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Original Research Article

A study of Iron store in Bone marrow and its correlation in various anemia

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ABSTRACT

Background: incidence of anaemia is a very common but prevalence of anaemia is disproportionately high in developing countries. With proper clinical history, haematological and bone marrow examination findings proper evaluation and management of patients with anaemia can be made. Microscopic examination of bone marrow aspirate is the gold standard for assessing marrow iron store. However, conventional Gale's method assesses iron in marrow fragments alone which provides little valuable information about functional iron deficiency seen in many chronic diseases.

Aim: This study is conducted to measure iron grading using perl's Prussian blue staining in various hematological conditions.

Materials and Methods: A descriptive study of Perl's Prussian blue stained bone marrow aspirate smears of 130 patients with anaemia. A CBC, Reticulocyte count and Bone marrow iron was assessed by the Gale's method in this study.

Result: After excluding 9 cases of dry tap, 121 cases were evaluated in our study. 78.57% of cases (61 cases) diagnosed as microcytic anaemia had iron store very low (grade 0 and 1). In patients with megaloblastic anaemia (15 cases), 46.7% of cases (7 cases) had iron store grade was normal, but in 8 cases iron grade was low. In patients with dimorphic anaemia (23 cases), 78.3% of cases (18 cases) had iron store grade was normal.

Conclusion: Bone marrow aspiration with application of Prussian blue stain is useful to investigate patients with suspected iron deficiency anaemia, anaemia of chronic disease, megaloblastic anaemia and acute leukemia.

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1. Introduction

Nutritional anemia, particularly iron deficiency, continues to be a major public health problem worldwide, particularly in the developing countries.¹ Various investigations like serum ferritin, serum iron, total iron binding capacity (TIBC), and percentage saturation of transferrin are routinely used to assess the iron status of an individual.² Serum ferritin reflects the total body iron store and a low level is indicative of a hypoferremic state.^{2,3} However, ferritin is also an acute phase reactant, it

may increase in infection, inflammation, liver disease, malignancy, hemodialysis, so interpretation of normal or high serum ferritin values difficult in such conditions.² The serum iron markers, therefore, may not differentiate between reduced iron stores and conditions associated with defective reticuloendothelial release of iron (functional iron deficiency).^{4,5}

The increasing prevalence of multiple comorbidities among anaemic patients with chronic kidney disease has made the use of serum ferritin and transferrin saturation more challenging in diagnosing iron deficiency.⁶ Hence, serum iron and transferrin saturation cannot be made

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the gold standard/absolute criteria for diagnosing iron deficiency anemia. Diseases associated with defective reticuloendothelial release of iron may be difficult to distinguish from the iron deficiency state since serum iron parameters may overlap. Assessment of the bone marrow iron store in bone marrow aspirate is the gold standard.⁷ However, the conventional Gale's method of assessing iron in marrow fragments alone provides little valuable information about the functional iron deficient state.⁸

This study is conducted to measure iron grading using perl's Prussian blue staining in various hematological condition and establish the fact that oldest procedure to measure iron storage is still the gold standard in the era of cytogenetics and molecular pathology.

2. Material and Methods

Present study was conducted at Department of Pathology, Government Medical College, Surat during the period of June 2018 to June 2020 after ethical clearance from institute. Total 130 patients are included in present study. Bone marrow examination is carried out in patients admitted with moderate to severe anemia and no history of transfusion in preceding 4 weeks, in whom a diagnostic bone marrow examination was requested by the clinician. Blood sample for complete blood count, Retic count and Bone marrow aspirate was obtained after written informed consent from the posterior superior iliac spine observing strict asepsis, spread onto a slide, air-dried, fixed with methanol, and stained with hematoxylin and eosin (H and E), observed microscopically and also simultaneously stained with Prussian blue stain as under. Equal volume of 2% of potassium ferrocyanide 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 distilled water counterstaining with saffranin for 30s. Then allowed to dry and then examined. The iron statuses of the 130 patients were assessed by both the Gale's method mentioned below.⁸

1. Nil: No iron granules seen
2. +1: Small granules in reticulum cells only under oil-immersion.
3. +2: Few small granules visible with low power lens(10X)
4. +3: Numerous small granules in all marrow particles.
5. +4: Large granules in small clumps.
6. +5: Dense large granules of clumps.
7. +6: Very large deposits obscuring the marrow cells.
 - (a) Grade 0: Iron deficiency (a minimum 7 bone marrow particles must be available before concluding that hemosiderin is absent)
 - (b) Grade 1: Diminished iron store
 - (c) Grade 2 & 3: normal iron store
 - (d) Grade 4,5& 6: Increased Iron store.

3. Results

In present study of 130 cases, bone marrow iron grading was not possible in 9 cases due to dry tap or bone marrow aspiration heavily diluted with blood. 121 cases evaluated in which 62 (51.23%) were males and 59 (48.76%) were females. In present study, out of 121 cases, majority of cases 27 (22.3%) were observed in 11-20 years of age group followed by 20 (16.52%) cases in 21-30 years. Out of 121 cases in present study, predominant hematological disorders were microcytic anaemia in 70 (57.8%) cases, megaloblastic anaemia in 15 (12.4%) cases and dimorphic anaemia in 3 (2.47%) cases. Other 8 cases unrelated to anemia were acute leukemia (2), chronic myeloid leukemia (2), multiple myeloma (1), granulomatous inflammation (1), idiopathic thrombocytopenic purpura (1) and essential thrombocythemia (1).

Our study showed 70 cases of microcytic anemia in which 57 (81.42%) cases showed marrow findings of erythroid hyperplasia with micronormoblastic maturation. All 15 cases of megaloblastic anemia in present study, marrow findings were erythroid hyperplasia with megaloblastic maturation and 11 out of 15 (73.33%) cases showed features of dyserythropoiesis along with megaloblastic maturation. Out of 23 cases of dimorphic anemia in present study, 18 (78.26%) cases show marrow findings of normoblastic erythropoiesis with 5 (21.73%) cases showed megaloblastic maturation. In present study, 3 cases of aplastic anemia noted, all showed hypocellular marrow.

4. Discussion

Examination of bone marrow is one of the diagnostic pillars of hematological practice and some non-hematological diseases. Bone marrow aspiration and trephine biopsy are the two procedures done for the diagnosis of hematological and non-hematological disorders. These procedures are also employed for follow up of patients on chemotherapy, bone marrow transplantation and other forms of treatment. The present study was carried out at tertiary health care centre aligned to medical college from the period of June 2018 to June 2020. Study includes 121 patients who were admitted at tertiary health care centre and referred by clinician for bone marrow examination.

The age group range in our present study was 1 year to 80 years with mean age of 30 years. Majority (27%) cases were affected during 11-20 years, next group (20%) was 21-30 years, third (19%) were <10 years and 31-40 years. In study by Pujara et al they found majority of cases (30%) in age group 21-30 years followed by (27%) in age group of 31-40 years.⁸ In study of Deka et al they found (31.3%) and (21.3%) cases in age group of 21-30 years and 31-40 years.⁹ 62 (51.23%) were males and 59 (48.77%) were females with male to female ratio of 1.05:1.

Table 1:

S.No.	Age in years Disease	<10	11-20	21-30	31-40	41-50	>50	Total Cases
1	Microcytic anemia	15	9	10	14	10	12	70 (57.7%)
2	Megaloblastic anemia	01	04	02	03	04	01	15 (12.4%)
3	Dimorphic anemia	02	9	05	02	02	03	23 (19%)
4	Aplastic anemia	00	03	00	00	00	00	03 (2.47%)
5	Thalassemia	00	01	00	00	00	00	01 (0.8%)
6	Sickle cell anemia	00	00	01	00	00	00	01 (0.8%)
7	Others	1	1	02	00	02	02	8 (6.6%)
Total		19(15.70%)	27 (22.3%)	20 (16.52%)	19 (15.7%)	18 (14.87%)	18 (14.87%)	121

Table 2: Predominant marrow findings and marrow iron storage.

Diseases	No. of cases	Marrow findings	Iron grade
Microcytic anemia	70	Mild erythroid hyperplasia with normoblastic and micronormoblastic maturation	0-3
Megaloblastic anemia	15	Erythroid hyperplasia with megaloblastic changes with feature of dyserythropoiesis	1-5
Dimorphic anemia	23	Normoblastic erythropoiesis	2-5
Aplastic anemia	03	Hypocellular marrow	1-5
Sickle cell anemia	01	Normoblastic erythropoiesis	4
Thalassemia	01	Normoblastic erythropoiesis	1
Others	08	Variable, depend on cases	1-5

Table 3: Distribution of iron grade in various type of anaemia

Iron Grade	Microcytic Anaemia	Megaloblastic Anemia	Dimorphic Anaemia	Aplastic Anaemia	Hemoglobinopathies
0	33(47.1%)	1(6.7%)	0	0	0
1	28(40%)	2(13.3%)	1(4.3%)	1(33.3%)	0
2	7(10%)	4(26.7%)	02(8.7%)	0	0
3	2(2.8%)	3(20%)	10(43.5%)	1(33.3%)	1(100%)
4	0	2(13.3%)	08(34.8%)	0	0
5	0	2(13.3%)	02(8.7%)	1(33.3%)	1(100%)
Total	70	15	23	3	2

In present study, 61(87.1%) cases of microcytic anaemia patients have iron grade of 0 and 1 which was observed in Pujara et al, Deka et al and Alexander et al.⁸⁻¹⁰ In present study, other 9 (12.85%) cases of microcytic anaemia have iron grade of 2 and 3, which received parenteral iron therapy before bone marrow examination. Jameson et al studied 4 cases of iron deficiency anemia which received parenteral iron before bone marrow examination and recorded iron storage grade of 2 and 3.¹¹

14 (93.33%) cases with macrocytic anaemia have iron grade ranges from 1-5 similar findings were observed in Pujara et al and Rajeev Saxena et al.^{8,12} while 1 (6.67%) case had iron grade of 0 which might have associated iron deficiency. Chanarin et al studied 127 cases

of megaloblastic anemia.¹³ Out of 127 cases, 37 (29.13%) cases showed absent iron stores. He quoted that many cases with nutritional cobalamine deficiency have iron deficiency.

In present study, we found 23 (32.85%) cases of dimorphic anemia in which iron grade ranges from 1-5. Rajeev Saxena et al found iron grade range of 1-4 in his study of 15 (13.63%) cases of dimorphic anemia.¹² Pujara et al found iron grade range of 2-4 in his study of 7 (9.58%) cases of dimorphic anemia.⁸ Reason behind slight variation is that less number of cases of dimorphic anemia in their study.⁸

In present study, 3 cases of aplastic anemia were recorded in which iron grade were 1, 3 and 5. Pujara et al and Deka et al found iron grade of 0-2 (3 cases) and 0-1 (2 cases)

respectively in their study.^{8,9}

1 (0.8%) case of thalassemia minor was studied in present study, in which iron store found to be 2. Pujara et al studied 1 (1.36%) case of thalassemia, in which iron storage grade was 3. Deka et al studied 16 (11.76%) cases of thalassemia and other hemoglobinopathies, in which patients have iron storage grade of 3 and 4.^{8,9} In our study we observed 1 (0.8%) case of sickle cell anemia was studied in present study, in which iron grade found to be 3. Koduri et al in his review article mentioned that iron deficiency is more common in patients with sickle cell anemia without any blood transfusion.¹⁴ According to him, deficient iron state is beneficial as it reduces sickling by decreasing MCHC-S amount in RBCs.

5. Conclusion

Bone marrow aspiration with application of Prussian blue stain is useful to investigate patients with suspected iron deficiency anemia, anemia of chronic disease, megaloblastic anemia and acute leukemia. Prussian blue stain is simple and helpful technique to measure body iron semi quantitatively. We can able to diagnosed coexistence of iron deficiency with other anaemia.

6. Conflict of Interest

The authors declare that there is no conflict of interest.

7. Source of Funding

None.

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