MAK HILL Publications Research Journal of Medical Sciences

Research Article
doi: 10.36478/makrjms.2024.12.247.250

OPEN ACCESS

Key Words
Bone marrow findings, hematological conditions, anaemic persons

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Received: 18 September 2024
Accepted: 10 November 2024
Published: 23 November 2024

Citation: G.K. Bharath and M.R. Manjunath, 2024. Bone Marrow Findings in Various Hematological Conditions in Anaemic Persons. Res. J. Med. Sci., 18: 247-250, doi: 10.36478/makrjms.2024.12.247.250

Bone Marrow Findings in Various Hematological Conditions in Anaemic Persons

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ABSTRACT
Cellular iron is found as either ferritin or hemosiderin. It is identified in cells by the Perls or Prussian blue reaction, in which ionic iron reacts with acid ferro cyanide to impart a blue color. The stain is best used on bone marrow aspirate smears but can also be used on blood films and aspirate clot tissue sections. Bone marrow smears and blood investigation report of patients age <18 years and of either sex who are referred to the Department of Pathology for bone marrow aspiration as part of diagnostic work up for anemia were included in this study. In our study majority of the bone marrow finding was erythroid hyperplasia with megaloblastic differentiation (12, 23.07%), followed by erythroid hyperplasia with micronormoblastic maturation (9,17.30%).

INTRODUCTION

Bone marrow is a semifluid and is aspirated through a needle. The iliac crest and body of the sternum are the most common sites for aspiration in adults. Local analgesia is usually employed. The procedure is performed in a sterile manner. Firstly, skin around the area should be cleaned. Local anesthetic is infiltrated into the skin, subcutaneous tissue and periosteum, overlying the selected site before the needle is penetrated perpendicularly into the bony cavity by pressure associated with boring movement/rotation[1]. After the cavity is penetrated., the stylet is withdrawn and a tightly fitting syringe is attached. Strong but brief suction yields about 0.2ml of bone marrow sample. Successful aspiration is accompanied by transient pain[2]. Films are prepared immediately because bone marrow clots faster than peripheral blood, by placing the aspirated material on a glass slide, sucking off most of the blood and preparing a film where the particles are drawn along by a spreader to leave trails of dislodged bone marrow cells. The remainder of the material then be delivered into a bottle containing an appropriate amount of ethylenediaminetetraacetic acid (EDTA) anticoagulant and used later to make more films. The air-dried films are then fixed in the manner appropriate for the desired staining procedure [3]. Cellular iron is found as either ferritin or hemosiderin. It is identified in cells by the Perls or Prussian blue reaction, in which ionic iron reacts with acid ferrocyanide to impart a blue color. The stain is best used on bone marrow aspirate smears but can also be used on blood films and aspirate clot tissue sections[4]. Perls stain is used to stain marrow iron which also helps in differentiating iron from malarial pigment. Following Perl's stain iron is graded from 0-6, with low normal being grade 1-3 and grade 4-6, with increased iron stores. Iron should be assessed only in marrow particles. Multiple fragments are needed since iron distribution is not uniform in all fragments[5]. Iron smear assessment using the intensive histological grading method was interpreted as 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)[6].

MATERIALS AND METHODS

Study Design: Descriptive cross sectional study.

Study Population: Bone marrow aspirate of patients age more than 18 years and of either sex, who are referred to the Department of Pathology, as part of diagnostic work up for anemia were included in this study.

Sampling Method: Consecutive samples as per the inclusion and exclusion criteria.

Inclusion Criteria: Bone marrow smears and blood investigation report of patients age more than18 years and of either sex who are referred to the Department of Pathology for bone marrow aspiration as part of diagnostic work up for anemia were included in this study.

Exclusion Criteria:

- Marrow sample found to be diluted with blood.
- Marrow smears having poor material or inadequate material.
- Marrow samples from those who undergoing blood transfusion one week prior to bone marrow aspiration.
- Marrow samples from those who are on parenteral or oral iron supplementation one week prior to bone marrow aspiration.

Data Collection: Data collection was done by collecting the bone marrow smears, which are obtained from bone marrow aspiration done in Department of pathology. These bone marrow smears will be stained by Perls' stain and assessed microscopically according to Gale's histological grading method and intensive histological grading method for iron stores. The blood investigation reports of complete hemogram, peripheral smear, serum ferritin, serum iron and serum TIBC from blood samples sent to central diagnostic laboratory was collected.

RESULTS AND DISCUSSIONS

In our study majority of the bone marrow finding was erythroid hyperplasia with megaloblastic differentiation (12, 23.07%), followed by erythroid hyperplasia with micronormoblastic maturation (9,17.30%). Whereas in a study conducted by Pujara[7] predominant bone marrow finding was mild to moderate normoblastic erythropoietin, followed by megaloblastic erythropoietin and normoblastic erythropoietin. In our study participants belonged to grades 0, 1, 2, 3 and 4 in 10 (19.2%), 10 (19.2%), 19 (36.5%), 8 (15.4%) and 5 (9.6%), respectively. In the current study, no participants belonged to Grades 5 or 6. A study conducted by Sharma S et al. showed grade 0,1 in 29 (47.54%), grade 2,3 in 23 (37.71%) and increased iron stores in 9 (14.75%) of cases. In another study conducted by Dharwadkar[8] grade 0, 1, 2 and 3, was seen in 8(14.54%), 35(63.63%), 8(14.54%) and 4(7.27%) cases respectively. In the present study, based on the intensive grading method of bone marrow, the majority of the participants were diagnosed with Iron store deficiency (36.5%) and combined functional and iron deficiency (38.5%). This was comparable to the study done by Singh[9] who had

Table 1: Bone Marrow Findings in Various Haematological Conditions

Diagnosis	Bone marrow finding	Total	Percentage
Pancytopenia (N=18)	•Erythroid hyperplasia with megaloblastoid differentiation.	06	33.3%
	•Erythroid hyperplasia with micronormoblastic maturation.	02	11.11%
	•Hypoplastic bone marrow.	01	5.55%
	•Hypocellular BM with supression of myelopoiesis.	01	5.55%
	•Normocellular BM with micronormoblastic and megaloblastic maturation.	02	11.11%
	•Myelodysplatic syndrome.	01	5.55%
	•Hypercellular BM with erythroid, megaloblastic and megakaryocytic hyperplasia.	01	5.55%
	•Erythroid and megakaryoblastic hyperplasia.	01	5.55%
	•Normocellular BM with normoblastic eryhthroid hyperplasia.	01	5.55%
	•Hypercellular BM with erythroid and myeloid hyperplasia.	01	5.55%
	•Non-Hodgkins Lymphoma.	01	5.55%
Dimorphic anaemia (N=11)	•Hypercellular BM with erythroid and megaloblastic differentiation.	02	18.18%
	•Erythroid hyperplasia with micronormoblastic maturation.	01	9.09%
	•Hypoplastic BM with erythroid predominance.	01	9.09%
	•Normocellular BM with micronormoblastic maturation.	01	9.09%
	•Normocellular BM with micronormoblastic and megaloblastic maturation.	02	18.18%
	•Hypoplasia of bone marrow.	01	9.09%
	•Hypocellular BM with supression of erythropoeisis and myelopoiesis.	01	9.09%
	•Erythroid and megakaryoblastic hyperplasia.	01	9.09%
	•Hypercellular BM with megaloblastic maturation.	01	9.09%
Macrocytic anaemia (N=9)	•Erythroid hyperplasia with megaloblastic maturation.	04	44.44%
	•Erythroid hyperplasia.	05	55.55%
Normocytic normochromic (N=6)	•Normocellular Bone marrow.	02	33.33%
	•Erythroid and megakaryocytic hyperplasia.	01	16.66%
	•Normocellular BM with megakayocytic hyperplasia and normal erythropoeisis and myelopoiesis.	01	16.66%
	•Erythroid hyperplasia with micronormoblastic maturation.	01	16.66%
	•Normocellular BM with megakaryocytic hypoplasia.	01	16.66%
Microcytic hypochromic (N=5)	•Erythroid hyperplasia with micro-normoblastic maturation.	05	100%
Normocytic hypochromic (N=3)	•Erythroid hyperplasia with micro-normoblastic maturation.	01	33.33%
	•Erythroid hyperplasia with megaloblastic maturation.	01	33.33%
	•BM dysplasia.	01	33.33%

Table 2: Distribution of Cases Based on Gale's Histological Grading of Bone Marrow

Gale's histological grading	Frequency	Percentage
0	10	19.2
1	10	19.2
2	19	36.5
3	8	15.4
4	5	9.6
5	0	0
6	0	0
Total	**52**	**100**

Table 3: Classification of Patients According to Intensive Grading Method

Intensive grading method	Frequency	Percentage
Normal	5	9.6
Functional iron deficiency	8	15.4
Iron store deficiency	19	36.5
Functional and iron store deficiency	20	38.5
Total	**52**	**100**

Table 4: Comparison of Patients According to Intensive Grading Method

Intensive grading method	Singh[9]	Present Study
Normal	37.76%	9.6%
Functional iron deficiency	14.68%	15.4%
Iron store deficiency	4.20%	36.5%
Functional and iron store deficiency	43.36%	38.5%

the majority of the participants diagnosed with functional and iron deficiency (43.36%) and Functional iron deficiency (14.68%)[10].

CONCLUSION

- Most common bone marrow finding being erythroid hyperplasia with megaloblastic differentiation seen in 12 cases (23.07%).
- Perl's stain was done on all the bone marrow aspirate smears and assessed microscopically using Gale's method of grading bone marrow iron stores.

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