Original Article

Biochemical Assessment of Serum Hepcidin, Ferritin and Transferrin Saturation in Pregnant Women with Iron Deficiency Anemia in Thi-Qar Province – Iraq

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ABSTRACT

Objective: To assess serum hepcidin, ferritin, and transferrin saturation levels in pregnant women with iron deficiency anemia, and to explore their interrelationships as well as their possible diagnostic value as a combined biomarker panel

Study Design: Case-control observational study

Place and Duration of Study: This study was conducted at the outpatient antenatal clinics of Al-Nasiriyah Teaching Hospital, Thi-Qar Province – Iraq, from 15th August 2025 to 30th June 2026.

Methodology: Ninety pregnant women in their second and third trimesters were recruited, 60 women with iron deficiency anemia and 30 healthy pregnant controls. Venous blood samples were taken for the estimation of complete blood count, serum iron, total iron binding capacity, ferritin and hepcidinby enzyme-linked immunosorbent assay. Transferring saturation was computed as serum iron/total iron binding capacity.

Results: Compared with controls, women with iron deficiency anemia exhibited significantly lower levels of hemoglobin, ferritin and transferring saturation, with markedly reduced hepcidin concentrations (P<0.001 for each). Hepcidin correlated well with ferritin (r=0.71) and transferring saturation (r=0.62) and moderately with hemoglobin. Receiver operating characteristic analysis showed hepcidin had an excellent discriminative capacity for iron deficiency anemia (AUC = 0.91).

Conclusion: The addition of hepcidin to the standard iron parameters and its potential as a supplementary marker to improve the diagnosis of iron deficiency anemia in pregnancy.

Key Words: Iron deficiency anemia, Transferrin saturation, Pregnancy, Iron homeostasis, Ferritin.

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INTRODUCTION

Iron deficiency anemia (IDA) is the most common nutritional anemia known to occur in pregnancy. The World Health Organization estimates that nearly 40% of pregnant women worldwide are anemic and the majority of these cases are, at least in part, due to iron deficiency.[1] The disease is not only affect maternal well-being, but has also been reported to be associated with premature delivery, low birth weight, impaired neurocognitive development of the offspring and an increased risk of peripartum mortality.[2] Pregnancy is a major physiological stress on iron storage, mostly due to plasma volume expansion, fetal requirements and placental needs that increase the daily iron requirements considerably beyond what most diets can readily provide.[3]

Ferritin and transferrin saturation (TSAT) are the most extensively used biochemical indicators of iron status, but both have important limits in pregnancy.[4] The levels of ferritin are affected by inflammation and the TSAT shows a marked circadian rhythm.[5] More recently, hepcidin, a short 25 amino acid hepatic peptide, has been identified as the key regulator of iron homeostasis, regulating intestinal iron absorption and the release of iron from macrophages through its

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interaction with ferroportin.[6] When iron availability is low, hepcidin expression is suppressed, in theory allowing greater absorption and mobilization of iron, while inflammation produces the opposite effect.[7]

The non-pregnant populations have demonstrated a fairly tight relationship between hepcidin and iron markers.[8] However, data in pregnant women, especially those with overt IDA, remains comparatively limited. Moreover, regional variations in dietary habits, parasitic burden, and supplementation practice make it desirable to generate locally relevant data.[9]

METHODOLOGY

A case–control observational study was carried out at the outpatient antenatal clinics of Al-Nasiriyah Teaching Hospital, Thi-Qar Province – Iraq from 15th August 2025 to 30th June 2026 letter No. 7/54/624 dated 3rd August 2025. A total of 90 pregnant women were enrolled, of whom 60 were diagnosed with IDA and 30 were age- and gestation-matched healthy pregnant women. The diagnosis of IDA was based on the WHO definition (hemoglobin<11 g/dL during pregnancy) accompanied by a serum ferritin value below 30 ng/mL.[10]

Inclusion criteria for both groups were maternal age between 18 and 40 years; singleton pregnancy in the second or third trimester (gestational age 14-36 weeks); and willingness to provide written informed consent. Exclusion criteria were: multiple pregnancy, known hemoglobinopathies (e.g. thalassemia, sickle cell disease), chronic inflammatory or infectious disorders, recent blood transfusion (within 3 months), recent iron therapy (within 1 month), hepatic or renal dysfunction, history of malignancy and any acute infection at the time of sampling. Women with overt obstetric problems like pre-eclampsia or gestational diabetes were also eliminated to reduce confounding.[11]

Venous blood (about 8 mL) was collected from each participant, generally between 08:00 and 10:00 following an overnight fast, to minimize diurnal variation of iron-related indicators. The sample was separated into two aliquots, 2 mL in an EDTA tube for complete blood count and the remainder 6 mL in a plain serum separator tube. After complete clotting at room temperature, the latter was centrifuged at 3,000 rpm for 10 minutes; serum was then separated, distributed into aliquots, and stored at −80°C until analysis. Samples were thawed only once to limit assay drift, and all analyses were carried out within three months of collection.[12]

Complete blood count (CBC), including hemoglobin (Hb), red blood cell count (RBC), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and red cell distribution width (RDW), was performed using an automated hematology analyzer (Sysmex XN-1000, Kobe, Japan). Serum iron and total iron-binding capacity (TIBC) were measured by the ferrozine colorimetric method on an automated chemistry analyzer. Transferrin saturation (TSAT, %) was calculated using the standard equation: TSAT=(serum iron/TIBC)×100. Serum ferritin was quantified by an immunoturbidimetric assay calibrated against the WHO international standard. Serum hepcidin-25 was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (DRG International, USA), strictly following the manufacturer's instructions. All assays were performed in duplicate; intra- and inter-assay coefficients of variation remained below 8% and 10%, respectively.[13]

Data were analyzed using SPSS-31. Shapiro-Wilk tested continuous variables for normality. Group comparisons were performed using the independent-samples t-test or the Mann-Whitney U test and categorical variables were examined using the chi-square test. Pearson or Spearman correlation coefficients were used between variables according to the distribution of data. The diagnostic contribution of hepcidin, ferritin and TSAT was simulated using multivariate logistic regression. Receiver operating characteristic (ROC) curves were utilized to evaluate the diagnostic performance of these markers alone and in combination as a panel, reporting the area under the curve (AUC) with 95% confidence intervals. Two-sided P-values <0.05 were significant.

RESULTS

The demographics of the participants and obstetric features were shown in Table 1. The hematological parameters are explained in Table 2. The serum iron, TIBC, ferritin and TSAT are summarized in Table 3. IDA significantly reduced serum hepcidin levels in pregnant women compared to controls (Table 3). Correlation analysis revealed that hepcidin was positively and significantly associated with ferritin, and inversely correlated with TIBC as shown in Table 4 and Figure 1.

ROC curve study analyzedhepcidin, ferritin, and TSAT diagnostic accuracy (Figure 2). Hepcidin had an AUC of 0.91 (95% CI: 0.85-0.96) with a cut-off of 7.8 ng/mL, sensitivity of 87% and specificity of 89%. Ferritin showed an AUC of 0.94 (95% CI: 0.89-0.98) at a cut-off of 18 ng/mL and TSAT 0.88. The AUC increased modestly to 0.96 as a panel using logistic regression, suggesting that the addition of hepcidin to ferritin and TSAT increases diagnostic value, especially in borderline or unclear cases. Hepcidin is not redundant, even if ferritin alone performed marginally better in this group. Its key strength is identifying women with inflammation who may have difficulty interpreting ferritin according to traditional cut-offs.

Table No. 1: Demographic and clinical characteristics of pregnant women with IDA and healthy pregnant controls

Characteristic	IDA (n = 60)	Control (n = 30)	P-value
Maternal age (years), mean±SD	27.1±7.4	26.4±4.7	0.71
Gestational age (weeks), mean±SD	23.5±6.3	24.6±5.2	0.63
BMI (kg/m²), mean±SD	23.8±1.7	23.2±2.6	0.66
Parity, median (range)	3 (0–4)	3 (0–5)	0.72
Primigravida, n (%)	14 (23.3)	8 (26.7)	0.71
Urban residence, n (%)	38 (63.3)	19 (63.3)	0.93
Previous iron supplementation, n (%)	22 (36.7)	13 (43.3)	0.54
Education ≥ secondary, n (%)	41 (68.3)	22 (73.3)	0.61

Table No. 2: Comparison of hematological parameters between pregnant women with IDA and healthy pregnant controls

Parameter	IDA (n = 60)	Control (n = 30)	P-value
Hemoglobin (g/dL)	7.9±0.9	12.8±1.1	<0.001
RBC count (×10⁶/µL)	3.68±0.57	4.31±0.36	<0.001
Hematocrit (%)	29.6±3.2	37.2±2.4	<0.001
MCV (fL)	74.6±6.2	88.4±4.7	<0.001
MCH (pg)	24.1±2.7	29.6±1.9	<0.001
MCHC (g/dL)	30.4±2.1	33.5±2.2	<0.001
RDW (%)	17.7±2.1	12.2±2.3	<0.001

Table No. 3: Serum iron status parameters in pregnant women with IDA and healthy pregnant controls

Parameter	IDA (n = 60)	Control (n = 30)	P-value
Serum iron (µg/dL)	38.1±12.4	84.6±18.3	<0.001
TIBC (µg/dL)	446±52	358±41	<0.001
Transferrin saturation (%)	8.6±3.4	23.8±6.1	<0.001
Ferritin (ng/mL), median (IQR)	8.2 (4.1-14.6)	38.4 (24.7-55.2)	<0.001
Hepcidin (ng/mL), median (IQR)	3.4 (1.8-5.9)	18.6 (12.4-25.3)	<0.001

Table No. 4: Correlation coefficients between serum hepcidin and selected hematological and iron parameters across the combined study population (n = 90).

Parameter	Correlation coefficient (r)	P-value
Hemoglobin (g/dL)	0.52	< 0.001
Serum ferritin (ng/mL)	0.71	< 0.001
Serum iron (µg/dL)	0.59	< 0.001
TIBC (µg/dL)	−0.41	< 0.001
Transferrin saturation (%)	0.62	< 0.001
MCV (fL)	0.44	< 0.001
RDW (%)	−0.39	< 0.001

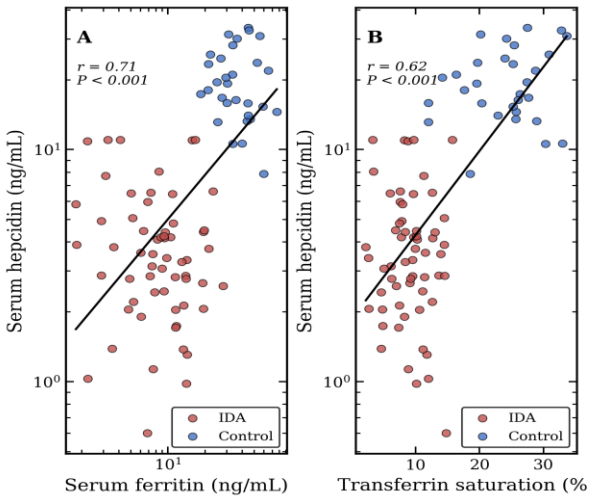


Figure No. 1: The relationship between serum hepcidin and (A) serum ferritin and (B) transferrin saturation in the combined study population. Black solid lines represent the linear regression fit (on log-transformed scales where appropriate).

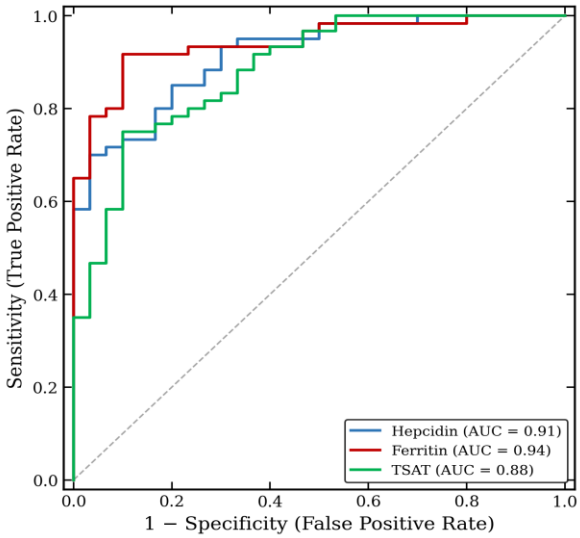


Figure No. 2: Receiver operating characteristic (ROC) curves for serum hepcidin, ferritin, and transferrin saturation in distinguishing pregnant women with iron deficiency anemia from healthy controls. The dashed diagonal indicates the line of no discrimination (AUC = 0.50). Corresponding AUC values are reported in the legend.

DISCUSSION

The present study revealed that hepcidin is reduced physiologically in IDA women compared to controls. Low hepcidin stabilizes ferroportin on the basolateral membranes of enterocytes, thereby permitting absorption of dietary iron and release of iron from macrophages and hepatocytes.[14] In women with minimal iron stores before pregnancy or in low-resource situations, inhibition of hepcidin alone is not

enough to restore iron status, and the woman moves from iron-depleted stores to iron-deficient erythropoiesis and anemia.[15] The extent of this suppression is consistent with the well-known notion that iron deficit downregulates hepcidin transcription, maximizing iron uptake and mobilization.[16] Many researchers believe that hepcidin is a better indicator of real iron shortage than ferritin, especially when systemic inflammation raises ferritin. The narrow interquartile range in the IDA group in our dataset shows that hepcidin suppression is a common aspect of pregnancy-related iron depletion, not a rare finding.[17]

The strength of association with ferritin closely mirrors what has been reported in non-pregnant populations and suggests that, even during pregnancy, ferritin remains an important determinant of hepcidin expression.[18] However, in a small subset of women in the IDA group with relatively higher ferritin (likely reflecting subclinical inflammation), hepcidin levels remained low; this implies that, at this stage of pregnancy, the iron-deficiency signal often outweighs the inflammatory drive on hepcidin regulation.[19]

In addition, iron status does not only affect hepcidin. Erythroferrone can be more inhibitory to hepcidin when erythropoiesis increases, which is essential in late pregnancy.[13] Inflammatory cytokines, such as IL-6, can counter these signals and increase hepcidin, leading to functional iron insufficiency even in the absence of a substantial reduction in total body iron.[20] The interplay of these competing inputs may explain some of the dispersion in our association study and is a reminder to always interpret hepcidin in a clinical situation.[21]

Our findings agree with many researchers found significantly lower hepcidin levels in iron-deficient pregnant women from European cohorts. Indian, Egyptian, Iranian, and sub-Saharan populations show similar patterns, but absolute hepcidin values vary between studies due to biological differences and methodological heterogeneity, especially in ELISA platform.[7-9] The cut-off of ~7.8 ng/mL in our cohort aligns with previously established limits, but should not be blindly applied to other labs.[22] This reinforces the idea that local reference intervals for hepcidin are needed before routine use. Hepcidin suppression with declining ferritin has also been found in neighboring nations, supporting the regulatory relationship.[23]

CONCLUSION

The pregnant women with iron deficiency anemia have lower serum hepcidin, ferritin, and transferrin saturation than healthy pregnant controls. Hepcidin was tightly associated with ferritin and TSAT and was an effective prenatal IDA diagnostic tool, the hepcidin's role as a central regulator of iron homeostasis and its potential as a supplementary biomarker for the iron panel. Hepcidin is particularly beneficial when ferritin interpretation is hampered by inflammation or borderline readings, which is typical in clinical practice. The integrating hepcidin measurement into prenatal anemia evaluation, but more prospective and interventional studies are needed to establish trimester-specific reference ranges and determine if it can predict iron therapy responsiveness. An integrated strategy may enhance diagnosis, treatment, and maternal and newborn outcomes.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Lina Abdullah Naser, Sadoun Abbas Alsalimi
Drafting or Revising Critically:	Lina Abdullah Naser, Idries Muhson Abeed Al-Mashkor
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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