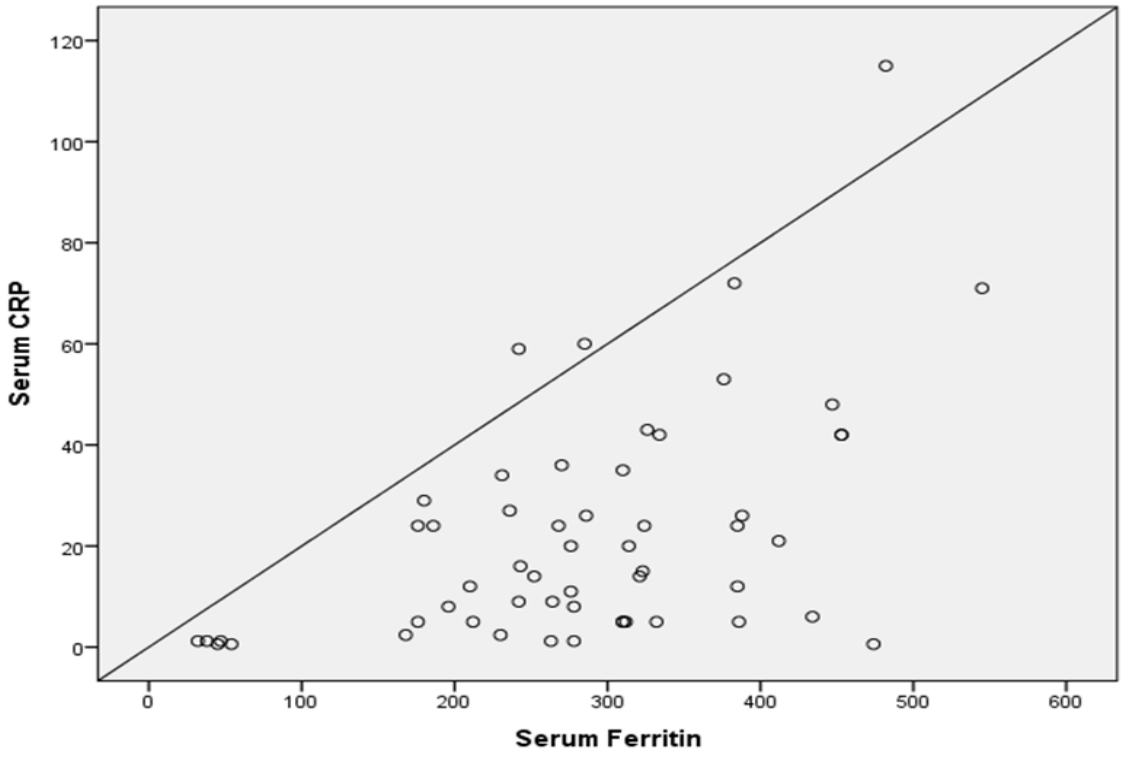

Fig. 3: Correlation between serum Ferritin & serum CRP [Scatter diagram]

Discussion

Iron deficiency is an important contributor to morbidity and mortality in ESRD. The cause is thought to be due to poor nutrition stemming from a predominantly vegetarian diet. Hepcidin, a regulator of body iron stores, has been postulated to play a critical role in the pathogenesis of anemia of chronic disease. Indian subjects have demonstrated to have micro inflammation and higher body fat percentage in healthy subjects as compared to Caucasians [8,9].

Hemoglobin levels in CKD-4/5 with/with out Iron intake shows less when compare with control subjects. Transferrin Saturation levels in CKD-5 with Iron intake shows high when compare with CKD-4 without Iron, CKD-5 without Iron and control subjects. Ferritin levels in CKD-5 with Iron intake shows very high when compare with CKD-4 without Iron, CKD-5 without Iron and control subjects. CRP levels in CKD-5 with Iron intake shows very high when compare with CKD-4 without Iron, CKD-5 without Iron and control subjects. Hepicidin levels in CKD-5 with Iron intake shows very high when compare with CKD-4 without Iron, CKD-5 without Iron and control subjects.

In our study there has been inflammatory activation with S.CRP levels increased in all study group. The cause of elevated mean CRP in subjects who did receive parenteral iron are on Hemodialysis is probably due to exposure to dialysis membranes, activation of immune cells (monocytes & T cells), or co-existing subclinical infection. A similar study done by V. Jha et al revealed inflammatory activation which was evident in 74 ESRD individuals has shown significantly higher CRP with (p value of < 0.001)[10]. Similar findings were noted in our study with significant correlation with hepcidin and serum CRP with significant p value (< 0.00).

In this study, the major finding was elevated hepcidin, in the study groups with subjects who did receive parenteral iron showed a higher S. hepcidin compared with subjects who didn't receive parenteral iron and control with significant (p value of < 0.01). A study done by Jha et al, revealed higher S. hepcidin levels in 74 ESRD patients with statistically significant (p value of < 0.12)[10]. Similar findings were noted in our study with increased serum hepcidin levels in ESRD individuals. Malyszko et al, revealed increased hepcidin level following I.V. iron therapy in Hemodialysis patients, but did not measure other inflammatory marker [11]. Comparing this study with our study group mean values of serum hepcidin was increased in CKD patients who were on haemodialysis and received i.v. iron therapy which was significant.

In our study S. ferritin was significantly elevated in subjects who did receive parenteral iron compared to individuals who didn't receive parenteral iron with statistically significant (p value of < 0.000). Ferritin was markedly increased due to iron overload in these subjects who did receive parenteral iron. V. Jha et al revealed elevated s. ferritin in individuals who did receive i.v. iron compared to individuals who didn't receive i.v. iron with p value (< 0.007)[10]. These findings were consistent with our study group individuals which showed significant statistically correlation between hepcidin and serum ferritin.

In our study there was no significant correlation between hemoglobin levels and s hepcidin levels. (p=0.138). Our study population consisted of only stage 4 and 5 CKD, the mean Hb levels were similarly low in all the groups of patients and had no relation to increasing s hepcidin levels. In our study, there was no significant correlation between hepcidin levels and TIBC (p=0.523). Serum Hepcidin & MAMC levels doesn't showed significant correlation (p=.240) could be established between these variables. Hepcidin & albumin levels did not show any correlation (p=0.511).

However in our study though CRP was significantly raised in the hemodialysis group, it is not correspondingly correlate with the elevated s hepcidin levels we had noted in this group.

Determination of hepcidin levels in CKD patients may not provide more diagnostic value than ferritin, but further studies are needed. Hepcidin and its regulatory pathways are potential therapeutic targets, which could lead to effective treatment of anemia of chronic disease and ESA hyporesponsiveness in CKD.

Atherosclerosis could induce an increase of the arterial IMT and arterial stiffening, and eventually lead to luminal obstruction with consequent ischemic events, such as myocardial infarction and stroke. Thus higher s hepcidin levels may be able to predict the subgroups of population with CKD who may be at higher risk for cardiovascular disease. This is an exciting area that needs to be further studied and may point the direction in which future strategies employing hepcidin as a diagnostic modality or as a target of directed therapeutic approaches.

Conclusion

There have been elevated serum hepcidin levels in ESRD patients more in those receiving Iron therapy.

High hepcidin levels would reveal functional iron deficiency. There was significant correlation between levels of hepcidin and iron status with inflammatory markers. The cause of the relatively greater degree of inflammatory activation as well as the relationship with parenteral IV iron administration needs further studies. Larger randomized multicenter studies could throw more light on the diagnostic and therapeutic potentials of using Hepcidin-25 levels in regular practice.

References

1. KDOQI; National Kidney Foundation. II. Clinical practice guidelines and clinical practice recommendations for anemia in chronic kidney disease in adults. Am J Kidney Dis. 2006; 47: S16–85.
2. Collins AJ, Li S, Ebben J, Ma JZ, Manning W. Hematocrit levels and associated medicare expenditures. Am J Kidney Dis. 2000; 36: 282–93.
3. MacDougall IC. Hyporesponsiveness to anemia therapy: What are we doing wrong? Perit Dial Int. 2001; 21: S225–30.
4. Kalantar-Zadeh K, Kopple JD, Humphreys MH, Block G. Comparing outcome predictability of markers of malnutrition-inflammation complex syndrome in hemodialysis patients. Nephrol Dial Transplant. 2004; 19: 1507–19.
5. Kalantar-Zadeh K, Rodriguez RA, Humphreys MH. Association between serum ferritin and measures of inflammation, nutrition and iron in hemodialysis patients. Nephrol Dial Transplant. 2004; 19: 141–9.
6. Feldman HI, Joffe M, Robinson B, Knauss J, Cizman B, Guo W, et al. Administration of parenteral iron and mortality among hemodialysis patients. J Am Soc Nephrol. 2004; 15: 1623–32.
7. Mehta BC. Iron deficiency. Prevalence and problems. J Assoc Physicians India. 1990; 38: 421–4.
8. Chambers JC, Eda S, Bassett P, Karim Y, Thompson SG, Gallimore JR, et al. C-reactive protein, insulin resistance, central obesity, and coronary heart disease risk in Indian Asians from the United Kingdom compared with European whites. Circulation. 2001; 104: 145–50.
9. Misra A. C-reactive protein in young individuals: Problems and implications for Asian Indians. Nutrition. 2004; 20: 478–81.
10. V. Jha , A. Jairam, R. Das,1 P. K. Aggarwal, H. S. Kohli, Iron status, inflammation and hepcidin in ESRD patients: The confounding role of intravenous iron therapy, Indian J Nephrol. 2010 July; 20(3): 125–13.
11. Malyszko J, Malyszko JS, Hryszko T, Pawlak K, Mysliwiec M. Is hepcidin a link between anemia, inflammation and liver function in hemodialyzed patients? Am J Nephrol. 2005; 25: 586–90.