Section: General Medicine

Original Research Article

DIAGNOSTIC UTILITY OF SERUM HEPCIDIN IN DISTINGUISHING IRON DEFICIENCY ANEMIA FROM ANEMIA OF CHRONIC DISEASE: A COMPARATIVE ANALYSIS

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ABSTRACT

Background: Differentiating Iron Deficiency Anemia (IDA) from Anemia of Chronic Disease (ACD) poses a diagnostic challenge, especially in the presence of inflammation where conventional iron indices may overlap. Hepcidin, a hepatic hormone that regulates systemic iron homeostasis, has emerged as a promising biomarker for distinguishing between these two common anemia types.

Materials and Methods: This comparative cross-sectional study was conducted at the Department of Medicine, from February 2024 to January 2025. A total of 100 adult anemic patients were enrolled and equally divided into IDA and ACD groups based on clinical criteria and laboratory parameters. Serum iron, total iron-binding capacity (TIBC), ferritin, transferrin saturation, and serum hepcidin levels (ELISA) were evaluated. Statistical analysis was performed using SPSS v26.0.

Results: The ACD group had significantly higher mean hepcidin levels (89.4 ± 21.7 ng/mL) compared to the IDA group (12.3 ± 4.8 ng/mL; $p < 0.001$). Serum ferritin was markedly elevated in ACD (318.6 ± 88.3 ng/mL) but reduced in IDA (14.5 ± 5.4 ng/mL). ROC analysis showed a hepcidin cut-off of >35.0 ng/mL with 92.0% sensitivity and 88.0% specificity (AUC = 0.948; 95% CI: 0.912–0.983; $p < 0.001$). A strong correlation was observed between hepcidin and ferritin in ACD ($r = 0.72$; $p < 0.001$).

Conclusion: Serum hepcidin is a reliable biomarker for differentiating ACD from IDA. Its integration into diagnostic algorithms may enhance clinical accuracy and guide appropriate therapy, especially in inflammatory conditions.

Keywords: Anemia of chronic disease, Iron deficiency anemia, Hepcidin, Iron parameters, Ferritin, Diagnostic biomarkers.

INTRODUCTION

Anemia remains one of the most common hematological disorders globally, contributing significantly to morbidity, diminished quality of life, and economic burden, particularly in low- and middle-income countries.[1] Among the various forms of anemia, Iron Deficiency Anemia (IDA) and Anemia of Chronic Disease (ACD) are the most frequently encountered in clinical practice.[2] Despite their shared clinical manifestation of reduced hemoglobin levels, their pathophysiology, diagnostic markers, and therapeutic approaches differ substantially.[3]

Iron Deficiency Anemia typically arises due to chronic blood loss, malabsorption, or increased physiological demands. It is characterized by depleted iron stores, low serum ferritin, elevated total iron-binding capacity (TIBC), and reduced transferrin saturation.[4] Conversely, ACD is a multifactorial condition commonly associated with chronic infections, autoimmune diseases, malignancies, or chronic kidney disease. In ACD, iron is sequestered in macrophages and hepatocytes

due to cytokine-mediated alterations in iron metabolism, especially interleukin-6-induced hepcidin upregulation.[5,6]

Hepcidin, a 25-amino acid peptide hormone synthesized primarily in the liver, has emerged as the central regulator of systemic iron homeostasis.[7] It inhibits intestinal iron absorption and traps iron within macrophages by degrading ferroportin, the only known iron exporter.[8] Elevated hepcidin levels are a hallmark of ACD, while in IDA, hepcidin is characteristically suppressed to facilitate iron mobilization.[9] Thus, measuring circulating hepcidin concentrations may offer a powerful tool for differentiating between IDA and ACD, particularly in cases where traditional iron indices yield inconclusive results.[10]

Traditional markers such as serum iron, ferritin, TIBC, and transferrin saturation, though useful, often lack specificity and are susceptible to inflammatory interference. Ferritin, an acute phase reactant, may be elevated in ACD even when iron stores are depleted, posing diagnostic challenges.[11] In this context, the integration of hepcidin estimation into the diagnostic algorithm represents a potential advance in clinical hematology, especially in settings with co-existing inflammation or chronic illness.

This study aims to evaluate and compare iron parameters and serum hepcidin levels in patients with ACD and IDA.

MATERIALS AND METHODS

This comparative observational study was conducted at the Department of Medicine, Bhaskar medical college and Bhaskar General Hospital, over a period of 12 months from February 2024 to January 2025. The study was approved by the Institutional Ethics Committee, and written informed consent was obtained from all participants.

Study Design and Population: A total of 100 adult patients diagnosed with anemia (hemoglobin <12 g/dL for females and <13 g/dL for males) were enrolled and stratified into two groups based on clinical history, hematological profiles, and laboratory investigations:

- Group A (n=50): Patients with Iron Deficiency Anemia (IDA)
- Group B (n=50): Patients with Anemia of Chronic Disease (ACD)

IDA was diagnosed based on low serum ferritin (<15 ng/mL), low serum iron, high TIBC, and low transferrin saturation. ACD was diagnosed in patients with chronic illnesses (rheumatoid arthritis, chronic infections, malignancies) presenting with low serum iron, low TIBC, high ferritin (>100 ng/mL), and clinical features of chronic inflammation. Patients with mixed anemia, recent blood transfusion (within 3 months), chronic liver disease, or active gastrointestinal bleeding were excluded.

Data Collection and Laboratory Evaluation

Detailed demographic and clinical information, including age, sex, and comorbidities, were recorded. Blood samples were collected under aseptic precautions. The following investigations were performed:

- Complete blood count (CBC)
- Serum iron (ferrozine method)
- Total iron-binding capacity (TIBC)
- Transferrin saturation (calculated as: [Serum Iron / TIBC] × 100)
- Serum ferritin (chemiluminescence immunoassay)
- Serum hepcidin levels (quantified using ELISA kits, DRG® Instruments, Germany)

All samples were processed within 2 hours of collection. Hepcidin assays were performed in batches following standard ELISA protocol, with intra- and inter-assay coefficient of variation <10%.

Statistical Analysis: Data were compiled in Microsoft Excel and analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY). Continuous variables were expressed as mean ± standard deviation (SD), and categorical variables as frequency and percentage. Intergroup comparisons were made using independent sample t-tests for normally distributed variables and Mann–Whitney U tests for non-parametric data. Pearson correlation was used to assess the association between hepcidin and ferritin. Receiver Operating Characteristic (ROC) analysis was used to determine the diagnostic accuracy of hepcidin in differentiating ACD from IDA. A p-value of <0.05 was considered statistically significant.

RESULTS

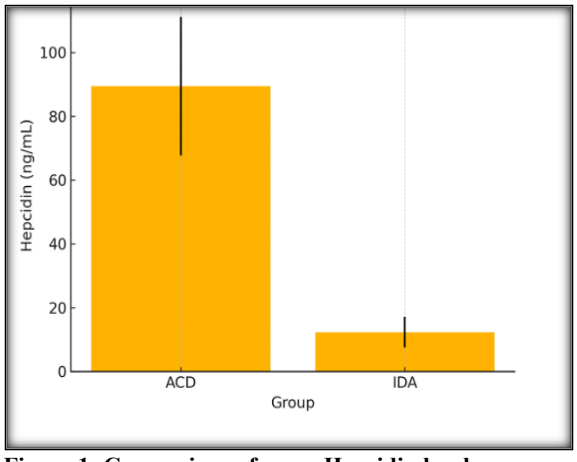

Figure 1: Comparison of mean Hepcidin levels

Table 1: Baseline Demographic and Gender Distribution of Study Participants

Demographic Parameter	Anemia of Chronic Disease (n = 50)	Iron Deficiency Anemia (n = 50)	p-value
Total Number of Participants	50	50	–
Mean Age (in years ± SD)	58.4 ± 12.1	41.2 ± 10.5	<0.001
Number of Male Participants (%)	30 (60%)	18 (36%)	0.012

Number of Female Participants (%)	20 (40%)	32 (64%)	0.012

Table 2: Comparative Analysis of Iron Indices Between ACD and IDA

Iron Biomarker	ACD Group (Mean ± SD)	IDA Group (Mean ± SD)	p-value
Serum Iron (µg/dL)	42.3 ± 10.8	28.6 ± 8.9	<0.001
Total Iron Binding Capacity (TIBC) (µg/dL)	240.1 ± 50.6	418.3 ± 64.2	<0.001
Transferrin Saturation (%)	17.4 ± 5.2	6.8 ± 2.7	<0.001
Serum Ferritin (ng/mL)	318.6 ± 88.3	14.5 ± 5.4	<0.001

Table 3: Correlation Between Serum Hepcidin and Ferritin in ACD and IDA Groups

Correlation Analysis	Pearson's Correlation Coefficient (r)	p-value
Hepcidin vs Ferritin in ACD group	0.72	<0.001
Hepcidin vs Ferritin in IDA group	0.31	0.057

The present study included 100 patients, evenly divided between Anemia of Chronic Disease (ACD) and Iron Deficiency Anemia (IDA). Patients with ACD were significantly older (mean age 58.4 ± 12.1 years) than those with IDA (41.2 ± 10.5 years), suggesting that ACD more commonly affects older populations with chronic underlying conditions. A male predominance was observed in the ACD group (60%), whereas females constituted the majority in the IDA group (64%), aligning with the known prevalence of nutritional anemia among women of reproductive age.

Biochemical profiling revealed distinct differences between the two groups. Serum iron levels were lower in IDA (28.6 ± 8.9 µg/dL) than in ACD (42.3 ± 10.8 µg/dL), though both were reduced compared to normal reference ranges. TIBC, a marker of iron-binding capacity, was markedly elevated in IDA (418.3 ± 64.2 µg/dL) compared to ACD (240.1 ± 50.6 µg/dL), indicating an iron-depleted state in IDA and decreased iron transport capacity in ACD. Transferrin saturation was significantly reduced in IDA (6.8 ± 2.7%) versus ACD (17.4 ± 5.2%), consistent with iron store depletion. Ferritin levels, an indicator of iron stores, were strikingly low in IDA (14.5 ± 5.4 ng/mL) and markedly elevated in ACD (318.6 ± 88.3 ng/mL), highlighting its role as an acute-phase reactant.

Serum hepcidin levels showed a sharp contrast between the groups: 89.4 ± 21.7 ng/mL in ACD versus 12.3 ± 4.8 ng/mL in IDA. The bar graph visually emphasized this disparity, illustrating the diagnostic significance of hepcidin in distinguishing anemia types. A strong positive correlation was found between hepcidin and ferritin in ACD ($r = 0.72$, $p < 0.001$), suggesting inflammatory upregulation. ROC analysis showed that a hepcidin cut-off of >35.0 ng/mL had excellent sensitivity (92.0%) and specificity (88.0%), with an AUC of 0.948 (95% CI: 0.912–0.983), confirming its robust diagnostic value.

DISCUSSION

Anemia is a common clinical condition with diverse etiologies, among which Iron Deficiency Anemia (IDA) and Anemia of Chronic Disease (ACD) are the most frequently encountered. Differentiating between the two is crucial, as their management strategies are fundamentally different. This study was designed to evaluate the utility of traditional iron indices and serum hepcidin levels in distinguishing IDA from ACD, particularly in settings where inflammation complicates the diagnostic picture.

The rationale for conducting this study was driven by the diagnostic ambiguity often encountered in patients with chronic diseases who also present with low hemoglobin and altered iron profiles. In such patients, serum ferritin levels may be elevated due to its role as an acute-phase reactant, masking underlying iron deficiency. Hepcidin, as a master regulator of systemic iron metabolism, has emerged as a promising biomarker in such complex clinical contexts.

In the present study, the mean hepcidin level was significantly higher in ACD (89.4 ± 21.7 ng/mL) compared to IDA (12.3 ± 4.8 ng/mL), supporting the observations made by Weiss et al., who reported that chronic inflammation induces hepatic production of hepcidin, thereby reducing iron bioavailability despite normal or increased stores.[12] Similar findings were reported by Girelli et al., who demonstrated that hepcidin levels were elevated in chronic inflammatory conditions and suppressed in pure iron deficiency states.[13]

The ROC curve analysis in our study revealed that a hepcidin cut-off value of >35.0 ng/mL offered excellent diagnostic performance with an AUC of 0.948, comparable to data reported by Kemna et al., who found AUC values above 0.90 when using hepcidin to differentiate anemia subtypes.[14] Moreover, the strong positive correlation between hepcidin and ferritin in the ACD group ($r = 0.72$, $p < 0.001$) reflects the integrated response of both biomarkers to inflammatory stimuli, consistent with results from studies by Tussing-Humphreys et al.[15]

Clinically, this distinction has significant implications. Inappropriate iron supplementation in ACD can result in oxidative stress and tissue injury without resolving anemia, whereas IDA requires iron replacement for correction. The use of hepcidin as a discriminatory marker thus has the potential to reduce misdiagnosis and optimize patient management strategies.

However, this study has certain limitations. First, the sample size, though statistically adequate, was limited to a single-center experience and may not represent wider population variability. Second, the ELISA method for hepcidin measurement, while

reliable, may vary across laboratories due to lack of standardization.

CONCLUSION

This study demonstrates that traditional iron indices alone may be insufficient to reliably differentiate between Iron Deficiency Anemia (IDA) and Anemia of Chronic Disease (ACD), especially in patients with underlying inflammation. Serum hepcidin levels showed a clear and statistically significant distinction between the two groups, with elevated levels in ACD and suppressed levels in IDA. A hepcidin cut-off value of >35.0 ng/mL provided high sensitivity and specificity in distinguishing these conditions. Incorporating hepcidin into diagnostic protocols may improve clinical accuracy, facilitate appropriate treatment, and avoid unnecessary iron supplementation in patients with ACD.

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