Research Article

Hepcidin A Potential Novel Biomarker for Iron Status in Chronic Kidney Disease

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Abstract
Background and objectives: Hepcidin is a key regulator of iron homeostasis, but its study in the setting of chronic kidney disease (CKD) has been hampered by the lack of validated serum assays. Design, setting, participants, & measurements: This study reports the measurements of bioactive serum hepcidin using a novel competitive ELISA in ٦٠ patients, ٢٠ of them (CKD ٤) and ٢٠ CKD ٥) patients, ٢٠ ESRD on dialysis and ٢٠ as a control group. **Results:** When compared with their respective controls hepcidin was significantly increased in CKD ٤, CKD ٥, and ESRD (٦٥٢.٤ ng/ml). Multivariate regression analysis was used to assess the relationship between hepcidin and indicators of anemia, iron status, inflammation, and renal function. There was a correlation between ferritin and hepicidin and a correlation between hepicidin and degree of anemia. Also there was correlation between elevated level of hepicidin and parathyroid hormone. **Conclusions:** These findings suggest that 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.

Keywords: Hepcidin, kidney disease**,** anemia

Introduction
Recombinant erythropoietin (rhEPO) has transformed anemia therapy in patients with chronic kidney disease (CKD). However, rhEPO resistance, often asso-ciated with iron deficiency and inflame-mation, remains a challenging problem [١–٤]. Current available iron indices do not reliably identify iron-restricted erythropoiesis, often a sequela of inflammation, or those patients who would likely benefit from parenteral iron therapy[٥–٧]. To address these issues, it is crucial to understand the molecular mech-anisms that link inflammation, iron balance, and erythropoiesis.

Hepcidin, an acute phase reactant protein produced in the liver, is a recently discovered key regulator of iron homeo-stasis. Hepcidin inhibits intestinal iron absorption and iron release from macrophages and hepatocytes[٨]. Because hepcidin production is increased by inflammation, and high hepcidin concen-trations limit iron availability for erythro-poiesis, hepcidin likely plays a major role in the anemia of inflammation and rhEPO resistance.

Due to the previous absence of an accurate serum assay, most studies of hepcidin in humans have been performed using a urinary assay. Because such an assay may not reliably reflect serum hepcidin levels in patients with CKD, previous studies have instead attempted to measure the serum levels of prohepcidin, the peptide precursor of hepcidin[٩–١١]. However, these studies have been difficult to interpret because the relationship between prohepcidin, hepcidin, and iron parameters remains was used to detect a positive correlation between serum hepcidin and ferritin levels in CKD[١٠,١٧], but this technique is limited by its semiquan-titative nature and requirement for equip-ment that is not widely available.

Because of its renal elimination[١٨,١٩] and regulation by inflammation[٢٠–٢٣], it is possi-ble that progressive renal in- sufficiency leads to altered hepcidin metabolism,

subsequently affecting enteric absorption of iron and the availability of iron stores. In this study, using a novel assay, we present quantitative measurements of bioactive serum hepcidin in patients with CKD[١٢–١٦].

Materials and Methods

This study included ٨٠ subjects; ٦٠ patients, In addition to ٢٠ apparently healthy subjects of matched age and sex chosen as control group. They were divided into the following groups:

Group I:

Included ٢٠ patients CKD stage ٤ GFR range between ١٥ ml/min to ٢٩ ml/min, which are calculated by MDRD, ٩ (٤٥ %) males and ١١ (٥٥ %) females, their ages ranged from ١٨ to ٦٥ years.

Group II:

Included ٢٠ patients with CKD stage ٥ but not on RRT: GFR below ١٠ml/min which measured by MDRD associated with anemia, ١٢ (٦٠٪) males and ٨(٤٠ %) females, their ages ranged from ٢٥ to ٦٥ years

Group III:

Included ٢٠ patients with ESRD GFR < ١٠ml/min with anemia, ١٣ (٦٥ %) males and ٧ (٣٥ %) females, their ages ranged from ١٨ to ٦٥ years.

Group IV:

Included ٢٠ apparently healthy subjects of matched age and sex; ٩ (٤٥٪) males and ١١ (٥٥٪) females, their ages ranged from ١٨ to ٦٥ years.

- Exclusion criteria:

١- Previously diagnosed non-renal cause of anemia

٢- Evidence of active or occult bleeding, blood transfusion within the past four months.

٣- History of malignancy, end-stage liver disease, or chronic hypoxia.

٤- Recent hospitalization or infection requiring antibiotics within the past four weeks.

- All participants were subjected to the following

١- History taking

Careful history taking with special stress on anemia manifestations; duration, blood transfusion and family history.

٢- Physical examination

General examination and abdominal examination

٣- Lab. Investigations

♦ **Routine investigations**:

١- Complete blood count (CBC).

٢- Renal function tests (blood urea, serum creatinine).

٣- Liver function tests (ALT, AST, serum albumin and total protein).

٤- HB Electrophoresis.

٥- Serum iron and serum ferritin assay.

٦- Erythrocytes sedimentation rate (ESR).

٧- C- reactive protein.

٨- HCV by enzyme immunoassay (EIA).

٩- Calcium , phosphorous and PTH.

١٠-Special investigations:

Hepcidin level assay by enzyme immune-assay (EIA).

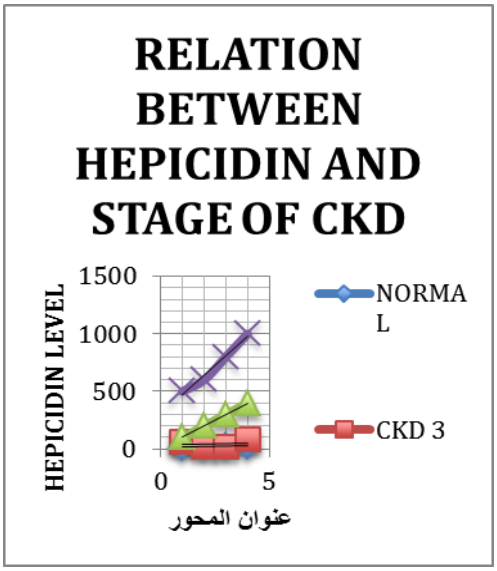
Statistical Analysis Of The Data Collected:

Analysis of data was done by IBM computer using SPSS (statistical program for social science version ١٢) as follow:

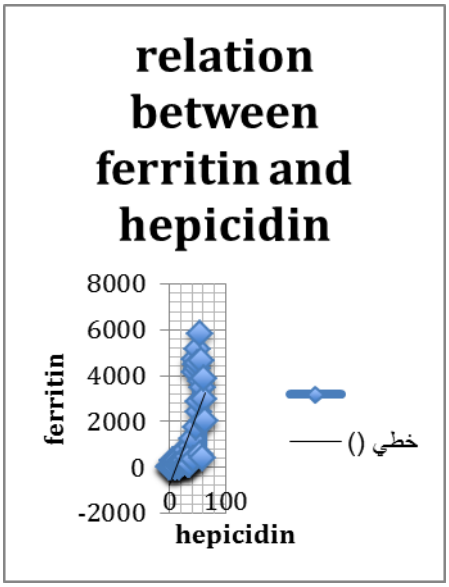
- **Description** of quantitative variables as mean, SD and range
- **Description** of qualitative variables as number and percentage
- **Chi-square** test was used to compare qualitative variables between groups.
- **Fisher exact test** was used instead of chi-square when one expected cell or more are less than ٥.
- **Unpaired t-test was** used to compare two groups as regard quantitative variable.
- **Paired t–test was used** to compare quantitative variables in the same group
- **One WAY ANOVA** test (analysis of variance) was used to compare more than two groups as regard quantitative variable.
- **Mann Whitney test was** used instead of unpaired t-test in non parametric data SD>٥٠٪ mean.
- **Spearman correlation** test was used to rank different variables positively or inversely.
- **ROC** (receiver operator characteristic curve) was used to find out the best cut of value and validity of certain variable.

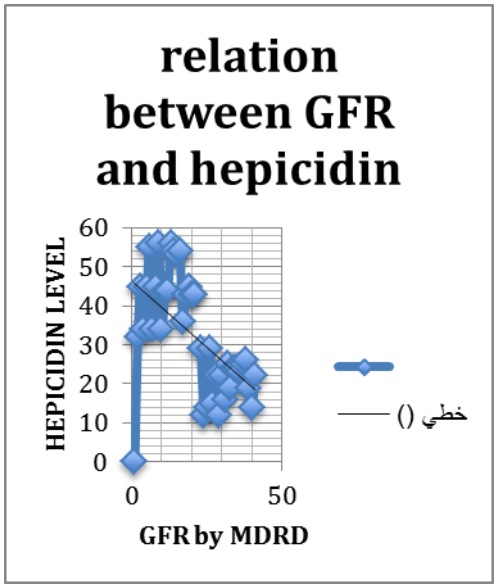
- Sensitivity = true ve +/true +ve + false – ve= ability of the test to detect +ve cases Specificity = true -ve/true-ve+ false +ve=

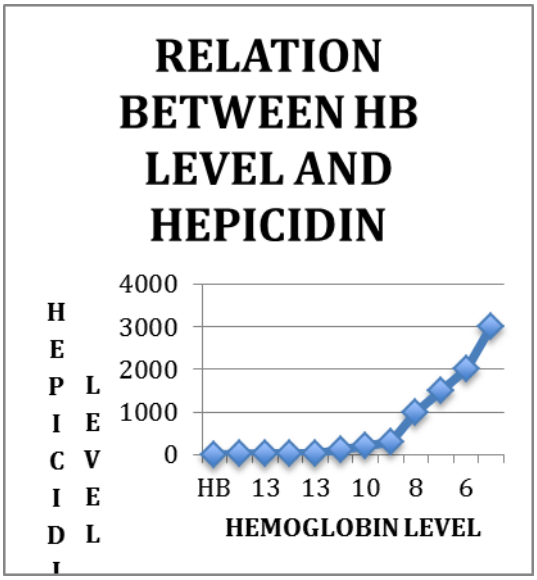
Results



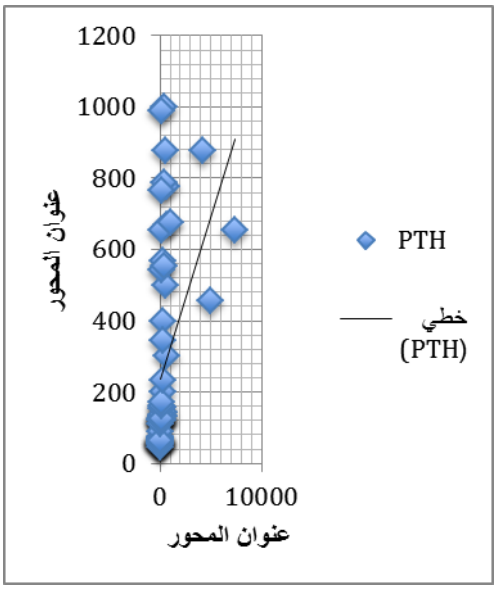
Our results show negative correlation between GFR and hepicidin and relation between hepicidin and stage of CKD also our results showed negative correlation between hemoglobin level and hepicidin. And positive coorelation between hepicidin and ferritin.







also we recorded positive correlation between parathyroid hormone and hepicidin.



Discussion

Using a novel validated assay (Ganz T et al., ٢٠٠٨) we report the quantitative measurements of bioactive serum hepcidin in adult non dialysis CKD and ESRD on dialysis.

In this study, we have demonstrated that serum hepcidin is progressively elevated across the spectrum of CKD. As expected from studies in other populations, the elevation of hepcidin appears multifactorial, particularly given its known regulation by iron stores, erythropoiesis, and inflammation (Ganz T. et al., ٢٠٠٧).

The most important finding in this study was the inverse association between GFR and hepcidin in different stages of CKD. Hepcidin is excreted in urine and metabolized by the kidney (Park et al., ٢٠٠١), (Swinkels DW et al., ٢٠٠٨), (Kulaksiz H et al., ٢٠٠٥).

The impairment of one or both of these processes may cause hepcidin accumulation as GFR decreases.

The highest levels of hepcidin were observed in the CKD ٥D group. Some elevation of hepcidin in this group would be expected given more intensive iron therapy reflected by higher average ferritin and percent iron saturation. Previous studies in populations with normal renal function have shown that oral or parenteral iron loading induces hepcidin production (Ganz T ٢٠٠٧), (Nemeth E et al., ٢٠٠٤), (Pigeon C et al., ٢٠٠١).

Elevated levels of hepcidin were present in the CKD٥D group despite the increased use of rhEPO to stimulate erythropoiesis, which typically inhibits hepcidin produ-ction (Pak M et al., ٢٠٠٦).

Finally, in the multivariate model that included all CKD stages, hepcidin was predicted by whether the patient had stage ٥D CKD versus stages ٢ to ٤ CKD. Although further studies are warranted, this finding adds additional support to the concept that worsening renal insufficiency leads to increased hepcidin levels.

Kulaksiz and his colleagues, ٢٠٠٥ measured Pro-hepcidin in healthy subjects and those with renal impairment. It was significantly increased in the serum of patients suffering from chronic renal insuffiency compared with that in the control group, but they gave no comment on Hb level, and they were not on regular HD but in our study we showed elevation in serum hepicidin in all stages of CKD and we gave a correlation between hemoglobin level and hepicidin level.

In all stages of CKD populations, a strong correlation was observed between hepcidin and markers of iron status by multivariate analysis, especially with ferritin, the primary storage molecule for cellular iron and a marker of tissue iron stores (Kalantar-Zadeh K et al., ٢٠٠٦), (Wish et al., ٢٠٠٦).

This is consistent with numerous studies in non-CKD populations that have documented a positive correlation between ferritin and hepcidin. (Ganz T ٢٠٠٧), (Swinkels DW et al., ٢٠٠٨), (Malyszko J. et al., ٢٠٠٧).

The serum hepcidin concentration exhibited a statistically significant correlation with serum ferritin concentrations in both patient subsets, but no statistically significant correlations were observed between serum hepcidin and other laboratory markers of iron status (Dallalio et al., ٢٠٠٣).

Similar to our results, CKD and dialysis-dependent groups had significantly increased hepcidin levels, with hepcidin inversely correlating with GFR. Two major types of hepcidin assays are becoming available. In the first, hepcidin peptides can be detected and measured by mass spectrometry, usually after a chromatographic or selective adsorption purification step. Internal standards are used to improve the accuracy of this type of assay. The second type of assay, used in this study and the study by (Ashby et al., ٢٠٠٩), uses an anti-hepcidin antibody in a competitive binding assay between a radiolabeled or tagged hepcidin and the sample.

Although the two types of assays correlate extremely well (un- published data), the

absolute values reported by the assays vary by as much as ten-fold.
The reason for this discrepancy may include hepcidinbinding factors in sera and urine, differing hepcidin standards and their state of aggregation, and the tendency of hepcidin to adsorb to assay surfaces. Provided that a suitable reference population is used for each study, these factors do not affect the conclusions reached.

Efforts are underway to resolve these differences and to provide technical standardization of the hepcidin assays.
We also found at our study a negative e correlation between serum albumin and hepicidin level.

Our result is corresponding to a cohort study of ٥٣ patients were followed for ١٢ months. Its association with albumin and hemoglobin levels at the start of the study and at ٦ and ١٢ months thereafter were looked for. In all patients, there was correlated positively between the albumin level and with the hemoglobin level (Teruel et al., ٢٠٠٥).

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