Research Article

Evaluation of the Iron Regulatory Hormone Hepcidin in Patients with Chronic Kidney Disease

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Abstract

Background: Hepcidin is a key hormone 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. **Aim of the work:** was to measure hepcidin-٢٥ levels in patients with chronic kidney disease (CKD) across the spectrum of the disease to determine its associations with some biochemical parameters in CKD patients and to explore its relation with iron status. **Patients and Methods**: Sixty patients were included, ٢٠ of them (CKD ٤) and ٢٠ patients with (CKD ٥) , ٢٠ patients with ESRD on hemodialysis (Group III) with an overall mean age of ٥٥.٦±١٤.٤ years and ٢٠ of matched age and sex as a control group. Analysis of urea, creatinine, hemoglobin were performed. GFR was estimated using MDRD formula. Serum ferritin, albumin, and parathormone were also measured. Measurements of bioactive serum hepcidin was done using a novel competitive ELISA assay. **Results:** When compared with their respective controls hepcidin was significantly increased in CKD ٤, CKD ٥, and ESRD compared to control (٣٦.٦±١٩.٠٦, ١٨١.١±١٠٥.١٥, ١١٩٦.٦٥±١٩٥.٣٨ and ٥٠.٦٥±٢٤.٨٣ respectively), (p<٠.٠٠١). Multivariate regression analysis was used to assess the relationship between hepcidin and indicators of anemia, iron status, and renal function (urea, creatinine and eGFR). Hepcidin was positively correlated with ferritin (r= ٠.٦٢, p<٠.٠٠١), and negatively with eGFR (r= -٠.٦, p<٠.٠٠١) and (Hb) degree of anemia (r= - ٠.٤, p<٠.٠٠١) and albumin (r= - ٠.٣, p<٠.٠٥). There was a positive correlation between elevated level of hepicidin and parathyroid hormone (r=٠.٧, p<٠.٠٠١). **Conclusions:** These findings suggest that hepcidin increased across the spectrum of CKD patients & Hepcidin-٢٥ levels increase as renal function deteriorates. Hepcidin was positively correlated with ferritin and creatinine and inversely correlated with eGFR, Hb and Albumin.

Keywords: Hepcidin, Chronic kidney diseases (CKD)**,** ESRD (End stage renal disease), Anemia, Iron

Introduction

Human hepcidin, a ٢٥–amino acid peptide made by hepatocytes, may be the long-sought iron-regulatory hormone[١]. It is a recently discovered key regulator hormone of iron homeostasis [٢]. It inhibits intestinal iron absorption and iron release from macrophages and hepatocytes[٣]. Hepcidin production is increased by inflammation, and high hepcidin concentrations limit iron availability for erythropoiesis, hepcidin likely plays a major role in the anemia of inflammation and rhEPO resistance and levels of the hormone is elevated in chronic low grade inflammation[٤]. Nakanishi et al., reported that hepcidin levels have been described in association with markers of inflammation (e.g. C-reactive protein and interleukin-٦), anaemia (e.g. haemoglobin and endogenous EPO) and most strongly with iron status (e.g. ferritin)[٢].

Both anaemia and chronic inflammation are frequently detected in CKD[٥]. Hepcidin levels also predict the progression of renal anemia in patients with non-dialysis chronic kidney disease[٦].

Several factors, such as iron stores, inflammation, anemia, and hypoxia, have been identified as potential regulators of hepcidin[٧]. Current available iron indices do not reliably identify iron-restricted erythropoiesis, often a sequel of inflammation, or those patients who would likely benefit from parenteral iron therapy[٨]. In anemia of inflammation, hepcidin production is increased up to ١٠٠-fold and this may account for the defining feature of this condition, sequestration of iron in macrophages[٩]. Numerous studies now show that hepcidin is elevated in CKD patients[١٠]. Mechanisms suggested to account for this are increased expression by inflammatory cytokines and reduced renal clearance. IL-١ may play a significant role in the anemia of inflammation by up-regulating hepcidin[١١]. Recent work has identified hepcidin excess as a main contributor to the disordered iron homeostasis and anemia of CKD by impairing dietary iron absorption and iron mobilization from body stores[١٢]. CKD is associated with increased serum hepcidin levels that may contribute to the developpment and severity of anemia and to resistance to erythropoiesis-stimulating agents[١٣]. Using a novel assay, quantitative measurements of bioactive serum hepcidin in patients with CKD had been done[١٤].

Materials and Methods

This study was done in the period from June ٢٠١٢ to November ٢٠١٣ and included ٨٠ subjects; ٦٠ patients attending nephrology outpatient clinic and dialysis unit in Minia university hospital, In addition to ٢٠ apparently healthy subjects of matched age and sex chosen as control group. Informed consent was taken from all participants and approval of the Ethical committee of Minia faculty of medicine, Minia university was given. 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 renal replacement therapy: GFR below ١٠ml/min, 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, 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: Previously diagnosed non-renal cause of anemia, Evidence of active or occult bleeding, blood transfusion within the past four months, History of malignancy, end-stage liver disease, or chronic hypoxia, 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; blood transfusion and family history with general and abdominal examinations

٢- **Laboratory Investigations: Routine investigations** included: Complete blood count (CBC) determined by automated cell counter Sysmex KX-٢IN (TAO Medical Incorporation, Japan). Renal function tests (blood urea, serum creatinine), Liver function tests (ALT, AST, serum albumin and total protein) were assayed using fully automated clinical chemistry auto-analyzer system Konelab ٢٠i (ThermoElectron Incorporation, Finland)**.** Serum ferritin quantitative assay is based on a solid phase enzyme linked immunosorbent assay (ELISA). Serum ferritin levels were measured using an automated Roche (Indianapolis, IN USA) Hitachi analyzer. Kits supplied by BioCheck Instruments Foster City, USA)**.** Calcium by Colorimetric Assay Kit from Biovision, phosphorous by column chromatography method and Parathyroid hormone (PTH) by Enzyme Immune Assay[١٥].

Special investigations: Hepcidin level was assayed using Enzyme Immune Assay (EIA) (The DRG Hepcidin hormone ELISA kit is a solid phase enzyme- linked immunosorbant assay (ELISA) based on the principle of competitive binding[١٦]. Hepcidin measurement is based on either mass spectrometry or antibody-based hepcidin detection. Competitive radioimmunoassay and ELISA tests have been developed for the antibody-

based methods. Two major types of hepcidin assays are becoming available[٩]. In the first, hepcidin peptides can be detected and measured by mass spectrometry[١٧], 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, uses an anti-hepcidin antibody in a competitive binding assay between a radio labeled or tagged hepcidin and the sample [١٨]. 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 hepcidin-binding factors in sera and urine, differing hepcidin standards and their state of aggregation, and the tendency of hepcidin to adsorb to assay surfaces. Efforts are underway to resolve these differences and to provide technical standardization of the hepcidin assays (Kits supplied by DRG Instruments GmbH, Germany)[١٩].

Statistical Analysis

Analysis of data was done by IBM computer using SPSS (statistical program for social science version ١٧) 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. 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.

Results

Data are expressed as mean ± SD. Difference is considered significant when *p* value is < ٠.٠٥. In patients with CKD and End stage renal disease (ESRD), Hepicidin is significantly higher than control (p<٠.٠٠١) (Table١). Hemoglobin is signify-cantly lower in patients than control. Moreover, hemoglobin is significantly lower in patients at group III with ESRD than other groups (p< ٠.٠٠١) (Table ٢)**.** Urea and Creatinine are significantly higher in Patient than Control groups (p< ٠.٠٠١). However, GFR is significantly low in patient groups than control (p< ٠.٠٠١) (Table ٣). Ferritin is significantly higher in patients than control (p< ٠.٠٠١). Moreover, ferritin is significantly higher in patients at group III with ESRD than other groups (p< ٠.٠٠١) (Table ٤). Hepcidin level is significantly high in patients with high parathyroid hormone (p< ٠.٠٠١) (Table ٥). There is positive Correlation between different stages of CKD and Hepcidin levels. Serum hepcidin is progressively elevated across the spectrum of CKD. There is a significant positive correlation between Ferritin and Hepcidin levels. (r = ٠.٥, p< ٠.٠٠١) (Fig ١). There is a significant negative correlation between eGFR and Hepcidin levels. (r = - ٠.٦, p<٠.٠٠١) (Fig ٢). There is also a significant negative Correlation between Hemoglobin level and Hepicidin, r = -٠.٤, p<٠.٠٠١ (Fig. ٣). There is negative correlation between Hepcidin and Albumin (r= - ٠.٣, p< ٠.٠٥) (Fig. ٤). There is a positive correlation between Hepcidin and Parathyroid hormone. (r = ٠.٦, p<٠.٠٠١) (Fig.٥).

Table (١): Comparison between Patients and Control regarding Hepicidin

	Group I CKD stage٤	Group II CKD ٥ ND	Group III ESRD	Group IV control	P value		
Hepcidin Range ng/ml M ± SD	(١٠-٨٢) ٣٦.٦±١٩.٠٦	(٩٠-٤٣٠) ١٨١.١±١٠٥.١٦	(١٠٥-٧٤٣٢) ١١٩٦.٦٥±١٩٥٤.٣٨	(١٥-٩٠) ٥٠.٦٥±٢٤.٨٣	<٠.٠٠١		
					I vs IV	**II vs IV**	**III vs IV**
					<٠.٠٠١	<٠.٠٠١	<٠.٠٠١

Table (٢): Comparison between patients and controls regarding Hb level

	Group I CKD stage ٤	Group II CKD stage ٥ ND	Group III stage ٥D ESRD	Group IV control	P value		
Hb:					<٠.٠٠١		
Range gm/dl M±SD	(١٠-١٢) ١١.٣٣ ± ٠.٦١	(٤.٩-١٠.٥) ٨.٣٨ ± ١.٦١	(٤-٩.٦) ٦.٩ ± ١.٧٥	(١٢.٦-١٦.٢) ١٤.٧±٠.٩٧	**I vs IV**	**II vs IV**	**III vs IV**
					<٠.٠٠١	<٠.٠٠١	<٠.٠٠١

Table (٣): Comparison between Patients and Control groups regarding Urea, Creatinine and GFR

	Group I CKD ٤	Group II CKD ٥ ND	Group III ESRD	Group IV control	P value		
Urea:					<٠.٠٠١		
Range mg/dl M ± SD	(١٥-٤٠) ٢٦.٩±٦.٤٤	(٥٠-١٠٠) ٧٤.٥٥±١٥.٠١	(١١١-٢٢١) ١٤٨.٩٥±٣٦.٩١	(٢١-٥٠) ٣٢.٤±٧.٧٤	**I vs IV**	**II vs IV**	**III vs IV**
					<٠.٠٠١	<٠.٠٠١	٠.٠٠١
Creatinine:					<٠.٠٠١		
Range mg/dl M ± SD	(١.٦-٢.٤) ١.٨٣±٠.٢٢	(٣-٧) ٥.١٢±١.١١	(٨-١٤) ١١.٠٥±١.٣٢	(٠.٥-٠.٨) ٠.٦٢±٠.٠٧	**I vs IV**	**II vs IV**	**III vs IV**
					<٠.٠٠١	<٠.٠٠١	<٠.٠٠١
GFR:					<٠.٠٠١		
M ± SD	٤٤.٧٥±٧.٩٣	٢٠.٦٣±٥.٤٦	٥.٧٥±٢.٥٧	١٣١.٦٥±٩.٨٧	**I vs IV**	**II vs IV**	**III vs IV**
					<٠.٠٠١	<٠.٠٠١	<٠.٠٠١

Table (٤): Comparison between Patients and Control Groups regarding Ferritin

	Gp I CKD٤	Gp II CKD٥	Gp III ESRD	Gp IV	P value		
					< ٠.٠٠١		
Ferritin Range ng/ml M ± SD	(٢٥-٢٨٩) ١٣٦.٦٥±٩٨.٩٣	(١٠٥-١٢٦٥) ٤٣٣.١±٣١.٨	(٤١٥-٥٨١٩) ٣٣٧٨.٤٥±١٤٧١.٨	(٢٠-٣٠٥) ١٢٨.٠٥±٩١.٧	I vs IV	II vs IV	III vs IV
					< ٠.٠٠١	< ٠.٠٠١	< ٠.٠٠١

Table (٥): Parathyroid in relation to Hepcidin levels.

	Hepcidin		P value	OR	CI (٩٥٪)	P value
	<١٠٠ N=٤٣	>١٠٠ N=٣٧				
PTH: pg/ml <١٠٠. >١٠٠.	٤٠ (٩٣٪) ٣ (٧٪)	٠ (٥٪) ٣٧(١٠٠٪)	< ٠.٠٠١*	٨٦٧.٨٦	(٤٣.٣٧-١٧٣٦٧.٨)	< ٠.٠٠١*

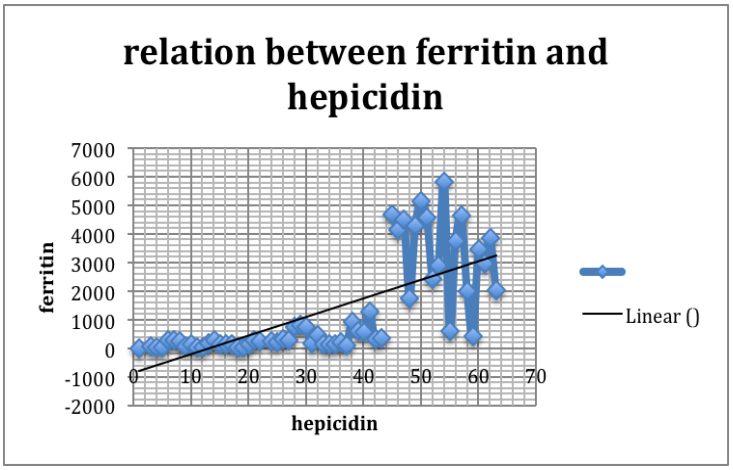

Fig (٢): Correlation between Ferritin (ng/ml) and Hepicidin (ng/ml) levels (r=٠.٥, p<٠.٠٠١)

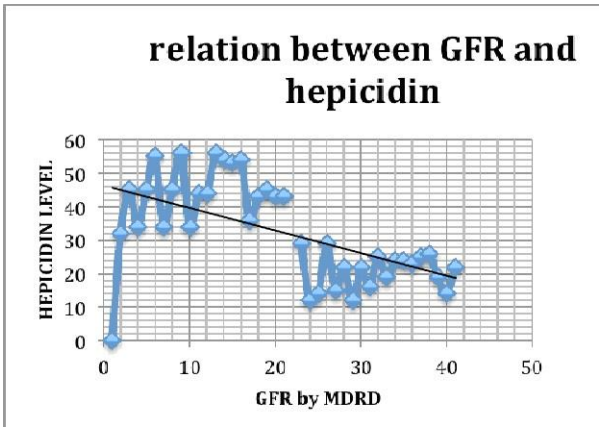

Fig. (٢): Correlation between Glomerular filtration rate (GFR) (ml/min) and Hepcidin (ng/ml) levels. (r= -٠.٦, p<٠.٠٠١)

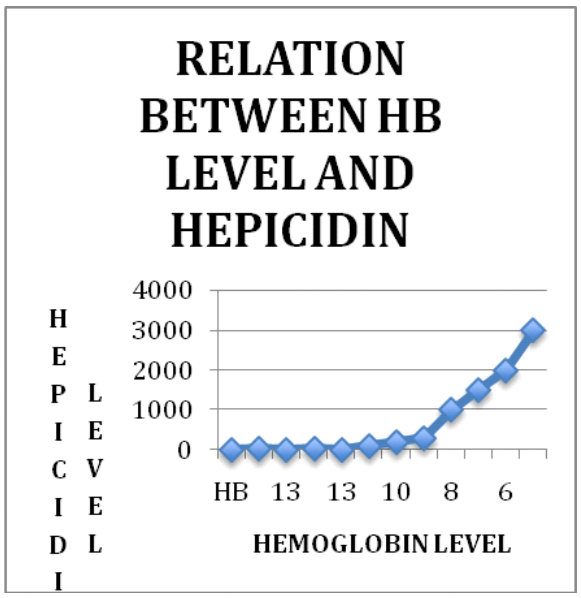

Fig. (٣): Correlation between Hemoglobin (gm/dl) and Hepcidin levels (ng/ml) (r= - ٠.٤, p<٠.٠٠١)

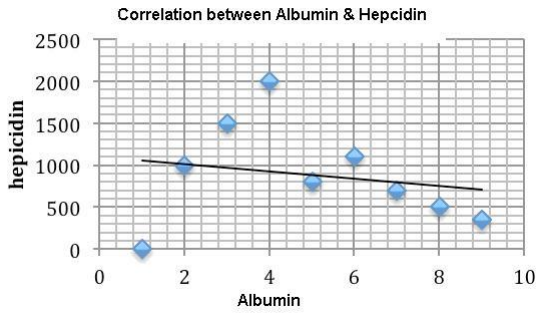

Fig. (٤): Correlation between Hepcidin (ng/ml) and Albumin (gm/dl). (r= - ٠.٣, p<٠.٠٥)

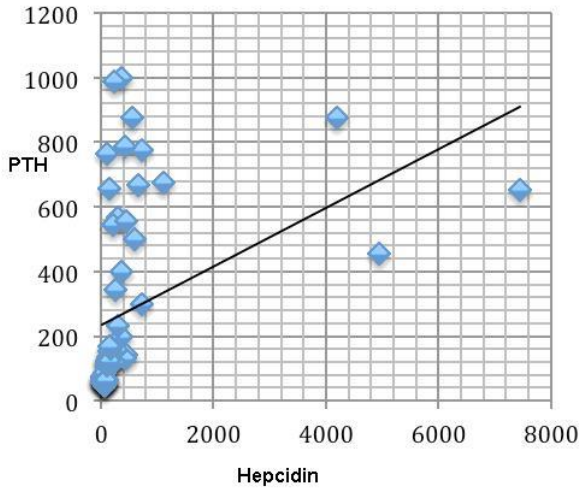

Fig. (٥). Correlation between Parathyroid hormone (pg/ml) and Hepcidin (ng/ml) levels. (r= ٠.٦, p< ٠.٠٠١)

Discussion

The ٢٥-amino acid active form of hepcidin (hepcidin-٢٥) is the master hormone regulating iron homeostasis in humans[٢٠]. Hepcidin-٢٥ is synthesized and secreted by the liver and controls dietary iron absorption and release of hepatic and macrophage iron stores by binding directly to the iron transport protein ferroportin, causing it to be internalized and degraded. This results in decreased iron transport across the cell membrane, which decreases iron availability for hemoglobin production and erythropoiesis[٣].

Using a novel validated assay which reported the quantitative measurements of bioactive serum hepcidin in adult non dialysis CKD and ESRD on dialysis[٩]. In the setting of CKD, increased circulating levels of hepcidin mediate a state of functional iron deficiency, resulting in insufficient iron being available for erythropoiesis despite adequate or even increased body iron stores as reflected by serum ferritin levels[٢١]. Higher hepcidin levels likely play a significant role in anemia and ESA resistance in CKD patients[٢٢]. In CKD, increased inflammation and possibly decreased clearance of hepcidin can lead to higher serum hepcidin levels, further contributing to iron-restricted erythropoiesis and rhEPO resistance[٢٣]. 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[٩]. 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[٢٤,١٧]. The impairment of one or both of these

processes may cause hepcidin accumulation as GFR decreases. Ashby et al., noted an inverse correlation between serum hepcidin and estimated GFR (eGFR) in adults with mild to moderate CKD[١٨]. The direct correlation of hepcidin-٢٥ with creatinine and its inverse correlation with eGFR suggest that hepcidin-٢٥ levels increase as renal function deteriorates, possibly due to decreased hepcidin-٢٥ renal clearance[٢٥]. However, another author found that Serum hepcidin-٢٥ levels in patients with chronic kidney disease are independent of glomerular filtration rate[٢٦]. It is unclear why our results were different from those of Peters et al., but one possibility may be that the populations of CKD patients differed in the respective studies. In our study the highest levels of hepcidin were observed in the CKD ٥D group. Elevated levels of hepcidin were present in the CKD٥D (stage ٥ on dialysis) group despite the increased use of rhEPO to stimulate erythropoiesis, which typically inhibits hepcidin production[٢٧]. 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 and this adds additional support to the concept that worsening renal insufficiency leads to increased hepcidin levels[٢٨]. Kulaksiz and his colleagues measured hepcidin hormone 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[٢٩], and in our study we showed elevation in serum hepicidin in all stages of CKD. In all stages of CKD populations, a strong correlation was observed between hepcidin and markers of iron status, especially with ferritin, the primary storage molecule for cellular iron and a marker of tissue iron stores[٨] and this is in accordance of our study. In states of hyperferritinemia, there is a linear relationship between serum ferritin and hepcidin[٢] and this also in accordance with our results. Numerous studies in non-CKD populations have documented a positive correlation between ferritin and hepcidin[٣,١٧,٣٠]. Serum hepcidin concentration exhibited a statistically significant correlation with serum ferritin concentrations in patient subsets, but no statistically significant correlations were observed between serum hepcidin and other laboratory markers of iron status[٣١]. Consistent positive correlations between hepcidin and serum ferritin levels have been demonstrated in adult and pediatric CKD patients as well as in healthy controls[٣٢]. In the setting of CKD, the direct relationship with ferritin may represent a protective effect of hepcidin against systemic iron overload. Our results reported hepcidin to be elevated and correlated with ferritin in CKD patients. It is known that hepcidin synthesis is induced by inflammation, a process that appears mediated by IL-٦[٣٣,٣٤]. Given that CKD is considered an inflammatory state, this positive correlation is expected. Our findings are in agreement with a report by Ashby *et al.* that in adult CKD and hemodialysis patients using a radioimmunoassay had significantly increased hepcidin levels, with hepcidin inversely correlating with GFR[١٨].

Recent reports have suggested a modest and significant correlation between hepcidin and hemoglobin[٣٥] and this is in accordance with our study. High hepcidin levels could predict the need for parenteral iron to help overcome hepcidin-mediated iron-restricted erythropoiesis and the need for relatively higher rhEPO doses to suppress hepcidin production. Conversely, patients with low hepcidin would be expected to respond better to oral iron. If so, hepcidin concentrations may become a unique biomarker to guide iron therapy in CKD[٣٦]. **Future perspectives**: In the future, it is possible that a hepcidin antagonist could be developed as a therapeutic tool in CKD. Specifically, lowering hepcidin levels or antagonizing its actions would reverse the negative effects of inflammation on erythropoiesis by allowing mobilization of stored iron and improved enteral iron absorption[٣٧].

Conclusions

Patients with CKD and ESRD, hepcidin levels were significantly elevated, positively correlated with ferritin and inversely correlated with the estimated glomerular filtration rate, hemoglobin and albumin.

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