Original Article

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Bone Marrow Iron Stores among Various Hematological Disorders

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Abstract

Background: Nutritional anemia, particularly iron deficiency, continues to be a major public health problem worldwide, particularly in the developing countries like India. Although mass spectrometry has been recently used to give a definitive determination of iron in tissue, microscopic examination of Prussian blue-stained bone marrow aspirate has been considered the practical "gold standard" for determining iron depleted states. *Aim:* To assess the bone marrow iron stores among the various haematological disorders. *Materials and Methods:* A cross sectional observational study was conducted by the department of pathology at Shri B.M. Patil Medical College, Hospital and Research centre, Vijayapur for a period of one year. A total of 110 cases of various hematological disorders were studied to assess the bone marrow iron stores. Bone marrow aspirate was obtained after written informed consent from the posterior superior iliac spine observing strict asepsis, spread onto slides, air dried, fixed with methanol, and stained with Giemsa Stain, observed microscopically and also simultaneously one smear stained with Prussian blue stain. Equal volumes of 2% of potassium ferrocynide and 2% hydrochloric acid solution are mixed in staining jar and slides are immersed in the solution for 15-20 min. Then removed and rinsed with tap water. Counterstaining was done with 1% neutral red for 30 seconds. *Results:* Of the various haematological disorders 61.7% of the RBC disorders and 60% of the combined disorders had shown decreased bone marrow iron store, whereas among WBC disorders and platelet disorders it was 45% and 33.3% respectively and there was no statistically significant association (p=0.461) between any of the haematological disorder and the reduced bone marrow iron stores. *Conclusion:* Performing a bone marrow study in all patients with haematological disorders should be made routine before starting iron replacement therapy.

Keywords: Bone marrow; iron stores; haematological disorder.

Introduction

Nutritional anemia, particularly iron deficiency, continues to be a major public health problem worldwide, particularly in the developing countries like India. Though haemoglobin estimation is used as a screening test for the assessment of anemia, a combination of surrogate markers, namely serum ferritin, serum iron, total iron binding capacity (TIBC), and percentage saturation of transferrin have been routinely employed in assessing the iron status of an individual.

Diseases associated with defective reticulo-endothelial release of iron will be difficult to distinguish from the iron deficient state as the serum iron parameters (serum iron, percentage saturation of transferrin < 15%) may usually overlap, so to solve this problem assessment of the bone marrow biopsy for the iron stores might often be necessary to come to a diagnosis. Microscopic examination of bone marrow aspirate remains to be the gold standard for assessing marrow iron store [1].

However, the conventional Gale's method of assessing iron in marrow fragments alone provides

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little valuable information about the functional iron deficient state [2]. Although mass spectrometry has been recently used to give a definitive determination of iron in tissue [3], microscopic examination of Prussian blue-stained bone marrow aspirate has been considered the practical "gold standard" for determining iron depleted states [4]. Conventionally, iron has been primarily assessed in marrow fragments which represent iron stores in the form of hemosiderin [5]. Although some studies have shown a reasonable correlation between histological iron grading and chemical iron concentration in bone marrow [6], others have not, raising questions about the validity of the histological grading [7].

Iron visualised in marrow fragments is from a meshwork of reticular cells which are usually undistinguishable. However, single "loose" macrophage may be inspected for iron and it is hypothesised to be particularly important when iron in fragments is absent and may signify the lowest level of iron stores depletion.

In areas where there is a high prevalence of inflammatory conditions, functional iron deficiency occurs more commonly. Erythroblast iron may be indicative of cellular iron utilisation and decreased in functional iron deficiency [8]; however there has been very little research done on the use of erythroblast iron as a marker of cellular iron availability [9]. Furthermore, in malaria endemic areas, interpretation of iron status is confounded by the presence of hemozoin [10].

Aim To assess the bone marrow iron stores among the various haematological disorders.

Methodology

A cross sectional observational study was conducted by the department of pathology at Shri B.M. Patil Medical College, Hospital and Research centre, Vijayapur for a period of one year. Patients with various hematological disorders in whom bone marrow aspiration was indicated were included for the study. In patients for whom the bone marrow aspirates was inadequate material or dry tap, patients receiving iron supplementation and the patients who had undergone recent blood transfusion (preceding four weeks) were excluded from the study. A total of 110 cases of various hematological disorders were studied to assess the bone marrow iron stores.

The study was approved by the Institution Ethical Committee. Bone marrow aspirate was obtained after written informed consent from the posterior superior iliac spine observing strict asepsis, spread onto slides, air dried, fixed with methanol, and stained with Giemsa stain observed, microscopically and also simultaneously one smear stained with Prussian blue stain. Equal volumes of 2% of potassium ferrocynide and 2% hydrochloric acid solution are mixed in staining jar and slides are immersed in the solution for 15-20 min. Then removed and rinsed with tap water for 3-4 minutes. Counterstaining was done with 1% neutral red for 30 seconds. Then allowed to dry and then examined. The marrow was rejected when it was found to be diluted with blood or poor material or inadequate material on Giemsa stain.

The grading of iron storage in the bone marrow was done by the following scoring (Galles) [11].

0 = No stainable iron

1+ = Small iron particles just visible in reticulum cell in oil immersion objective

2+ = Small, sparse iron particles in low power field

3+ = Numerous small granules in all marrow particles

4+ = Large granules in small clumps

5+ = Dense large clumps of granules

6+ = Very large deposits obscuring marrow details.

Results

The age and sex wise distribution of the study population shows that majority of the patients were in the age group of 21 – 30 years (51.8%) and the gender wise distribution shows that there is almost equal number of males and females in the study population, and the mean age among the males and females was 28.45 and 29.14 respectively (Table 1).

The various haematological disorders that were distributed among the study population was shown in Table 2. It is inferred from the table that the major haematological disorder among the study subjects was RBC disorders (55.5%) comprising of various types of anemias followed by WBC disorders (18.2%) and platelet disorders (5.5%). There were 10 patients with combined (RBC, WBC and platelet) disorders.

The bone marrow iron stores were graded based on the Galles scoring (0 – 6) which were further grouped into three groups. The score with 0–1 grade are considered as diminished, grade 2 and 3 are normal and the grades with 4 – 6 were considered as increased iron stores in bone marrow. In our study majority of the study subjects had decreased iron stores (54.5%) and about 27% of them had normal iron stores and 18% had increased iron stores in the bone marrow

(Table 3).

The distribution of the bone marrow iron stores in the various haematological disorders is shown in Table 4. Of the various haematological disorders 61.7% of the RBC disorders and 60% of the combined disorders had shown decreased bone marrow iron store, whereas among WBC disorders and platelet disorders it was 45% and 33.3% respectively and there was no statistically significant association (p=0.461) between any of the haematological disorder and the reduced bone marrow iron stores. Similarly the normal bone marrow iron stores was present in almost all the haematological disorders in equal numbers with slightly more among the patients with platelets disorders (50%) but the difference was not statistically significant (p=0.384). Increased bone marrow iron stores was found in more patients with WBC disorders (35%) than the other haematological disorders but it was not found to be statistically significant (p=0.318).

The Figures of the various grades of iron stores were given below (Figure 1 -6).

Table 1: Age and sex wise distribution of the study population

Age group	Male	Female	Total
10 – 20	10 (18.5%)	9 (16%)	19 (17.2%)
21 – 30	27(50%)	30 (53.5%)	57 (51.8%)
31 – 40	15(27.7%)	17 (30.3%)	32 (29%)
>40	2 (3.7%)	0	2 (1.8%)
Total	54 (100%)	56 (100%)	110 (100%)
Mean (SD)	28.45 (3.2)	29.14 (3.8)	

Table 2: Distribution of the various haematological disorders among the study population

Disorders	Frequency	Percentage (%)
RBC disorder	61	55.5
WBC disorder	20	18.2
Platelet disorder	6	5.5
Combined	10	9.1
Others	13	11.8
Total	110	100

Table 3: Distribution of the bone marrow iron stores among the study population

Bone marrow iron stores	Frequency	Percentage
Decreased	60	54.5%
Normal	30	27.2%
Increased	20	18.1%
Total	110	100%

Table 4: Bone marrow iron stores and the various haematological iron stores among the study population

Haematological Disorders	Bone marrow iron stores		
	Decreased	Normal	Increased
RBC disorder (n=61)	37 (61.7%)	17 (27.8%)	7 (11.4%)
WBC disorder (n=20)	9 (45%)	4 (20%)	7 (35%)
Platelet disorder (n=6)	2 (33.3%)	3 (50%)	1 (16.6%)
Combined (n=10)	6 (60%)	3 (30%)	1 (10%)
Others (n=13)	5 (38.4%)	4 (30.7%)	4 (30.7%)
Total (n=110)	60 (54.5%)	30 (27.2%)	20 (18.1%)
P value (Chi square test)	0.461	0.384	0.318

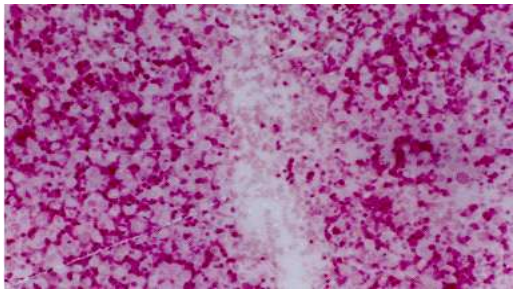

Fig. 1: Grade 1 iron stores

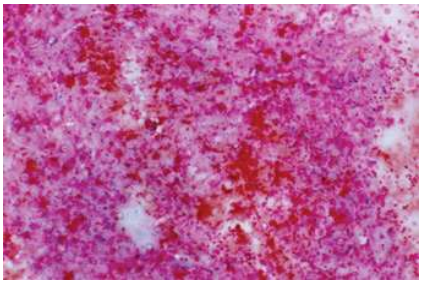

Fig. 2: Grade 2 iron stores

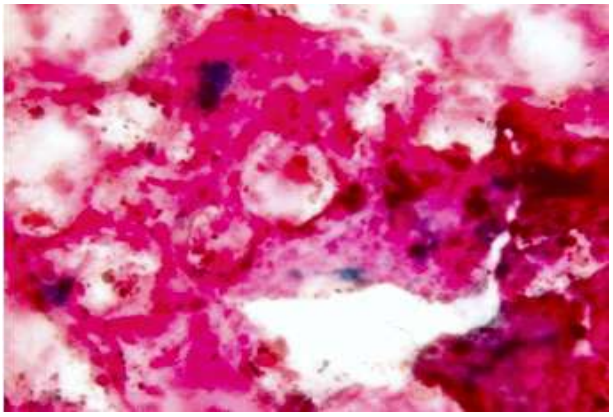

Fig. 3: Grade 3 iron stores

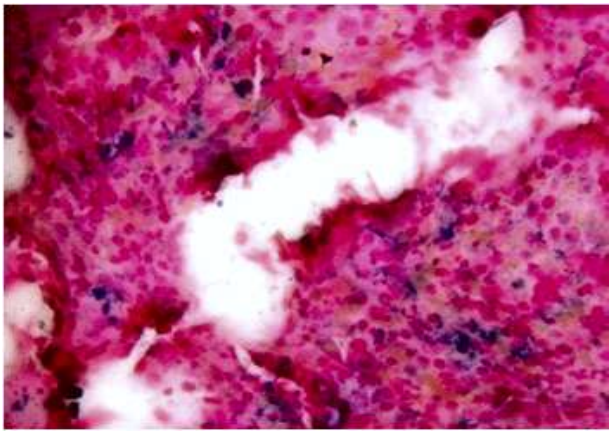

Fig. 4: Grade 4 iron stores

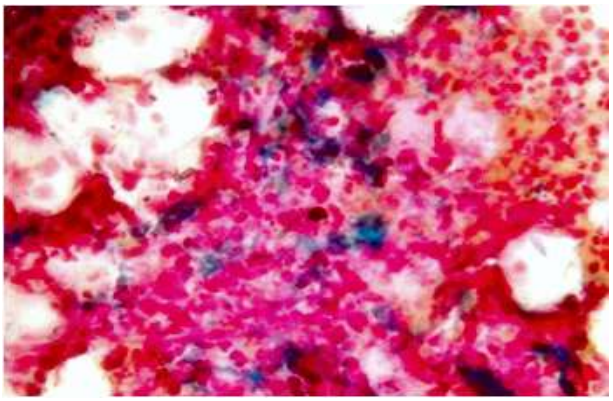

Fig. 5: Grade 5 iron stores

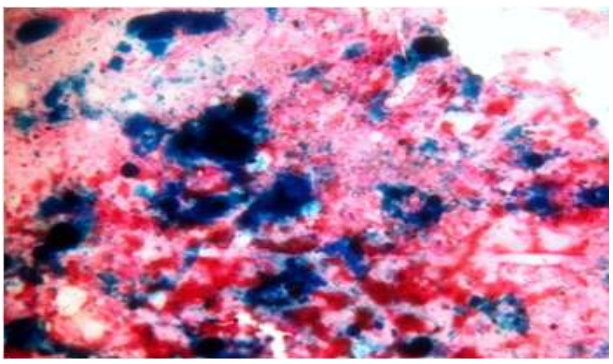

Fig. 6: Grade 6 iron stores

Discussion

Various workers in the diagnostic field have emphasized the importance of iron storage by Prussian blue method. Being very old technique many old studies are there, but now a days it has gained a firm stand, being a simple technique with temptation and enthusiasm to know and see what our ancients have founded. This short study is an attempt to put forth and establish the importance of bone-marrow iron stores in clinical ground and to assess its result with a wish to continue as a routine practice in hematology.

The present study had shown that majority of the study subjects were in the age group of 21 – 30 years and both the males and females were almost in equal numbers. In par with our results the study done by Krupal M Pujara et al [11] had shown in their study that majority were in the 20 – 30 years age group, while males were more affected during first and second decade of life and females in third and fourth decade of life.

Our study had shown that among the various haematological disorders the most common was RBC disorder followed by WBC and platelet disorders and the similar type of results was quoted in study done by Bableshwar RS [12], where he also mentioned iron deficiency anemia was the most common among the RBC disorders.

A study done by Nielsen et al [13] and Donald et al [14] had shown in their results that majority of their study subjects with haematological disorders had reduced levels of iron stores in the bone marrow and our results are also exactly in par with those studies. Whereas a little contradictory to our study a study done by Jain et al [15] and Tripathi et al [16] had normal iron stores in majority of their patients with hematoligical disorders and in another study done by Coenen et al [17] it was shown that majority of the patients had increased iron stores in the bone marrow.

From the results of our study, it is inferred that the bone marrow iron stores was decreased in majority of the patients with red blood cell disorders but it was also reduced among the patients with other blood cell disorders (WBC's and platelets)and so bone marrow iron stores is not specific for RBC disorders and similar type of results was also quoted in a study done by Neal S. Young et al [18] and Rajajee S et al [19]. Prussian blue positive iron stained granules were seen in most of the cases in macrophage and as free particles. Of all the various haematological disorders, iron deficiency anemia is the commonest form which when not diagnosed and treated properly would lead on to various complications like impaired growth,

development and cognition among children, poor pregnancy outcome in antenatal mothers, frequent falls and higher incidence of developing renal and cardiac diseases among the elderly. So bone marrow studies would help to diagnose the condition much early and intervention in form of iron therapy would reverse the condition and prevent further complications due to iron deficiency anemia. Study of bone-marrow iron stores in all haematological disorders is very much essential as it helps to decide whether iron therapy would be of any use or not.

Conclusion

This study has proven that bone marrow iron stores will be reduced not only in red blood cell disorders but also in all other blood disorders, so performing a bone marrow study in all patients with haematological disorders should be made routine before starting iron replacement therapy.

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