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Dysregulation of iron metabolism in chronic kidney disease – hepcidin as a diagnostic biomarker in Iraqi adults – a case-control study

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ABSTRACT

Introduction and aim. Chronic kidney disease (CKD) is a global health burden, with iron deficiency (ID) being a prevalent but under-diagnosed comorbidity. Traditional biomarkers, such as serum ferritin and transferrin saturation, are confounded by inflammation, which limits diagnostic accuracy. Hepcidin, a key regulator of iron homeostasis, offers potential as a reliable biomarker; however, data from low- and middle-income countries (LMICs), including Iraq, remain scarce. This study assessed the diagnostic utility of hepcidin for CKD and its association with iron dysregulation in Iraqi adults, addressing regional gaps in biomarker validation and clinical application.

Material and methods. This case-control study (September 2024–February 2025) recruited 60 patients with CKD (stages 2–4) and 40 age-and sex-matched healthy controls. The exclusion criteria were recent transfusions, infections, or iron therapy. Serum hepcidin, ferritin, iron, Total Iron-Binding Capacity (TIBC), transferrin, and renal function indices were analyzed via enzyme-linked immunosorbent assay and standardized assays.

Results. CKD patients exhibited significantly elevated hepcidin (39.8±23.1 vs. 13.7±4.8 pg/mL, $p<0.001$) and ferritin (246±128 vs. 160±61 ng/mL, $p<0.001$) but lower serum iron (12.38±3.1 vs. 118±33.5 μg/dL, $p<0.001$), TIBC (347±109 vs. 385±62 μg/dL, $p=0.004$), and transferrin levels (224±74 vs. 258±39 mg/dL, $p=0.005$) than controls. Hepcidin levels increased progressively with CKD stage ($p<0.001$) and correlated strongly with eGFR ($r=-0.800$, $p<0.001$) and serum creatinine ($r=0.702$, $p<0.001$). At a cut-off of >25 pg/mL, hepcidin demonstrated 80% sensitivity and 100% specificity for CKD diagnosis, with an AUC of 0.86 (95% CI: 0.78–0.92, $p<0.001$).

Conclusion. Hepcidin demonstrated high diagnostic accuracy for CKD and was a superior indicator of iron metabolism dysregulation compared to traditional biomarkers. Its utility in resource-limited settings, such

as Iraq, could enhance early CKD detection and guide management strategies, thereby addressing critical gaps in LMIC healthcare.

Keywords. chronic kidney disease, diagnostic biomarker, hepcidin, iron deficiency

Introduction

Chronic kidney disease (CKD) is a worldwide public health burden, affecting approximately 10% of the population. CKD is a progressive condition that leads to irreversible decline in renal function and is associated with cardiovascular diseases, anemia, and elevated mortality. iron deficiency (ID) is a frequently overlooked comorbidity in CKD.[1,2] Inappropriate dietary intake, chronic inflammation, and systemic and renal signs of impaired iron utilization contribute to the pathogenesis of ID in CKD. ID leads to anemia of chronic inflammation, a frequently occurring condition in patients with CKD, contributes to reduced oxygen supply, worsens weariness, and deteriorates quality of life.[3–5]

The coexistence of CKD and ID is unacceptably high. Studies have reported that the prevalence of ID among patients with non-dialysis CKD ranges from approximately 25% to 50%, with the proportion increasing as CKD progresses to more advanced stages.[6] For example, one study found that 47.1% of non-dialysis CKD patients had ID, and another study identified ID anemia in 38.8% of pre-dialysis CKD patients. The prevalence of anemia, often due to ID, was nearly 40% among non-dialysis-dependent CKD patients in a large European cohort, and the rates increased with worsening CKD stage.[7] In low- and middle-income countries (LMICs), including Iraq, the clinical burden of this dual pathology is exacerbated by limited access to advanced diagnostics and effective therapies.[8] The Middle East and North Africa (MENA) region, for example, continues to experience a high burden of ID, with more than 88 million cases reported in 2021 and little improvement in recent decades.[9]

In healthy individuals, iron homeostasis is a tightly regulated process primarily orchestrated by the hepatic peptide hormone hepcidin. Hepcidin, a 25-amino-acid peptide produced by the liver, serves as the master regulator of systemic iron availability.[10] It maintains the iron balance by binding to and inducing the degradation of the iron exporter ferroportin, which is present on the surface of enterocytes in the intestine, macrophages in the reticuloendothelial system, and hepatocytes. This action reduces both intestinal iron absorption and the release of recycled iron from macrophages, thereby lowering plasma iron levels.[11]

Modern biomarkers, such as hepcidin, allow potential improvement of diagnostic accuracy and therapeutic monitoring when dealing with ID associated with CKD.[12] The main problem with traditional markers such as serum ferritin and transferrin saturation (TSAT) is that the former is severely affected by inflammation, while TSAT does not provide the necessary specificity, inevitably leading to misclassification of the iron status.[13] In contrast, hepcidin directly represents the body iron-regulatory experience and level of inflammation. Consequently, hepcidin is a reliable marker of functional ID in CKD patients.[14] Modern

research has revealed that elevated levels of hepcidin indicate the pathology of the filtration rate, and the patient will be affected by an inability to react to iron therapy.[15] Existing research on hepcidin's role in iron metabolism dysregulation in CKD predominantly stems from high-income settings, with limited data from LMICs, such as Iraq, where diagnostic constraints and high clinical burden coexist.[8,16] Traditional biomarkers (e.g., serum ferritin and TSAT) are confounded by inflammation and lack specificity, underscoring the need for contextually validated alternatives.

Aim

This study aimed to assess the diagnostic utility of hepcidin as a biomarker for CKD and its associated iron dysregulation in Iraqi adults, address the regional research gap, and explore its potential to enhance diagnostic accuracy and therapeutic strategies in resource-limited settings.

Material and methods

This case-control study was conducted between September 2024 and February 2025, recruiting participants from the nephrology outpatient clinics of a Diwaniyah Teaching Hospital. A total of 60 adult patients (aged 18–80 years) with non-dialysis CKD stages (2–4) , as defined by the KDIGO 2012 Clinical Practice Guideline based on estimated glomerular filtration rate (eGFR)[17], and 40 age- and sex-matched healthy controls were enrolled. Patient eligibility required stable renal function (no greater than a 25% change in eGFR over the preceding 3 months). Key exclusion criteria for the CKD group included a history of blood transfusions within the past 3 months, iron supplementation or erythropoiesis-stimulating agent (ESA) use within the past 4 weeks, and the presence of active infection or significant inflammation (C-reactive protein ≥10 mg/L). Patients with end-stage renal disease (ESRD) on dialysis, recent kidney transplants (<6 months), or polycystic kidney disease were also excluded. The sample size was determined as a convenience sample based on patient availability and logistical constraints during the study period.

Healthy controls, rigorously age- and sex-matched to the CKD participants, underwent screening to confirm normal kidney function (eGFR ≥90 mL/min/1.73 m² without proteinuria or hematuria), adequate hemoglobin levels, and the absence of chronic illnesses, inflammation, or iron abnormalities, with health assessments conducted according to STROBE guidelines; this screening was expanded to specifically require CRP levels ≤5 mg/L, normal iron status (ferritin >30 ng/mL and TSAT >20%), no chronic comorbidities (including diabetes, cardiovascular disease, or liver disease), and a BMI <30, while lifestyle factors such as smoking and alcohol use were recorded but were only exclusionary if deemed clinically significant.

Biochemical assessments

Venous blood samples (approximately 8 mL) were collected from all participants in the morning after an overnight fast (10–12 hours) into plain serum separator tubes. Samples were allowed to clot at room temperature for 30 minutes and were subsequently centrifuged at 3000 rpm for 10 minutes. The separated serum was aliquoted into sterile cryovials and stored at -80°C until analysis. All samples were analyzed within one month of collection and underwent a single freeze-thaw cycle to preserve analyte integrity.

Serum urea, creatinine, iron, ferritin, TIBC, and high-sensitivity C-reactive protein (hs-CRP) were analyzed using a Roche Cobas c501 automated analyzer (Roche Diagnostics, Basel, Switzerland). Transferrin levels were quantified using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Human Transferrin ELISA Kit, Elabscience®, Catalog No: E-EL-H6298). Serum hepcidin was measured using a commercially available competitive ELISA kit (Human Hepcidin ELISA Kit, Elabscience®, Catalog No: E-EL-H6202) with a detection range of 0.78–500 pg/mL and a sensitivity (limit of detection, LOD) of 9.4 pg/mL. Hemoglobin levels were determined using a Sysmex XN-1000 automated hematology analyzer (Sysmex Corporation, Kobe, Japan).

All assays were performed in duplicate according to the manufacturers' instructions. Internal quality control materials provided by the respective kit manufacturers were included in each assay run. The intra- and inter-assay coefficients of variation (CV) for the hepcidin ELISA were 4.8% and 7.3%, respectively. The laboratory participates in an external quality assurance scheme for routine biochemistry parameters.

Anemia was defined as a hemoglobin concentration <13 g/dL for men and <12 g/dL for women. Absolute iron deficiency (ID) was defined as a serum ferritin level <100 ng/mL. Functional iron deficiency (FID) was defined as a serum ferritin level between 100–500 ng/mL combined with a transferrin saturation (TSAT) <20%.

Ethical approval

This study was conducted in accordance with the ethical rules of medical research at the University of Al-Qadisiyah, collage of medicine. Before sampling, the consent of the patient or his companion was taken. The study protocol, subject Information and approval form were reviewed and approved by the clinical chemistry unite of the laboratory in al Diwaniyah Teaching Hospital accordance with Document No. 472130 dated (29/10/2024) to obtain this approval.

Statistical analysis

Statistical analyses of the demographic and clinical characteristics of the individuals diagnosed with CKD were performed using GraphPad Prism® (version 9.0 GraphPad Software, San Diego, CA, USA). Continuous variables were analyzed using independent-sample t-tests (a), whereas categorical variables were evaluated using the Chi-square test (b). For datasets comprising more than two groups, one-way

analysis of variance (ANOVA) was implemented, followed by Tukey's post-hoc test for specific pairwise comparisons.

Results

Table 1 compares the demographic features age, sex, and BMI and key clinical biomarkers hemoglobin, CRP, serum creatinine, blood urea, and eGFR between 60 CKD patients and 40 healthy controls. CKD patients exhibited significantly lower hemoglobin, higher CRP, elevated serum creatinine/blood urea, and reduced eGFR than controls ($p<0.001$). The CKD stages were distributed as follows: stage 2 (21.6%), stage 3a (27%), stage 3b (23.4%), and stage 4 (28%).

Table 1. Demographic and clinical characteristics of CKD patients and healthy controls*

Demographic & biomarker features		**CKD patients (n=60)**	**Healthy control (n=40)**	**p**
Age (years)	Mean±SD	53.50±19.4	51.46±17.3	0.6[a]
Gender	Male	31	22	0.4[b]
	Female	29	18	
BMI (kg/m^2)	Mean±SD	25.24±4.6	26.3±3.9	0.3[a]
Hemoglobin (g/dL)	Mean±SD	9.72±0.85	14.1±1.2	<0.001[a]
CRP (mg/L)	Mean±SD	29±13.4	2.6±1.7	<0.001[a]
Serum creatinine (mg/dL)	Mean±SD	4.1±1.6	0.91±0.2	<0.001[a]
Blood urea (mg/dL)	Mean±SD	108.9±38.6	30.1±7.5	<0.001[a]
eGFR (mL/min/1.73 m²)	Mean±SD	45.4±22.6	108±7.9	<0.001[a]
Stages among patients	Stage 2	13 (21.6)	-	-
	Stage 3a	16 (27)	-	-
	Stage 3b	14 (23.4)	-	-
	Stage 4	17 (28)	-	-

* a – independent sample t-test, B – Chi-square

Table 2 highlights the differences in iron-related biomarkers between patients with CKD and the controls. CKD patients had significantly higher ferritin ($p<0.001$) and hepcidin ($p<0.001$) levels, but lower serum iron ($p<0.001$), TIBC ($p=0.004$), and transferrin ($p=0.005$) levels than healthy individuals, indicating dysregulated iron homeostasis in CKD (Fig. 1).

Table 2. Iron metabolism biomarkers in CKD patients and healthy controls*

Demographic and biomarker features		CKD patients (n=60)	Healthy control (n=40)	p
Ferritin (ng/mL)	Mean±SD	246±128	160±61	<0.001 [a]
Serum iron (µmol/L)	Mean±SD	12.38±3.1	118±33.5	<0.001 [a]
TIBC (µg/dL)	Mean±SD	347±109	385±62	0.004 [a]
Transferrin (mg/dL)	Mean±SD	224±74	258±39	0.005 [a]
Hepcidin (pg/mL)	Mean±SD	39.8±23.1	13.7±4.8	<0.001 [a]

* a – independent sample t-test

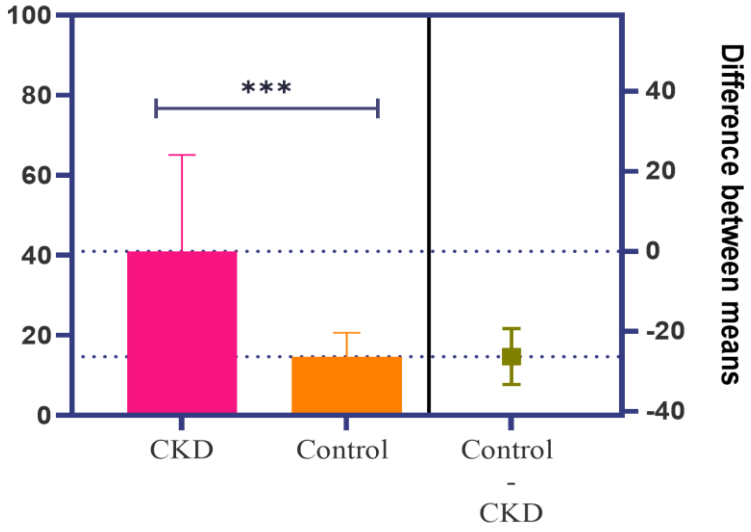

Fig. 1. Bar chart showing comparison of mean serum hepcidin among control group and CKD group

Biomarker levels (ferritin, serum iron, TIBC, transferrin, and hepcidin) were stratified according to CKD stage (2–4). Ferritin levels decreased with advancing stage (p=0.003), whereas hepcidin levels increased significantly (p<0.001). TIBC and transferrin levels declined progressively (p<0.001), reflecting worsening iron availability and inflammation. Post hoc analysis (Tukey's test) identified distinct inter-stage differences (denoted by superscript letters, Table 3, Fig. 2).

Table 3. Variation of serum biomarkers across CKD stages[#]

Serum biomarkers and CKD		Stage of CKD				p
		Stage 2 (n=13)	Stage 3a (n=16)	Stage 3b (n=14)	Stage 4 (n=17)	
Ferritin (ng/mL)	Mean±SD	328±107^{A}	206±132^{B}	219±141^{B}	155±116^{C}	0.003
Serum iron (µmol/L)	Mean±SD	11.2±2.7^{A}	10.9±2.7^{A}	7.4±1.6^{B}	10.4±3.0^{A}	0.04

TIBC (µg/dL)	Mean±SD	417±68^{A}	371±85^{B}	271±63^{C}	247±98^{C}	<0.001
Transferrin (mg/dL)	Mean±SD	293±58^{A}	268±61^{B}	203±44^{C}	181±71^{C}	<0.001
Hepcidin (pg/mL)	Mean±SD	13.8±3.9^{A}	35.8±8.2^{B}	29.6±6.8^{B}	68.6±15.8^{C}	<0.001

[#] O – one-way ANOVA, * – significant at p<0.05, *** – significant at p<0.001, statistical analysis was performed using one-way ANOVA with post-hoc Tukey's test for pairwise comparisons, superscript letters (A, B, C) denote the results of these pairwise comparisons, groups that do not share a common letter are significantly different from each other (p<0.05)

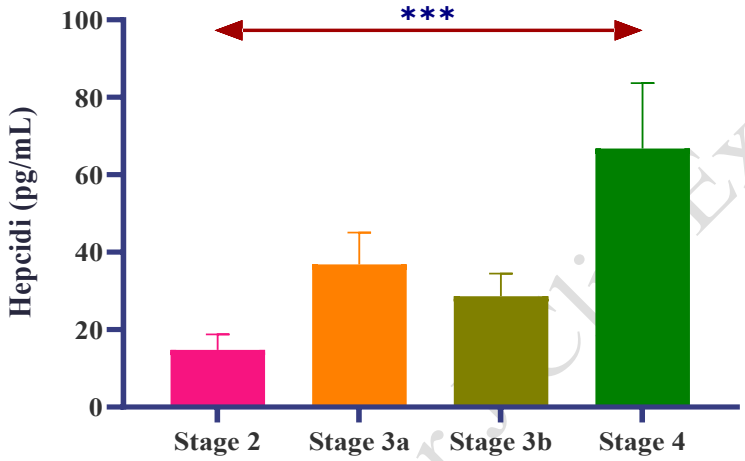

Fig. 2. Bar chart presenting the distribution of hepcidin levels among various stages of CKD

Hepcidin demonstrated high diagnostic accuracy for CKD at a cut-off of >25 pg/mL, with 80% sensitivity, 100% specificity, 100% PPV, 80% NPV, and an AUC of 86% (p<0.001). This finding supports the potential of hepcidin as a biomarker for CKD (Table 4, Fig. 3).

Table 4. Diagnostic performance of hepcidin for CKD detection*

Biomarkers	Cut-off value	Sens %	Spec %	PPV	NPV	Accuracy	AUC (95% CI)	p
Hepcidin (pg/mL)	>25	80	100	100	80	0.79	86 (0.78 to 0.92)	<0.001

* Sens – sensitivity, Spec – specificity, PPV – positive predictive value, NPV – negative predictive value, AUC – area under the curve

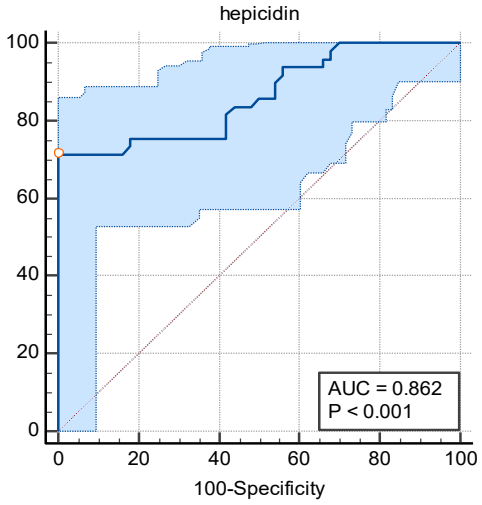

Fig. 3. ROC curve chart of hepcidin in CKD

This correlation matrix revealed associations between iron markers (transferrin, TIBC, ferritin, and hepcidin) and renal function indices (eGFR and serum creatinine). Hepcidin levels were strongly correlated with eGFR (r=-0.800, p<0.001) and serum creatinine (r=0.702, p<0.001), whereas transferrin and TIBC were positively correlated with eGFR (r=0.433, p<0.001). Ferritin showed weak associations with the other parameters (Table 5).

Table 5. Correlation between iron metabolism biomarkers and renal function parameters in CKD

Transferrin (mg/dL)	1	1.000 p<0.001	0.201 p=0.045	0.222 p=0.026	0.433 p<0.001	-0.036 p=0.725	-0.519 p<0.001	-0.311 p=0.001
TIBC (µg/dL)	1.000 p<0.001	1	0.201 p=0.045	0.222 p=0.026	0.433 p<0.001	-0.036 p=0.725	-0.519 p<0.001	-0.311 p=0.001
Hemoglobin (g/dl)	0.201 p=0.045	0.201 p=0.045	1	0.834 p<0.001	0.763 p<0.001	-0.498 p<0.001	-0.504 p<0.001	-0.679 p<0.001
Serum iron (µmol/L)	0.222 p=0.026	0.222 p=0.026	0.834 p<0.001	1	0.811 p<0.001	-0.458 p<0.001	-0.563 p<0.001	-0.691 p<0.001
eGFR (mL/min/1.73 m²)	0.433 p<0.001	0.433 p<0.001	0.763 p<0.001	0.811 p<0.001	1	-0.277 p=0.005	-0.800 p<0.001	-0.804 p<0.001
Ferritin (ng/mL)	-0.036 p=0.725	-0.036 p=0.725	-0.498 p<0.001	-0.458 p<0.001	-0.277 p=0.005	1	0.068 p=0.501	0.268 p=0.007
Hepcidin (pg/ml)	-0.519 p<0.001	-0.519 p<0.001	-0.504 p<0.001	-0.563 p<0.001	-0.800 p<0.001	0.068 p=0.501	1	0.702 p<0.001
Serum creatinine (mg/dL)	-0.311 p=0.001	-0.311 p=0.001	-0.679 p<0.001	-0.691 p<0.001	-0.804 p<0.001	0.268 p=0.007	0.702 p<0.001	1

	Transferrin (mg/dL)	TIBC (µg/dL)	Hemoglobin (g/dl)	Serum iron (µmol/L)	eGFR (ml/min/1.73 m²)	Ferritin (ng/mL)	Hepcidin (Pg/ml)	Serum creatinine (mg/dL)

Discussion

These findings revealed notable changes in iron metabolism biomarkers across the progressive stages of CKD, characterized by a gradual reduction in serum ferritin and transferrin concentrations. These trends underscore the multifactorial pathophysiology of disruption of iron homeostasis in advancing renal dysfunction, reflecting the interplay between regulatory mechanisms and disease progression. Current clinical guidelines, including KDIGO-2012 and ERBP 2013, advocate routine assessment of iron status in patients with CKD, predominantly through ferritin and TSAT measurements.[18] However, emerging evidence has challenged the reliability of these biomarkers. A clinical investigation reported that only 12% of hemodialysis patients exhibited normal transferrin levels, with 88% displaying varying degrees of malnutrition (5% severe, 30% moderate, and 53% mild). This malnutrition-associated hypotransferrinemia may artificially elevate TSAT values, potentially obscuring the true iron status in CKD populations.[19]

The interpretation of serum ferritin is further complicated by its dual role as an acute phase reactant. Multiple studies corroborate its elevation during inflammatory states, a common comorbidity in patients with CKD, thereby limiting its specificity as an iron storage marker.[20–22] Rashid and Al-Rubaie reinforced this limitation in an ESRD cohort, where 72.5% exhibited hyperferritinemia (≥300 ng/mL); however, these levels correlated more strongly with systemic inflammation than iron stores. Their analysis demonstrated that the combined TSAT-ferritin assessment achieved only a 47.5% diagnostic accuracy for iron status determination in ESRD.[23] These diagnostic challenges are further exemplified in the Al-Mashdali case report, where tissue iron quantification via T2* magnetic resonance imaging (MRI) revealed significant hepatic iron overload despite chronically elevated serum ferritin levels (>1000 µg/L) without clinical correlates.[24] The apparent paradox of declining ferritin in advanced CKD stages, contrary to expected inflammation-driven elevations, may reflect superimposed true ID from multifactorial etiologies, including malnutrition, occult gastrointestinal bleeding, or dialysis-related iron losses. Concurrent reductions in transferrin levels align with the established pathophysiology of impaired hepatic synthesis in uremia, exacerbated by chronic inflammation and protein energy wasting.[25]

The present study indicated that patients with CKD demonstrated significantly elevated hepcidin levels compared with healthy individuals, with a pronounced increase as kidney dysfunction advanced. Hepcidin concentrations progressively increased from the early to late CKD stages, correlating strongly with the disease severity. A cross-sectional study of 199 non-dialyzed patients with CKD was conducted to evaluate the relationship between hepcidin and iron disorders, inflammation, and hemoglobin levels. They found that hepcidin levels increased as GFR declined, with median values rising from 23.3 ng/mL in early stage

CKD to 36.1 ng/mL in advanced CKD. Absolute ID (defined as TSAT <20% and ferritin <40 ng/mL) was associated with significantly lower hepcidin levels (5.0 ng/mL), whereas inflammation combined with normal iron status led to higher hepcidin concentrations (34.5 ng/mL). The study concluded that hepcidin levels in CKD are independently influenced by iron status and inflammation, with renal clearance playing a secondary role.[26]

Hepcidin demonstrates significant potential as a diagnostic biomarker for CKD-associated iron dysregulation. In the present study, a hepcidin threshold (>23.2 pg/mL) yielded robust performance metrics for distinguishing CKD patients from healthy controls: 80% sensitivity, 100% specificity, 100% PPV, 85% NPV, and an AUC of 86%. These findings contribute to the growing body of evidence exploring hepcidin role in iron homeostasis in CKD. While our study design did not include a formal analysis against predefined ID thresholds (e.g., KDIGO criteria: ferritin <100 ng/mL or TSAT <20%) to directly compare diagnostic accuracy for ID, the observed strong inverse correlations between hepcidin and markers of iron availability (serum iron, TIBC, transferrin) support its central role in iron metabolism dysregulation. Notably, prior studies such as that by Gao et al. (2023) have reported that hepcidin outperformed ferritin/TSAT combinations for identifying functional iron deficiency, with high AUC values (0.94) and specificity (87%), particularly in distinguishing true functional iron deficiency from inflammation-driven hyperferritinemia.[14] The 2023 KDIGO Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease also highlights the limitations of traditional markers and acknowledges the emerging role of hepcidin, though it notes that more evidence is needed before routine clinical use can be recommended.[27]

Emerging evidence underscores the critical role of hepcidin dysregulation in CKD progression and associated complications. A pivotal study demonstrated that patients in the highest hepcidin quartile (25.1–80.0 ng/mL) exhibited a 1.37-fold increased risk of ESKD compared with those in the lowest quartile. This association followed a linear dose-response relationship, persisting even after adjustment for traditional risk factors, positioning hepcidin as a novel prognostic biomarker for CKD progression and as a potential tool for risk stratification.[28] The regulation of hepcidin is further influenced by dialysis modality, as highlighted by Malyszko et al. Their comparative analysis revealed that peritoneal dialysis (PD) patients had significantly elevated hepcidin/ferritin ratios, 2.5-fold higher than non-dialysis (ND) and hemodialysis (HD) cohorts, likely due to sustained intraperitoneal inflammation and impaired clearance mechanisms. These findings suggest that PD-associated inflammation exacerbates iron sequestration, emphasizing modality-specific pathways in hepcidin dysregulation.[29]

Notably, elevated hepcidin levels are closely associated with functional iron deficiencies (FID) and subclinical inflammation. In patients with FID, hepcidin levels in the highest quartile correlated strongly with hs-CRP levels, implicating iron dysregulation as both a consequence and driver of inflammatory processes. Importantly, early CKD stages showed that hepcidin elevation predicts cardiovascular morbidity,

advocating routine monitoring in early stage patients to mitigate downstream complications.[30] Furthermore, reinforcing this interplay, hepcidin was positively correlated with IL-6, a key inflammatory cytokine, and inversely correlated with hemoglobin and glomerular filtration rate (GFR). These associations underscore inflammation-driven hepcidin upregulation as a central mechanism in iron-restricted erythropoiesis, worsening anemia, and FID in CKD patients.[31]

Collectively, these studies delineate hepcidin as a multifunctional mediator that links iron metabolism, inflammation, and CKD progression. This elevation reflects a vicious cycle of inflammation-driven iron sequestration, exacerbation of anemia, and accelerated renal decline. These insights advocate the integration of hepcidin assessment into clinical practice, particularly for early risk stratification and personalized iron therapy, while highlighting the need for modality-specific management strategies for dialysis-dependent patients.

Study limitations

A key limitation of this study, common to many CKD biomarker investigations, is the absence of a universally accepted non-invasive gold standard for functional iron deficiency; while hepcidin demonstrated superior specificity and stronger correlations with renal function decline compared to current clinical biomarkers (ferritin, TSAT components), definitive validation against a reference standard like bone marrow iron was not feasible in this clinical setting. Furthermore, the study's cross-sectional design did not account for longitudinal variations in factors such as dietary iron intake and inflammatory status over time, which may influence iron biomarker levels and introduce bias. The use of a single commercial ELISA kit for hepcidin measurement, without inter-assay calibration against a standardized reference, may also contribute to measurement variability and limit the direct comparability of our absolute values with those from other studies. Future studies incorporating such reference validation methods where possible, accounting for dietary and socioeconomic variables, and conducted on a larger scale, particularly in LMICs, are warranted to definitively validate hepcidin diagnostic utility, establish its prognostic value, and determine its integration into CKD management protocol.

Conclusion

This study established hepcidin as a robust diagnostic biomarker for iron metabolism dysregulation in CKD, particularly in Iraqi populations. Elevated hepcidin levels correlate strongly with advanced CKD stages and renal dysfunction, demonstrating superior specificity compared to conventional markers. These findings underscore the clinical relevance of hepcidin in regions with limited diagnostic resources and offer a viable tool for early ID detection and therapeutic monitoring.

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Declarations

Funding

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Author contributions

Conceptualization, MAJT and HAJA; Methodology, MAJT; Software, MAJT; Validation, MAJT and HAJA; Formal Analysis, MAJT; Investigation, MAJT; Resources, MAJT; Data Curation, MAJT; Writing – Original Draft Preparation, MAJT; Writing – Review & Editing, MAJT; Visualization, HAJA; Supervision, HAJA; Project Administration, MAJT; Funding Acquisition, HAJA

Conflicts of interest

The authors declare that they have no competing interests.

Data availability

The datasets generated and analyzed during the current study are not publicly available due to participant privacy and confidentiality concerns but are available from the corresponding author on reasonable request.

Ethics approval

The study protocol, subject Information and approval form were reviewed and approved by the clinical chemistry unite of the laboratory in al Diwaniyah Teaching Hospital accordance with Document No. 472130 dated (29/10/2024) to obtain this approval.

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