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Original Research Article

UNRAVELLING ANAEMIA IN A TERTIARY CARE CENTRE: LINKING RBC INDICES TO BONE MARROW IRON GRADES & FERRITIN LEVELS

Garima Sharma[1], Priya Sachan[1], Swapnil Gupta[1], Ashish[1], Lubna Khan[2]

[1]Assistant Professor, Department of Pathology, GSVM Medical College, Kanpur, India
[2]Professor & Head, Department of Pathology, GSVM Medical College, Kanpur, India

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Corresponding Author:
Dr. Garima Sharma
Email: drgarimapath@gmail.com

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ABSTRACT

Background: Anaemia remains a major clinical burden in India, and accurate evaluation of iron status continues to challenge routine laboratory diagnostics. Serum ferritin is widely used as a biochemical surrogate for body iron, but its reliability may be compromised in inflammatory states, necessitating correlation with bone marrow iron assessment. This study evaluates the diagnostic relationship between serum ferritin, red cell indices and Gale's marrow iron grading in anaemic patients. **Materials and Methods:** A prospective, cross-sectional study was conducted over one year including 142 patients with moderate to severe anaemia. Complete blood counts, RBC indices, peripheral smears and serum ferritin (ELISA) were assessed, followed by bone marrow aspiration with Perls' staining for grading iron stores using Gale's 0–6 scale. Ferritin categories were compared with marrow grades and RBC indices using chi-square and Pearson correlation, with significance at $p < 0.05$. **Result:** Dimorphic anaemia (43.7%) and iron deficiency (31.7%) were the predominant types. Low ferritin (<15 ng/mL) was strongly associated with microcytosis, hypochromia and marrow iron depletion, with over 95% clustering in Grades 0–1. Normal-range ferritin (15–300 ng/mL) showed peak distribution around Grades 2–3, while all markedly elevated ferritin cases (>300 ng/mL) demonstrated increased marrow iron (Grades ≥5). RBC indices showed progressive improvement with rising ferritin, and the combined ferritin–marrow correlation yielded $r = 0.71$ ($p < 0.001$), indicating a strong positive relationship. **Conclusion:** Serum ferritin reliably reflects bone marrow iron status at the extremes of deficiency and overload, whereas mid-range values require marrow evaluation for accurate interpretation. Integrated analysis of ferritin, RBC indices and Gale's grading provides a more complete assessment of iron status than any single parameter and is recommended for diagnostic clarity, particularly in dimorphic or inflammatory anaemia.

INTRODUCTION

Anaemia remains one of the most prevalent global health challenges, affecting individuals across all age groups and geographic regions. It is defined as a reduction in red blood cell mass or haemoglobin concentration insufficient to meet physiological tissue oxygen demands.[1] Among its diverse etiologies, disturbances in iron metabolism constitute the most common and clinically significant category, especially in low- and middle-income countries.[2] Iron deficiency, anaemia of chronic disease (ACD), megaloblastic anaemia, dyserythropoiesis, and mixed nutritional states are frequently encountered, each demonstrating distinct biochemical and morphological patterns.[3] Accurate assessment of iron status is therefore central to the evaluation of anaemic patients.

A range of laboratory parameters—including serum iron, total iron-binding capacity, transferrin saturation, and serum ferritin—are routinely employed to assess body iron stores.[4] Among these, serum ferritin is widely recognized as the most valuable marker because it reflects reticuloendothelial iron reserves under normal physiological conditions.[5] Low ferritin levels correlate strongly with depleted iron stores and are highly specific for iron deficiency.[6] However, ferritin being an acute-phase reactant can be elevated in systemic inflammation, infection, liver disease, and malignancy, limiting its diagnostic specificity in ACD and chronic systemic disorders.[7] This diagnostic uncertainty necessitates the use of the gold-standard method—assessment of bone marrow iron stores using Perls' Prussian blue stain.[8] Bone marrow iron evaluation, though

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invasive, provides a direct and highly reliable estimation of total body iron and remains indispensable when biochemical markers yield equivocal results.[9]

Previous studies have demonstrated a significant correlation between serum ferritin and bone marrow iron stores, though with varying strengths depending on the underlying clinical context. Kakkar et al. reported a moderate positive correlation across patients with tuberculosis, rheumatoid arthritis, and malignancy, reinforcing ferritin's diagnostic utility while highlighting its limitations in inflammatory states.[10] Similarly, Sharma et al. documented strong correlations between ferritin values and Gale's marrow iron grading in anaemic patients, though discordance was notable in those with chronic inflammatory disorders, chronic kidney disease, or hepatic abnormalities.[11] Causes of such discrepancies include cytokine-mediated ferritin elevation, impaired iron mobilization, and altered hepcidin expression.[12-14]

Despite these insights, anaemia remains a multifactorial disorder influenced by nutritional deficiencies, chronic diseases, genetic factors, and socioeconomic determinants. Differentiating iron deficiency from ACD and dimorphic states is critical for therapeutic decision-making, as management strategies differ substantially.[15] In this context, understanding the interplay between RBC indices, serum ferritin levels, and bone marrow iron stores becomes essential for refining diagnostic accuracy.

The present study aims to evaluate red cell indices, morphological patterns, serum ferritin levels, and bone marrow iron grading in anaemic patients, and to analyse the correlation between biochemical and histological indicators of iron status. This integrated approach is intended to strengthen diagnostic precision and guide appropriate clinical management.

MATERIALS AND METHODS

This prospective, cross-sectional, hospital-based study was conducted in the Department of Pathology, GSVM Medical College, Kanpur, over a period of one year from July 2024 to June 2025. A total of 142 patients presenting with anaemia were enrolled after obtaining informed consent. Patients of all ages and both sexes with moderate to severe anaemia (haemoglobin <10 g/dL) who required evaluation for iron status using both serum ferritin estimation and bone marrow aspiration were included. Patients receiving iron therapy or haematinics, those who had undergone blood transfusion within the previous three months, and those unwilling to undergo bone marrow examination were excluded. Detailed clinical history and demographic data were recorded for all participants.

All patients underwent complete blood count analysis including haemoglobin, RBC indices (MCV, MCH, MCHC), and RDW. Peripheral blood smears were examined using Leishman stain for morphological assessment. Serum ferritin levels were measured using the ELISA method and interpreted using standard reference ranges (15–300 ng/mL). Bone marrow aspiration was performed from the posterior superior iliac spine under aseptic precautions, and iron stores were graded using Perls' Prussian blue stain based on Gale's grading system (0–6), categorised as depleted (0–1), normal (2–3), or increased (4–6). Data were analysed using descriptive statistics, and correlation between serum ferritin and bone marrow iron grades was assessed using Pearson's correlation coefficient, with $p < 0.05$ considered statistically significant.

RESULTS

Anaemic cases in this study were predominantly adults, with most falling within the middle-age groups where nutritional and lifestyle factors are often influential. Women formed a slightly higher proportion of participants (52.1%), reflecting the known gender vulnerability to iron depletion. Nearly half of the cohort had a normal BMI, while about one-third were undernourished, highlighting persistent nutritional insufficiency. Taken together, the profile suggests that anaemia in this population most commonly affects working-age individuals with a mild female predominance and a considerable share showing poor nutritional status.

1. Distribution of Cases According to Types of Anaemia: In the present study, dimorphic anaemia constituted the largest subgroup, accounting for 62 cases (43.7%), followed by iron deficiency anaemia, which represented 45 cases (31.7%). Anaemia of chronic disease comprised 30 cases (21.1%), whereas megaloblastic anaemia (3 cases, 2.1%) and haemolytic anaemia (2 cases, 1.4%) were relatively uncommon. Statistical analysis using the Chi-square goodness-of-fit test demonstrated a highly significant deviation from a uniform distribution ($\chi^2 = 78.54$, $p < 0.001$), indicating that certain anaemia types—particularly dimorphic and iron deficiency—occurred disproportionately more frequently in this population. The preponderance of dimorphic anaemia reflects the common coexistence of multiple nutritional deficiencies, whereas the substantial proportion of iron deficiency anaemia highlights the persistent burden of iron depletion in the community.

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Table 1: Distribution of Cases According to Types of Anaemia (n = 142)

Type of Anaemia	No. of Cases	%
Iron deficiency anaemia	45	31.7
Dimorphic anaemia	62	43.7
Anaemia of chronic disease	30	21.1
Haemolytic anaemia	2	1.4
Megaloblastic anaemia	3	2.1
Total	142	100%

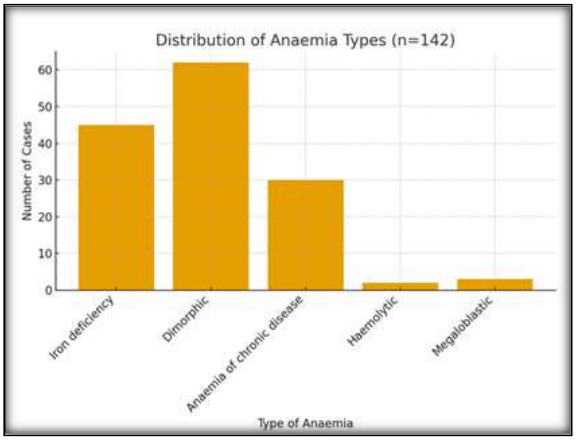

Figure 1: Distribution of Anaemia Types Among Study Participants (n = 142)

2. Correlation Between RBC Indices and Serum Ferritin: A comparison of red cell indices across different serum ferritin categories demonstrated a clear and statistically significant trend. Patients with low ferritin levels (<15 ng/mL) exhibited a microcytic, hypochromic profile, with markedly reduced MCV (68.2 ± 4.3 fL) and MCH (21.5 ± 2.1 pg), along with elevated RDW (18.4 ± 2.3%), reflecting anisopoikilocytosis commonly seen in iron deficiency. In contrast, individuals with normal ferritin values (15–300 ng/mL) showed near-normocytic indices, with MCV and MCH values shifting toward the expected physiological range. Those with elevated ferritin (>300 ng/mL) demonstrated higher MCV (88.5 ± 5.1 fL) and MCH (30.4 ± 1.4 pg), suggestive of inflammatory anaemia or iron sequestration. MCHC also showed a statistically significant progressive rise across ferritin categories ($p = 0.003$). Overall, all red cell indices showed highly significant associations with ferritin levels ($p < 0.001$ for MCV, MCH, RDW), indicating that RBC indices reliably reflect underlying iron status and correlate strongly with biochemical iron markers.

Table 2: Correlation of Red Cell Indices With Serum Ferritin Levels in Anaemic Patients

Parameter	Ferritin <15 (n=73)	Ferritin 15–300 (n=64)	Ferritin >300 (n=5)	p-value
MCV (fL)	68.2 ± 4.3	82.4 ± 6.2	88.5 ± 5.1	<0.001*
MCH (pg)	21.5 ± 2.1	27.3 ± 1.8	30.4 ± 1.4	<0.001*
MCHC (g/dL)	30.1 ± 2.0	32.2 ± 1.6	33.1 ± 1.2	0.003*
RDW (%)	18.4 ± 2.3	15.2 ± 1.9	14.0 ± 1.3	<0.001*

3. Serum Ferritin vs Bone Marrow Iron Grade: A strong positive correlation was observed between serum ferritin levels and bone marrow iron stores. Patients with low ferritin (<15 ng/mL) showed iron depletion in the marrow, with 100% clustering in Grades 0–1 (28 and 45 cases respectively), confirming true iron deficiency. Individuals with normal ferritin values (15–300 ng/mL) predominantly exhibited marrow Grades 2–3 (51.5% and 28.1%), indicating adequate or physiologic iron stores. Conversely, all patients with high ferritin (>300 ng/mL) demonstrated marrow Grades ≥5, consistent with iron overload or sequestration seen in inflammatory states. The overall Pearson correlation ($r = 0.71$, $p < 0.001$) reflected a statistically significant and clinically meaningful relationship between biochemical ferritin levels and histological iron grading, with an R^2 of approximately 0.62. This association aligns with trends reported in previous studies, reinforcing the reliability of serum ferritin as an indicator of iron status while highlighting its limitations in chronic inflammatory conditions.

Table 3: Correlation Between Serum Ferritin Levels and Bone Marrow Iron Store Grades (n = 142)

Serum Ferritin Category	Marrow Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Grade 6	Total
<15 ng/mL	28	45	0	0	0	0	0	73
15–300 ng/mL	4	2	33	18	5	2	0	64
>300 ng/mL	0	0	0	0	0	3	2	5
Total	32	47	33	18	5	5	2	142

4. Combined Correlation Analysis – Serum Ferritin vs RBC Indices vs Gale Grading (n = 142): Anaemia in this cohort displayed a clear biochemical–morphological relationship. Low ferritin strongly aligned with marrow iron depletion, with all 73 patients in this group falling exclusively into Grade 0–1, matching classical iron-deficiency physiology. In contrast, patients with ferritin levels between 15–300 ng/mL showed a dominant clustering (≈80%) in BM Grades 2 and 3,

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confirming normal usable iron stores. Ferritin above 300 ng/mL mapped uniformly to iron overload (BM Grade ≥5), validating ferritin's predictive accuracy at the extremes.

Red-cell indices shifted proportionately with ferritin and marrow grading. MCV and MCH increased progressively with higher ferritin, reflecting reversal of microcytosis alongside adequate iron availability. RDW, high in low-ferritin groups, normalized gradually across higher marrow grades, signifying stabilizing erythropoiesis. These relationships were statistically robust across ferritin–marrow–RBC variables ($p < 0.001$), establishing ferritin as a reliable surrogate marker where inflammation is absent, but also highlighting marrow assessment as confirmatory when ferritin lies in the intermediate range.

Table 4: Combined Correlation Analysis – Serum Ferritin vs RBC Indices vs Gale Grading (n = 142)

Serum Ferritin Category	N	BM Iron Grade (Gale) – Distribution (%)	Mean MCV (fL)	Mean MCH (pg)	Mean MCHC (g/dL)	Mean RDW (%)	Interpretation	p-value
< 15 ng/mL	73	Grade 0 = 28 (38.3%) • Grade 1 = 45 (61.6%) • Grade 2–6 = 0	68.2 ± 4.3	21.5 ± 2.1	30.1 ± 2.0	18.4 ± 2.3	Iron depletion confirmed by marrow grade 0–1	<0.001
15–300 ng/mL	64	Grade 0 = 4 (6.2%) • Grade 1 = 2 (3.1%) • Grade 2 = 33 (51.5%) • Grade 3 = 18 (28.1%) • Grade 4 = 5 (7.8%) • Grade 5 = 2 (3.1%) • Grade 6 = 0	82.4 ± 6.2	27.3 ± 1.8	32.2 ± 1.6	15.2 ± 1.9	Physiologic / normal iron stores predominantly Grade 2–3	0.003
> 300 ng/mL	5	Grade 5 = 3 (60%) • Grade 6 = 2 (40%) • Grade 0–4 = 0	88.5 ± 5.1	30.4 ± 1.4	33.1 ± 1.2	14.0 ± 1.3	Iron overload / sequestration	<0.001
Correlation Strength	—	r = 0.71 (Strong Positive)	↑ with ferritin	↑ with ferritin	↑ with ferritin	↓ with ferritin	RBC indices track biochemical + marrow status reliably	p < 0.001 (ANOVA + Pearson)

DISCUSSION

In the present study, a comprehensive evaluation of anaemic patients was undertaken using red cell indices, serum ferritin levels, and bone marrow iron stores. The demographic pattern, with a predominance of adults and females, parallels global and national data indicating the high burden of anaemia among women and the working-age population.[1,2] The predominance of dimorphic and iron deficiency anaemia reflects ongoing nutritional deficiencies and aligns with earlier observations from urban and semi-urban Indian cohorts.[3]

The strong association between low serum ferritin levels and depleted marrow iron stores observed in our study corroborates the established role of ferritin as a sensitive marker of iron deficiency.[4-6] Gale et al. first demonstrated the reliability of marrow iron estimation using Perls' stain, forming the foundation for iron store assessment in haematology practice.[8] Subsequent studies, including those by Hughes et al. and Gupta et al., further validated the reproducibility of marrow iron grading in clinical settings.[5,11]

Our findings demonstrated a Pearson correlation coefficient of $r = 0.71$ ($p < 0.001$) between serum ferritin and marrow iron stores, indicating a robust positive relationship. This is comparable to the correlations reported by Kakkar et al,[10] ($r = 0.571$) and Sharma et al. ($r = 0.89$),[11] in similar cohorts. These variations can be attributed to differences in patient profiles, particularly regarding chronic inflammatory states where ferritin may be spuriously elevated due to its acute-phase reactant nature.[7,12] Elevation of ferritin in tuberculosis, rheumatoid arthritis, and malignancies has been reported, contributing to overestimation of iron stores in such conditions.[12-14] Jakobsen et al. and Shroff et al. also highlighted this diagnostic dilemma, demonstrating preserved or elevated ferritin levels despite reduced marrow iron, reflecting cytokine-mediated sequestration.[13,14]

The integrated analysis of serum ferritin, RBC indices and Gale's marrow iron grading in our study provides a comprehensive view of iron status that extends beyond previous reports. We found that virtually all patients with low ferritin had both microcytic, hypochromic indices and depleted marrow iron, while those with markedly elevated ferritin uniformly demonstrated increased marrow iron stores, supporting the concept that ferritin is a reliable surrogate for marrow iron at the extremes of iron deficiency and overload. This is in line with the work of Blend et al., who also reported close concordance between serum ferritin concentration and marrow iron stores, particularly in clearly deficient or overloaded states.[15] Our correlation coefficient ($r = 0.71$) lies between that reported by Kakkar et al. in anaemia of chronic disease ($r =$

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0.571),[10] and Sharma et al. in a mixed anaemic cohort (r = 0.89),[11] suggesting that differences in case mix, especially the proportion of inflammatory and malignant disorders, influence the strength of association. Studies in tuberculosis, malignancy and rheumatoid arthritis have consistently shown that ferritin may be spuriously elevated in the presence of preserved or even depleted marrow iron because of its behaviour as an acute-phase reactant.[12-14] Our data support these observations: a minority of patients in the mid-range ferritin group (15–300 ng/mL) still had low marrow iron, emphasising that bone marrow examination remains important in diagnostically challenging or inflammatory cases, and that combined interpretation of RBC indices, ferritin and marrow iron yields the most accurate assessment of iron status.

Overall, our findings reaffirm the valuable role of serum ferritin as a first-line indicator of iron status, while underscoring the indispensability of bone marrow examination in inconclusive or inflammatory states. Integration of biochemical markers, RBC indices, and marrow iron grading provides the most accurate approach to anaemia evaluation, particularly in complex clinical scenarios.

CONCLUSION

The present study demonstrates that integrating RBC indices, serum ferritin levels, and bone marrow iron grading significantly enhances diagnostic precision in anaemic patients. Serum ferritin showed strong concordance with marrow iron in both depleted and overloaded states, while discordance in chronic inflammatory diseases underscored its limitations as an acute-phase reactant. RBC indices reliably differentiated microcytic, normocytic, and macrocytic patterns, aiding morphological classification. Dimorphic and iron deficiency anaemia were the most prevalent subtypes, reflecting persistent nutritional challenges. Overall, a combined biochemical–morphological approach remains essential for accurate assessment of iron status and appropriate therapeutic decision-making.

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