

# Bone marrow iron and serum profile in patients of iron deficiency anemia – A correlation study

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**Introduction:** Anemia is not a disease, but a manifestation of many underlying disease. Iron deficiency anemia is the most common nutritional anemia. Incidence of anemia is very common, but the prevalence of anemia is proportionately high in developing countries with proper clinical, hematological, and bone marrow examination findings proper evaluation and management of patients with anemia can be made. Microscopic examination of bone marrow aspirate is the gold standard for assessing iron stores. **Aims and Objectives:** The study is conducted to measure iron grading using bone marrow iron stores and serum iron profile. **Materials and Methods:** A descriptive study of Perl's Prussian blue-stained bone marrow smear of 37 patients with iron deficiency anemia was performed at the Department of Pathology, RMCH, Bareilly. Bone marrow was assessed by Gale's method and correlation with serum iron profile. **Results** Statistical data proved that *P* value of bone marrow iron grading is <0.001 with both serum ferritin and total iron binding capacity (TIBC), however overall correlating best with serum ferritin as compared to TIBC and serum iron.

**KEY WORDS:** Bone marrow iron stores, serum ferritin, serum iron, total iron binding capacity

## INTRODUCTION

Anemia is defined as a reduction of the total circulating red cell mass below normal limits.<sup>[1]</sup> Anemia is hemoglobin below two standard deviations of the mean for the age and gender of the patient. Iron deficiency anemia continues to be a major public health problem worldwide, particularly in the developing nations. It is a especial catastrophe crisis of South East Asia and Africa and its dominant cause is nutritional deficiency. In developed countries, the prevalence of iron deficiency anemia varies between 2% and 8%. The trend in availability of iron in diet has been deteriorating, and thus, a enormous public health crisis throughout the world.<sup>[2]</sup> The causes of iron deficiency anemia may be due to insufficient iron consumption, decreased

absorption of iron and chronic blood loss, low dietary intake, increased demand for iron like as in state of pregnancy, and decreased absorption of iron as in coeliac diseases. Parasitic infestations are commonly seen to cause iron deficiency anemia in developing countries.

Disordered iron metabolism manifests by deranged serum iron parameters such as low serum iron, decreased serum transferrin, and increased reticuloendothelial iron stores. Serum ferritin reflects the total body iron store and a low-level indicative of a hypoferremic state.<sup>[3]</sup> A combination of surrogate makers, namely, serum ferritin, serum iron, and total iron binding capacity (TIBC) are routinely employed to assess the iron status of an individual. Serum ferritin is an acute phase reactive protein, whereby concentrations increase during infection, inflammation and malignancy, thereby rendering the interpretation of normal or high serum ferritin values.

Bone marrow examination is universally accepted used for diagnosis of several hematological illnesses.<sup>[2]</sup> Gold standard examination is bone marrow microscopy. Bone marrow aspiration and trephine biopsies are to aid cytological, immunophenotypical

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and molecular genetic. It is done to evaluate cellular morphology, to look for extent of disease and to do special tests on the marrow such as flow cytometry and cytogenetics studies. In bone marrow aspiration, we look for bone marrow iron stores.

The present study is carried out to evaluate and compare the serum iron profile and bone marrow iron stores in iron deficiency anemia. In this study, we will grade the bone marrow iron stores and try to correlate the dynamics of metabolic changes in the conditions, by bone marrow aspiration and trephine biopsies.

## MATERIALS AND METHODS

This prospective observational study was place in a hospital. It was done at the pathology department of the Rohilkhand Medical College and Hospital, a tertiary care facility in Bareilly, Uttar Pradesh's Rohilkhand region. The trial lasted for a full year, from November 1, 2020, to October 31, 2021. Thirty-seven participants had hematological testing, including measurements of hemoglobin, total leukocyte count, platelet count, reticulocyte count, and differential leucocyte count on peripheral blood examination (Leishman's stain). Serum levels of ferritin, iron, and TIBC were measured using biochemical assays. The anterior cubital vein was used to extract 4–5 mL of venous blood, which was then separated by centrifugation at room temperature for 10 min at 1000 rpm. Leishman's stain and Prussian blue for iron stain were used to examine the bone marrow (based on the reaction of hemosiderin that is ferric form with potassium ferrocyanide leading to formation of a blue colored compound ferri-ferrocyanide).<sup>[4]</sup> Under a microscope, bone marrow smears were examined for bone marrow pieces. The iron stockpiles will be graded in accordance with Gale's [Table 1].

### Statistical Analysis

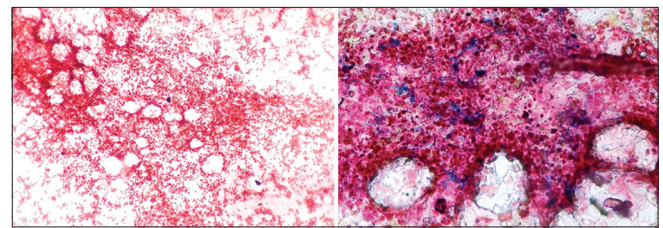
All the data will be applied using SPSS software (version 23.0). Coding, entry of the data, its clearing and compiling will be done in excel sheets. The data will be entered using Microsoft Excel sheets, tabulated, and analyzed. Depending on the distribution and type of data, quantitative data and expressed in means will be analyzed by *t*-test and proportions through Chi-square test.  $P < 0.05$  will be considered statistically significant.

## OBSERVATIONS AND RESULTS

The present study was undertaken to correlate findings of serum iron profile and bone marrow iron stores in iron deficiency anemia in 37 consecutive cases.

## DISCUSSION

The word "Anemia" is derived from Greek word "Anemia" not having blood. Anemia is functionally defined as an insufficient RBCs mass to adequately deliver oxygen to peripheral tissues. Iron deficiency anemia is one of the most common anemia worldwide. Iron has a major role in human body, the WHO



**Figure 1:** Bone marrow aspiration showing grade 0 and grade 2+ of Perl's stain

**Table 1:** Bone marrow iron grading<sup>[5]</sup>

Grade	Criteria
Grade 0	No iron granules observed
Grade 1+	Small granules in reticulum cells, seen only in oil-immersion lens
Grade 2+	Few small granules visible with low-power lens
Grade 3+	Numerous small granules in all marrow particles
Grade 4+	Large granules in small clumps
Grade 5+	Dense, large clumps of granules
Grade 6+	Very large deposits, obscuring marrow detail

**Table 2:** The demographic data

Characteristics	Total number cases
Gender	
Male	22
Female	15
Age	
10–20	15
21–30	7
31–40	4
41–50	6
51–60	2
61–70	2
71–80	1
Bone marrow morphological findings	
Erythroid hyperplasia with micronormoblast	29
Erythroid hyperplasia with normoblast	08
Perl's stain findings	
Grade 0	20
Grade 1	13
Grade 2	02
Grade 3	02
Grade 4	00
Grade 5	00
Grade 6	00

estimates near two billion people suffering from anemia and approximately 50% cases are due to iron deficiency anemia. Anemia is common in all phases of life. Adolescents age group are most commonly affected population.

**Table 3:** Serum iron profile distribution among the patients with iron deficiency anemia

Serum iron	Frequency	Serum ferritin	Frequency	TIBC	Frequency
10–20 mcg/dL	3	20–30 mcg/L	8	200–400 mcg/dL	16
21–30 mcg/dL	6	31–40 mcg/L	16	401–600 mcg/dL	17
31–40 mcg/dL	15	41–50 mcg/L	13	601–800 mcg/dL	3
41–50 mcg/dL	13	51–60 mcg/L	0	801–1000 mcg/dL	1
Total	37	Total	37	Total	37

**Table 4:** Correlation among serum ferritin level, serum iron level and total iron binding capacity versus bone marrow iron stores in the patients with iron deficiency anemia using Pearson correlation coefficient

	Bone marrow iron	Bone marrow iron	Bone marrow iron
Serum Ferritin Level			
Pearson Correlation	1	-0.112	0.104
Sig. (2-tailed)		0.040	0.041***
N	37	37	37
Serum Iron Level			
Pearson Correlation	-0.112	1	-0.776*
Sig. (2-tailed)	0.340		0.002
N	37	37	37
Total Iron Binding Capacity (TIBC)			
Pearson Correlation	0.104	-0.776**	0.001**
Sig. (2-tailed)	0.003	0.000	
N	37	37	37

\*\*Correlation is significant at the 0.01 level (2-tailed).

In the present study of 37 cases, the commonly affected gender was 60% male and 40% which were female patients [Table 2]. Similar gender prevalence was observed in many other studies. Ankur *et al.* conducted study on 47 cases of anemia, the male preponderance was more than is 27% and female was 20%. This study had more number of male patients with anemia compare to female patients. Bableshwar *et al.*<sup>[6]</sup> conducted the study on subjects with moderate to severe anemia. More number of cases were found in males 60% and 40% of cases were found to be female patients. Krause and Stolc<sup>[7]</sup> conducted a study of 91 patients. The findings were commonly seen in males compare to females. Discordance was observed with the study done by Mawani *et al.*,<sup>[8]</sup> in which 100 cases were taken as subjects, more commonly females were involved than males.

Thirty-seven cases in this study included wide variation of age 10–80 years group was observed. The most common age group observed in the study was between 11 and 20 years that is second to third decade of life. The maximum percentage observed was 40% between 11 and 20 years, 18% in 21–30 years of age group, 16% in 41–50 years of age group, 11% in patients in age group of 31–40 years, 6% in 61–70 years of age group, 5% in 51–60 years of age group, and 4% in 71–80 years of age group. Similar age variation was observed in similar study done by

Kumari *et al.*<sup>[9]</sup> conducted a study on a iron deficiency anemia in adolescent age group, 50% of girls found to be anemic. The most common age group involved in the study was 12–15 years. The highest prevalence was between 12 and 15 years of age group when requirement are at peak. Another study done by Patel *et al.*<sup>[10]</sup> conducted study on 1–80 years of age group.

In our study, serum iron levels of majority of the cases have 31 to 40 mcg/dl, with 13 cases ranging between 41 to 50 mcg/dl, with 6 cases between 21 to 30 mcg/dl and 3 cases between 10 to 20 mcg/dl [Table 3]. Study with similar findings was done by Christensen<sup>[11]</sup> who had maximum number of cases with serum iron range in between 31 and 50 mcg/dL. Other concordance study with similar finding given by Zaini<sup>[12]</sup>

In the present study of 37 cases, TIBC was estimated, majority of the cases were ranged between 401 and 600 mcg/dL that is in 17 cases, followed by 16 cases ranged between 200 and 400 mcg/dL, three cases ranged between 601 to 800 mcg/dL, and only one cases ranged between 801 to 1000 mcg/dL [Table 3]. Similar findings were seen in the study conducted by Khan and Said<sup>[13]</sup> with increased TIBC in maximum number of cases.

In this study, serum ferritin was assessed among 37 patients of iron group, majority of 16 cases were having serum ferritin between 31 and 40 mcg/L, followed by 13 cases ranged between 41 and 50 mcg/L [Table 3], similar findings were observed in the study done by Nasir *et al.*,<sup>[14]</sup> which showed maximum number of cases had serum ferritin ranged between 31 and 40 mcg/L.

As of morphological features of bone marrow aspiration among 37 cases, 29 cases (78%) majority of aspiration showed iron deficiency anemia and eight cases (22%) showed erythroid hyperplasia with normoblasts (Table 2). Similar findings were seen in the study done by Tevatia and Patel<sup>[15]</sup> with erythroid hyperplasia with micronormoblasts in all cases of iron efficiency anemia and similar result was seen in study done by Parajuli and Tuladhar<sup>[16]</sup>

Bone marrow aspiration with application of Prussian blue stain is useful to investigate patients with suspected iron deficiency anemia. In the current study, Perl Prussian blue stain was applied on 37 cases, majority of the cases 20 showed no haemosiderin granules( Grade 0), with 13 cases showed Grade 1, 2 cases showed Grade 2 and the remaining 2 cases showed Grade 3 [Figure 1]. Concordance study with similar findings was done by Birare *et al.*,<sup>[17]</sup> which showed that maximum number of cases had Grade 0 on Perl's stain. Nirmala and Ramana<sup>[18]</sup> showed discordance results.

BM iron grading is correlates best with serum ferritin, TIBC, and serum iron. The minus sign indicates this inverse relationship. In the study serum ferritin, iron and TIBC values were correlated (using Pearson Correlation coefficient) with bone marrow iron grading (0–VI) in 37 consecutive cases [Table 4]. Serum iron is directly proportional and indirectly proportional relationship of bone marrow iron grade with the serum ferritin and TIBC, respectively. The majority of the time and serum ferritin levels are a valid predictor of the presence of iron reserves in the bone marrow; consequently, using this information to estimate iron stores will lessen the need for invasive BMA procedures. The detection of the early iron deficiency anemia is another area where blood ferritin levels are extremely helpful.

## CONCLUSION

Anemia is common in all phases of life. Adolescent age group is most commonly affected population. Anemia is the most common hematological disorder affecting humanity and is usually observed in chronic disease states as a non-specific complains to physicians. This present study concludes that the primary hematological investigation along with the bone marrow examination is useful to make diagnosis at the early stage of iron deficiency anemia. Commonly, iron deficiency anemia manifests in females, while, in this study, male preponderance was observed; hence, proper screening should be done at every level to find the exact cause. Patients presenting mainly with clinical features of iron deficiency anemia mask the underlying cause of anemia of chronic disease, so general blood picture assessment should be incorporated in routine investigation for diagnosing and classifying the cause of anemias.

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