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Original Article

Correlation of Bone Marrow Iron Storage with Different Types of Anemia

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Abstract

Introduction: Nutritional anemia, particularly iron deficiency, continues to be a major public health problem worldwide, particularly in the developing nations. A combination of surrogate markers, namely serum ferritin, serum iron, total iron binding capacity, and percentage saturation of transferrin are routinely employed to assess the iron status of an individual.

Materials and Methods: It was a cross-sectional study conducted in the Department of Pathology, Rajendra Institute of Medical Sciences (RIMS), Ranchi. Air dried smears prepared from a total of 50 subjects refereed to the Pathology Department for investigation, the peripheral blood and bone marrow from different patients obtained from the Pathology Department of RIMS.

Results: About 24% cases had ferritin <14 ng/ml with the bone marrow iron stores Grade 0-2. 12% had ferritin 15-25 ng/ml with bone marrow iron store between Grades 0 and 2. About 6% of cases had ferritin between 45 and 100 ng/ml with bone marrow iron stores between Grades 2 and 4. Nearly 8% of cases had ferritin between 100 and 200 ng/dl with bone marrow iron store between Grades 1 and 3. 18% cases had ferritin between 200 and 500 ng/ml with bone marrow iron stores between Grades 1 and 3. 20% of cases had ferritin 500-2000 ng/ml with bone marrow iron stores between Grade 2 and 4. 20% cases had ferritin >2000 ng/ml with bone marrow iron stores Grade 3-4.

Conclusion: Microscopic examination of stainable iron in bone marrow is a reliable technique in assessing iron stores. In the present study, it has been found that all cases of iron deficiency anemia had low iron stores. Serum ferritin value when <14 ng/dl is diagnostic of iron deficiency anemia while raised serum ferritin does not exclude iron deficiency anemia. Serum iron correlates well with the bone marrow iron stores level.

Key words: Anemia, Bone marrow, Gales criteria, Perls staining

INTRODUCTION

According to the World Health Organization, anemia is defined as a condition in which the hemoglobin content is below normal. This situation occurs because of different patho-physiological mechanisms. The most prevalent types of anemia are due to nutritional deficiencies (malnutrition and iron, vitamin B12, and folic acid deficiencies) and chronic diseases (such as cancer, kidney disease, and congestive heart failure).[1,2]

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Nutritional anemia, particularly iron deficiency, continues to be a major public health problem worldwide, particularly in the developing nations.[3] A combination of surrogate markers, namely serum ferritin, serum iron, total iron binding capacity, and percentage saturation of transferrin are routinely employed to assess the iron status of an individual.[4-6]

Anemia is a major health problem in India. In the 2005-2006 National Family Health Survey (NFHS-3), a household survey aimed at having national and state representative data on population health and nutrition; the prevalence of anemia was 70% in children aged 6-59 months, 55% in females aged 15-49 years, and 24% in males aged 15-49 years.[7] Although the NFHS-3 showed that the prevalence of anemia was higher in rural areas, there is a paucity of data about the epidemiology of anemia in rural settings.[8]

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In order to characterize the type of anemia and formulate a differential diagnosis, the work-up should include physical exams and laboratory tests, such as evaluations of hematocrit, hemoglobin, and red blood cell (RBC) indices. The RBC indices should include the cell count, mean cell volume, mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), and red cell distribution width (RDW).[9]

MATERIALS AND METHODS

It was a cross-sectional study conducted in the Department of Pathology, Rajendra Institute of Medical Sciences (RIMS), Ranchi. Air dried smears prepared from a total of 50 subjects refereed to the Pathology Department for investigation, the peripheral blood, and bone marrow from different patients obtained from Pathology Department of RIMS.

Both peripheral blood and bone marrow smears were stained by Leishman stain to diagnose the type of anemia. The cases diagnosed as anemia were included in the study and bone marrow iron staining along with serum ferritin levels was performed. Iron staining of the bone marrow smears was done by Perl's method.[10]

In all case of anemia the following routine investigation were done: Hemoglobin estimation, total RBC count, white blood cell count, differential count, hematocrit, mean corpuscular volume, MCH, MCHC, platelet count, Red cell distribution width - standard deviation (RDW-SD), Red cell distribution width - coefficient of variation (RDW-CV).

Bone marrow aspirate was obtained after informed consent from the posterior iliac spine and ant. Iliac spine observing strict asepsis, spread on to a slide; air dried, fixed with methanol at the same setting for hemoglobin and serum ferritin level estimation. Grading was done according to Gale's method of bone marrow iron grading.[11]

Procedure

In the first step, sample and anti-ferritin coated paramagnetic micro-particles are combined. Ferritin present in the sample binds to anti-ferritin coated paramagnetic micro particles. After washing anti-ferritin, acridinium coated labeled conjugate to add in the second step. Pretrigger and trigger solution are then added to the reaction mixture, the resulting chemiluminescent reaction is measured in relative light units. A direct relationship exists between the amount of ferritin in the sample and relative light units detected by the architect optical system.

Data Analysis

Data were entered and analyzed using Microsoft Excel 2007.

RESULTS

The Table 1 shows the relative frequency of different types of anemia in present study. Among all the anemia, iron deficiency anemia was the most common anemia constituting of 40% of cases followed by anemia of chronic disease 26%; aplastic anemia 16%; others 6%, megaloblastic anemia 6%; hemolytic anemia 6%.

The Table 2 shows age wise distribution of different types of anemia; 12% of cases were between 1 and 10 years. 32% of cases were between 10 and 20 years of age. About 40% of cases were between 20 and 40 years; 12% cases were between 40 and 60 year of age; and 4% of cases were above 60 years.

The Table 3 shows sex wise distribution of different types of anemia. About 12% of males were affected from iron deficiency anemia, while 28% of female were affected from iron deficiency anemia. About 12% males were affected from anemia of chronic disease while 14% of female were affected from anemia of chronic disease. About 10% of males were affected from aplastic anemia, while 6% males were affected from aplastic anemia. Around 6% of males were affected from megaloblastic anemia 2% of females were affected from hemolytic anemia, while 4% of males were affected from hemolytic anemia. About 4% of females were affected from others (anemia of leukemia, Myelodysplastic syndrome [MDS]), while 4% of males were affected from others (anemia of leukemia, MDS).

The Table 4 shows the correlation of serum ferritin with bone marrow iron stores. About 24% cases had ferritin <14 ng/ml with the bone marrow iron stores Grade 0-2. About 12% had ferritin 15-25 ng/ml with bone marrow iron store between Grades 0 and 2. About 6% of cases had ferritin between 45 and 100 ng/ml with bone marrow iron stores between Grades 2 and 4. About 8% of cases had ferritin between 100 and 200 ng/ml with bone marrow iron store between Grades 1 and 3. About 18% cases had ferritin between 200 and 500 ng/ml with bone marrow

Table 1: Relative frequency of different types of anemia

Types of anemia	Number of cases	Percentage
Iron deficiency anemia	20	40
Aplastic anemia	8	16
Anemia of chronic disease	13	26
Hemolytic anemia	3	6
Megaloblastic anemia	3	6
Others (associated with leukemia, MDS etc.)	3	6
Total	50	100

MDS: Myelodysplastic syndrome

Table 2: Age wise distribution of different types of anemia

Types of anemia	0-10 years	10-20 years	20-40 years	40-60 years	>60 years
Iron deficiency anemia	1	8	7	3	1
Anemia of chronic disease	2	5	5	1	0
Aplastic anemia	0	2	5	1	0
Megaloblastic anemia	1	1	0	1	0
Others	1	0	1	0	1
Hemolytic anemia	1	0	2	0	0

Table 3: Sex wise distribution of different types of anemia

Types of anemia	Female	Male
Iron deficiency anemia	14	6
Anemia of chronic disease	7	6
Aplastic anemia	3	5
Megaloblastic anemia	0	3
Hemolytic anemia	1	2
Others	2	1

Table 4: Relationship of serum ferritin with bone marrow iron stores

Serum ferritin (ng/dl)	Number of cases	Bone marrow iron stores
<14	12	Grade 0-2
15-25	2	Grade 0-2
25	0	-
45-100	3	Grade 2-4
100-200	4	Grade 1-3
200-500	9	Grade 1-3
500-2000	10	Grade 2-4
>2000	10	Grade 3-4

iron stores between Grades 1 and 3. About 20% of cases had ferritin 500-2000 ng/ml with bone marrow iron stores between Grade 2 and Grade 4. About 20% cases had ferritin >2000 ng/ml with bone marrow iron stores Grade 3-4.

DISCUSSION

In the present study, Perl's stain was done with serum ferritin in 50 cases which were diagnosed as anemia hematologically.

In the present study, 40% of cases were of iron deficiency anemia, 16% cases were of aplastic anemia, 36% cases were of anemia of chronic disease; 6% cases were of hemolytic anemia, 6% cases were of megaloblastic anemia, 6% cases were of others (anemia of MDS; leukemia). Pujara *et al.* (2014) reported 60% microcytic anemia; 14.2% megaloblastic anemia; 3% hemolytic anemia, others 7.2% aplastic anemia 4.3%.[12]

In the present study, the majority of cases were from 20 to 40 years (42%) of age group. Next group was 10-20 years (28%) of age group. Iron deficiency anemia was more common in females and anemia of chronic disease was more common in males. Similar findings were observed in a study conducted by Pujara *et al.* in 2014.[12]

Predominant Marrow Findings in Different Types of Anemia

In the present study, predominant bone marrow finding in iron deficiency anemia was mild to moderate normoblastic erythroid hyperplasia with bone marrow iron stores of Grade 0-2, which correlated with study done by Pujara *et al.* and Bableshwar *et al.*[12,13]

In the present study, 16% cases had serum ferritin <14 ng/ml with bone marrow iron stores from Grade 0-2. About 12% cases had ferritin 15-25 ng/ml with bone marrow iron stores between Grades 0 and 2. About 6% cases had ferritin between 45 and 100 ng/ml with bone marrow iron stores between Grades 2 and 4. *P* value was <0.03 when compared with the bone marrow iron stores.

Studies	Serum ferritin (ng/dl)	Perxentage of cases	Bone marrow iron stores
Pujara	<14	17	Grade 0-2
	15-25	24	Grade 0-2
	45-100	11	Grade 0-4
Present study	<14	16	Grade 0-2
	15-25	12	Grade 0-2
	45-100	6	Grade 2-4

There was also the presence of siderotic granules in erythroblasts in variable number in megaloblastic anemia. The study correlates with study of Bableshwar *et al.* in study of 80 patients and Krause and Stolc in a study on 104 patients.[13,14]

CONCLUSION

Microscopic examination of stainable iron in bone marrow is a reliable technique in assessing iron stores. In the present study, it has been found that all cases of iron deficiency anemia had low iron stores. Serum ferritin value when

<14 ng/dl is diagnostic of iron deficiency anemia while raised serum ferritin does not exclude iron deficiency anemia. Serum iron correlates well with the bone marrow iron stores level.

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