

Role of intensive method of bone marrow iron assessment and serum Ferritin in prediction of iron deficiency: a study of 143 patients

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Abstract

Introduction: Iron deficiency anemia is a major health problem in developing countries like India. The situation is even more grim in economically backward areas of our country like Bundelkhand region. The increasing prevalence of co-existence of iron deficiency anemia with chronic diseases makes the use of biochemical markers like serum ferritin, challenging in diagnosing cases of inflammation and functional iron deficiency. Microscopic evaluation is the “gold standard” method for assessment of iron stores. In this study, we tried to correlate the marrow iron stores with serum ferritin so as to facilitate the distinction of iron store deficiency from functional iron deficiency.

Materials and Methods: We selected 143 adult patients with anemia. In all the patients diagnostic bone marrow examination was done. Serum ferritin assay, CBC and peripheral blood smear examination was also performed. Assessment of bone marrow iron was done by both Gale’s method and the intensive method and was correlated with serum ferritin.

Results: The Conventional Gale’s grading revealed hypoferremic state in 46.85% cases and normal iron stores in 53.15% cases. The new Intensive method of grading, classifies anemias according to iron status. It showed that most common was combined deficiency (functional & iron stores) (43.36%), followed by normal stores, functional iron deficiency and lastly iron stores deficiency in Bundelkhand region. We also found a positive correlation of microcytic hypochromic blood picture with S.Ferritin and iron deficiency anemia (Spearman’s Rho correlation, p value=0.0001). The proposed cutoff value of S.ferritin as a surrogate marker for iron deficiency used in our study is <60ng/ml.

Conclusion: The new Intensive Bone Marrow Grading Method has greatly improved iron assessment because it provides a useful iron status classification which is of high clinical importance, especially in areas with high prevalence of inflammatory conditions co-existing with iron deficiency anemia, like Bundelkhand region and remains the gold standard method.

Keywords: Anemia, Ferritin, Iron, Bone marrow.

Introduction

Anemia is a major health problem worldwide especially in developing countries like India. According to a survey conducted in India, the prevalence of anemia was found to be over 70% in pregnant females and children while it was ~25% in adults.^{1,2} Iron deficiency is one of the commonest cause of anemia, where iron supplement is necessary for treatment. In many inflammatory conditions even in presence of sufficient iron store, a functional iron deficient state develops because of impaired transport of iron to the target tissue.³ This is attributed to the release of various cytokines during inflammation.⁴

To assess the storage iron, Prussian blue staining is considered to be the gold standard, despite of the newer methods to determine iron in tissue like mass spectrometry.⁵⁻⁸ In Perl’s staining the iron in form of hemosiderin is assessed in the bone marrow fragments.^{9,10} Iron can also be assessed in erythroblast and macrophages apart from fragments. Erythroblast iron indicate cellular iron and its depletion show functional iron deficiency however only few studies have been done on this.¹¹

The conventional Gale method throws light only on true deficiency of storage iron without any information

of functional iron deficiency. However intensive iron grading assesses iron in erythroblasts, macrophages along with bone marrow fragments.¹² It is imperative to know about the functional iron deficiency so as to prevent the patient from unnecessary iron treatment.

The serum ferritin value correlates with the iron store in the bone marrow. But being an acute phase reactant its levels increase in inflammatory conditions. A discrepancy can be found in serum ferritin levels in case of iron deficiency anemia in inflammatory conditions which are quite common in developing countries like India.¹³

This study was conducted with aim to distinguish the functional iron deficiency from storage iron deficiency by intensive bone marrow study and to see the correlation between serum ferritin and marrow iron stores.

Materials and Methods

A total of 143 adult patients with anemia admitted in the IPD of Medicine department, MLB medical College, Jhansi over a period of 12 months, were selected. In all the cases bone marrow examination was advised before any blood transfusion. We conducted a descriptive study on these patients after getting Ethical

clearance from Institutional ethical committee and informed and written consents from these patients.

Blood samples of these patients were collected by Venipuncture in EDTA and plain vials. EDTA samples were used for CBC and GBP examination. The plain sample was subjected to Serum Ferritin assay using Architect Ferritin kit by Chemiluminescent Microparticle Immunoassay (CMIA).

After doing Bone Marrow aspiration, slides were fixed with methanol and stained with Prussian blue stain along with positive control for each batch. Marrow smears were subjected to microscopic examination.

Bone Marrow smears were first assessed according to Gale's histological grading method¹⁰ which assesses only marrow fragments (table 1). Iron grade 0(none) and grade 1(very slight) were taken as iron deficiency.

Those smears are again evaluated for iron stores by intensive histological grading method,¹² in which apart

from fragments (Gale's method), iron was also assessed at two other sites- macrophages and erythroblasts.¹² For macrophage iron, 20 fields around the marrow fragments were assessed while for erythroblastic iron a minimum of 100 erythroblasts were examined. Presence of <30% of erythroblast with visible cytoplasmic iron granules was taken as erythroblast iron deficiency.

Four categories was assigned to assess the iron store by intensive histological grading method - Normal status (normal iron stores and normal erythroblast iron); Functional iron deficiency (normal iron stores and deficient erythroblast iron); Iron stores deficiency (depleted iron stores and normal erythroblast iron); and Combined functional iron and Iron stores deficiency (depleted iron stores and deficient erythroblast iron; table 3).¹²

Table 1: Histological grading for bone marrow iron status according to Gale et al¹⁰

| | | |
|----------------|------------------|---|
| Grade 0 | None | No visible iron under high power magnification (X1000) |
| Grade 1 | Very Slight | Small iron particles just visible in few reticulum cells under (X1000) |
| Grade 2 | Slight | Small sparsely distributed iron particles just visible under low power magnification (X100) |
| Grade3 | Moderate | Numerous small iron particles present in reticulum cells throughout the marrow fragment (X100) |
| Grade 4 | Moderately heavy | Larger iron particles throughout the fragment with tendency to aggregate into clumps (X100) |
| Grade 5 | Heavy | Dense large clumps of iron throughout the fragment(X100) |
| Grade 6 | Very Heavy | Very large deposits of iron ,both intra- and extra-cellular obscuring cellular details in the fragment (X100) |

Table 2: Bone marrow iron status classification using the intensive grading method¹²

| Fragment iron | Macrophage iron | Erythroblast iron | Iron status category |
|---------------|-----------------|-------------------|--------------------------------------|
| Present | Present | Present | Normal |
| Present | Absent | Present | |
| Present | Present | Absent | Functional iron deficiency |
| Present | Absent | Absent | |
| Absent | Present | Present | Iron store deficiency |
| Absent | Absent | Present | |
| Absent | Present | Absent | Functional and iron store deficiency |
| Absent | Absent | Absent | |

Table 3: Summarises the predominant bone marrow findings and iron stores

| Anemia | No. | Predominant marrow findings | Gale's grading |
|--------------------------------|-----|---|----------------|
| Iron deficiency anemia | 67 | Cellular marrow,erythroid hyperplasia with late erythroblastic maturation | 0-1 |
| Megaloblastic anemia | 45 | Hypercellular marrow, erythroid hyperplasia with early erythroblastic maturation with giant myelocytes and metamyelocytes (few) | 2-4 |
| Anemia of chronic disease | 22 | Cellular marrow,erythroid hyperplasia with late erythroblastic maturation | 1-2 |
| Normocytic normochromic anemia | 9 | Cellular marrow with intermediate erythroblastic maturation | 2-3 |

Statistical Analysis

All the statistical analysis was performed using SPSS software. Frequency, cumulative percent, mean and Standard deviation was calculated. The accuracy and diagnostic performance of S.Ferritin was calculated and a Receiver operating characteristic (ROC) curve was plotted for the index test. Difference in means was evaluated using Student t test and difference in proportions, using the χ^2 test. Correlation was analyzed using Spearman's Rho Correlation test and 95% Confidence interval was calculated.

Results

In our study, out of 143 cases, 54.5% were male, 45.5% were female (M:F= 1.2:1). The mean age of presentation was found to be 31.93yrs (SD 15.19), whereas majority of cases affected in 15-24yrs age group (39.16%), followed by 25-34yrs age group (23.77%). We also noted that the mean age of iron deficiency anemia was 31.32yrs (SD 16.63) with minimum age of 15yrs and maximum of 82 yrs.

After bone marrow examination and iron assessment by conventional Gale's method 67 cases of iron deficiency anemia were identified. The rest of the cases were of megaloblastic anemia (45/143), anemia of chronic disease (31/143).

In Iron deficiency anemia, female preponderance was seen (M: F=1:1.8) unlike megaloblastic anemia as well as anemia of chronic disease where ratio was reversed (M: F=2.2:1 & 4.5:1) respectively. Majority of the cases of iron deficiency anemia were Hindus (86.6%) and belonged to rural area (70.1%). We noticed that majority of cases of iron deficiency anemia and anemia of chronic disease were symptomatic.

The Conventional Gale's grading revealed hypoferremic state in 46.85% cases and normal iron stores in 53.15% cases.

Intensive new method of grading, which classifies above anemias according to iron status revealed that the most common condition in Bundelkhand region was combined deficiency (functional & iron stores) (43.36%), followed by normal stores (37.76%), functional iron deficiency (14.68%) and lastly iron stores deficiency (4.20%). This indicates that the load of iron deficiency presenting with chronic disease is highest in Bundelkhand region. Low levels of S.ferritin were found in iron stores deficiency, whereas functional iron deficiency presented with highest levels of S.ferritin.

Maximum number of patients of microcytic hypochromic blood picture showed S. Ferritin level < 15 ng/ml (36.98%) followed by 15-24 ng/ml (27.39%). This was found to be highly significant statistically (p value<0.001). A positive Correlation of microcytic hypochromic blood picture with S.Ferritin and iron deficiency anemia was found. (Spearman's Rho correlation, R value =0.954, p value=<0.001). (Graph 1)

In the present study, an attempt was also made to propose a cutoff value of S.ferritin to recognize the iron deficiency anemia. Statistically, in cases of iron deficiency anemia, specificity of 100% was seen for S. ferritin levels upto 30ng/ml. The sensitivity increases by increasing levels, highest at <100 ng/ml, however the false positive burden (negative predictive value) was also high (90.76%) at the same ferritin level (Table 4). Whereas, the true positive cases (positive predictive value) decreases with increasing levels and found to be 100% upto 30ng/ml. The ROC curve (Curve 1) shows the left shift and largest area under the curve. The level of S.ferritin at the optimal positive likelihood ratio (12.92) for diagnosing iron deficiency was found to be < 45ng/ml. The accuracy and performance of the test was highest at <60 ng/ml level.

Graph 1: Graphical presentation of correlation of microcytic hypochromic blood picture with S.Ferritin and iron deficiency anemia

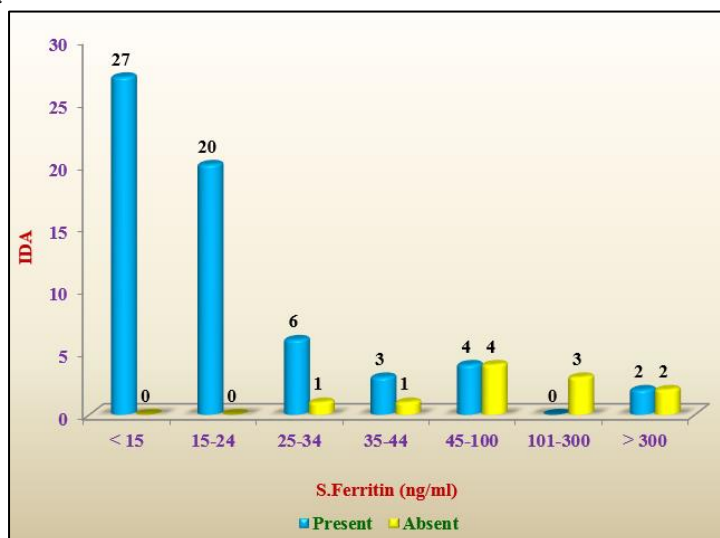


Table 4: Diagnostic performance of S.Ferritin as a surrogate marker for Iron deficiency anemia

| S. ferritin (ng/ml) | <15 | <30 | <45 | <60 | <100 |
|---------------------------|-------|-------|-------|-------|-------|
| Cumulative Frequency | 27 | 50 | 57 | 59 | 61 |
| Sensitivity | 40.29 | 74.62 | 85.07 | 88.05 | 91.04 |
| Specificity | 100 | 100 | 93.42 | 92.10 | 77.63 |
| Positive predictive value | 100 | 100 | 91.93 | 90.76 | 78.2 |
| Negative predictive value | 65.51 | 81.72 | 87.65 | 89.74 | 90.76 |
| Accuracy | 72.02 | 88.11 | 89.51 | 90.2 | 83.91 |
| Youden's J index | 40.29 | 74.62 | 78.49 | 80.15 | 68.67 |
| Positive likelihood ratio | NA | NA | 12.92 | 11.14 | 4.06 |
| Negative likelihood ratio | 0.59 | 0.25 | 0.15 | 0.12 | 0.11 |

* -NA stands for Not Applicable.

Curve 1: Receiver operating curve showing relationship of sensitivity and specificity of serum ferritin at different levels

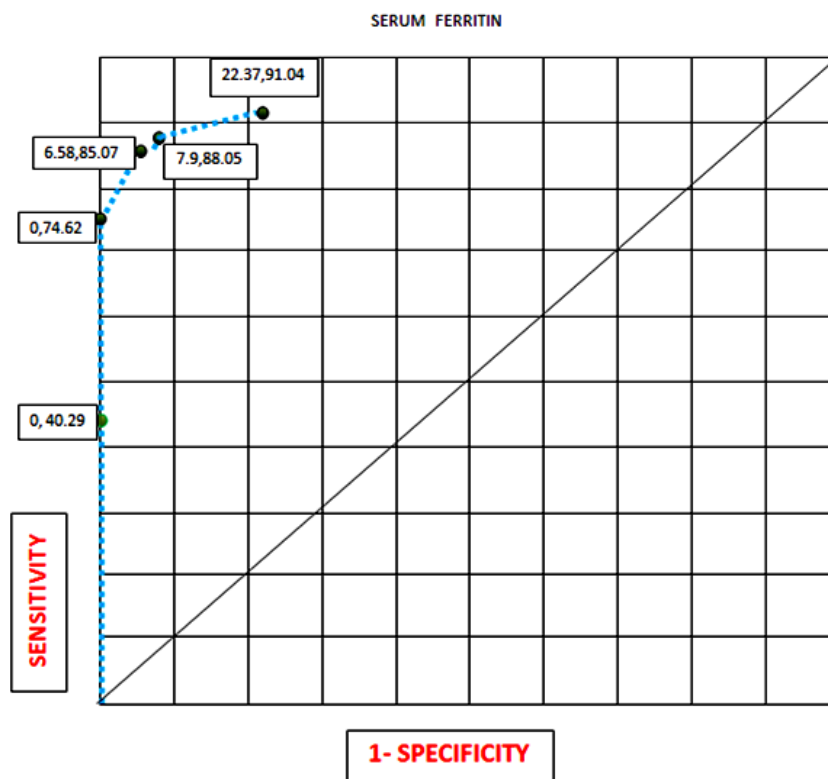


Table 5: Mean S. ferritin of iron stores status using conventional Gale's & intensive grading method and their correlation

| Index Test | | Gale's Grading Method | | Intensive Grading Method | | | |
|---------------------|-------------|-----------------------|------------------------|--------------------------|-----------------------------------|-----------------------------|---|
| S. Ferritin (ng/ml) | | Normal (n=76) | Iron deficiency (n=67) | Normal (N=54) | Functional iron deficiency (n=21) | Iron store deficiency (n=6) | Functional and Iron store deficiency (n=62) |
| Frequency | | 53.15 | 46.85 | 37.7 | 14.7 | 4.2 | 43.4 |
| Mean+/- SD | | 212.15 +/- 236.9 | 53.24+/-171.3 | 213.83 231.6 | 212.62 258.35 | 39.1 36.76 | 54.4 178.05 |
| 95% CI | Upper bound | 158.03 | 11.6 | 150.64 | 95.37 | 2.38 | 9.19 |
| | Lower bound | 266.27 | 95.01 | 277.01 | 329.86 | 75.82 | 99.60 |
| t value | | | 4.54 | | 0.01 | 1.83 | 4.18 |

| | t value | P value |
|--|---------|---------|
| Functional iron deficiency α Iron store deficiency | 1.61 | 0.118 |
| Functional iron deficiency α Functional & Iron store deficiency | 3.11 | 0.002 |
| Iron store deficiency α Functional & Iron store deficiency | 0.20 | 0.835 |

Discussion

The present study was aimed to compare the conventional Gale's method of bone marrow iron assessment with intensive grading method with an emphasis to find out the advantage of intensive grading method in iron deficiency anemias associated with chronic inflammatory condition.

The conventional Gale's method gives an idea about bone marrow fragment only however in intensive grading method used by Phiri et al,¹² additional information about macrophage as well as erythroblast iron can be achieved. Fragment and macrophage iron reflect iron stores. Since erythroblast iron represents utilizable iron which is usually reduced in functional iron deficiency, the intensive method gives an added advantage to distinguish between functional iron deficiency and iron store deficiency.

The findings of bone marrow iron grading were similar to findings pointed out by other studies Table 2. Phiri et al¹² and Bableshwar et al¹⁴ found functional iron deficiency as the commonest finding in contrast to our study where combined deficiency (functional iron + iron store) was the commonest. This shows that here in Bundelkhand region most cases of iron deficiency anemia are associated with chronic inflammatory conditions.

Mean S.ferritin in combined deficiency in comparison to normal iron stores, which was taken as control, found to be statistically significant (p value < 0.005). The correlation between functional iron deficiency and combined deficiency was found to be statistically significant (p value =0.002). Whereas, S.ferritin was found to be ineffective in differentiating iron stores deficiency from functional deficiency (p value =0.118) and from combined deficiency (p value=0.835). No statistical significance was found between functional iron deficiency and normal stores. (Table 5)

Serum ferritin plays a pivotal role in assessment of body iron stores but being an acute phase reactant, S.ferritin is not always found to be consistent with the status of iron stores. In our study the accuracy and the performance of the test was found to be best at <60 ng/ml in patients with dual iron store as well as functional iron deficiency. So a cutoff value of < 60ng/ml of S.ferritin as a surrogate marker for iron deficiency can be proposed.

Conclusion

Perl's method for grading of bone marrow iron is still regarded as the "Gold Standard". This simple technique is cost effective and efficient and should be used as a routine procedure where advance equipments are not available.

It can be inferred from this study that intensive bone marrow iron grading method scores over conventional iron grading method and can be used as an important tool to differentiate between true iron deficiency from functional iron deficiency by bone marrow examination. This could be of high clinical importance in areas like in Bundelkhand region where the rates of inflammatory conditions co-existing with iron deficiency anemia are high.

S.ferritin will be helpful where bone marrow examination facility is not available or not possible but being an acute phase reactant its value can be misleading in chronic diseases with iron deficiency. For such conditions we found that S. Ferritin level of <60ng/ml can be used as a surrogate marker of iron deficiency anemia.

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