Section: Pathology

Original Research Article

BONE MARROW IRON STORAGE IN VARIOUS CASES OF SEVERE ANAEMIA: A CROSS-SECTIONAL STUDY AT A TERTIARY CARE CENTRE

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ABSTRACT

Background: Severe anaemia is a significant public health problem with diverse etiologies requiring accurate diagnosis for appropriate management. Bone marrow aspiration with Perl's Prussian blue staining remains the gold standard for evaluating marrow iron stores despite the widespread availability of biochemical iron studies. This study evaluated bone marrow iron storage patterns in patients with severe anaemia and correlated iron stores with different hematological diagnoses. **Materials and Methods:** A hospital-based cross-sectional study was conducted in the Department of Pathology, G.R. Medical College and J.A. Group of Hospitals, Gwalior. Patients presenting with severe anaemia who underwent bone marrow aspiration were included. Clinical details, complete blood counts, peripheral blood smear findings, bone marrow morphology, and marrow iron stores assessed by Perl's Prussian blue staining were analyzed. Iron stores were graded and correlated with the underlying diagnosis. **Results:** Bone marrow examination demonstrated a broad spectrum of hematological disorders associated with severe anaemia. Iron deficiency anaemia showed markedly reduced or absent marrow iron stores, whereas megaloblastic anaemia generally demonstrated normal to increased iron stores. Patients with anaemia of chronic disease exhibited preserved or increased marrow iron, while cases of myelodysplastic syndrome frequently showed increased iron deposition. Bone marrow aspiration combined with Perl's staining effectively differentiated iron deficiency from other causes of severe anaemia. **Conclusion:** Bone marrow aspiration with Perl's Prussian blue staining remains a reliable and cost-effective method for assessing iron stores in patients with severe anaemia. The procedure provides valuable diagnostic information, facilitates differentiation of various etiologies of anaemia, and supports appropriate therapeutic decision-making, particularly in complex hematological disorders.

INTRODUCTION

Anaemia remains one of the most common hematological disorders worldwide and continues to be a major public health problem, particularly in developing countries. According to the World Health Organization, severe anaemia contributes substantially to morbidity and mortality across all age groups. Iron deficiency is the leading cause of anaemia; however, vitamin B12 deficiency, folate deficiency, anaemia of chronic disease, aplastic anaemia, myelodysplastic syndromes, leukemia, and other bone marrow disorders are also important etiologies. Accurate identification of the underlying cause is essential because treatment strategies vary considerably depending on the diagnosis.[1]

Although peripheral blood examination and biochemical investigations such as serum ferritin, serum iron, total iron-binding capacity, and transferrin saturation are routinely used in the evaluation of anaemia, these tests may be influenced by inflammation, infection, liver disease, and malignancy. Consequently, assessment of bone marrow iron stores remains the reference standard for evaluating body iron reserves, particularly in diagnostically challenging cases.[2]

Bone marrow aspiration is a simple, safe, and minimally invasive procedure that provides valuable information regarding marrow cellularity, erythropoiesis, myelopoiesis, megakaryopoiesis, infiltrative disorders, and iron stores. Perl's Prussian blue staining enables direct visualization and grading of hemosiderin deposits within marrow macrophages and erythroblasts, thereby allowing reliable

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assessment of iron status. In addition to differentiating iron deficiency anaemia from other causes of anaemia, bone marrow examination plays a pivotal role in diagnosing hematological malignancies, myelodysplastic syndromes, aplastic anaemia, plasma cell disorders, and other marrow pathologies.[3]

Several studies have demonstrated that bone marrow examination has high diagnostic utility in patients presenting with unexplained anaemia, pancytopenia, thrombocytopenia, leukopenia, and suspected hematological malignancies. Nevertheless, regional variations exist in the spectrum of bone marrow disorders and iron storage patterns because of differences in nutritional status, socioeconomic conditions, and disease prevalence. Therefore, institution-specific data remain valuable for guiding clinical practice.[4]

The present study was undertaken to evaluate bone marrow iron stores in patients with severe anaemia using Perl's Prussian blue staining and to correlate marrow iron status with various hematological disorders diagnosed on bone marrow aspiration. The study also aimed to assess the diagnostic utility of bone marrow examination in the evaluation of severe anaemia at a tertiary care teaching hospital in Central India.[5]

MATERIALS AND METHODS

Study Design and Setting: A hospital-based cross-sectional observational study was conducted in the Department of Pathology, G.R. Medical College and J.A. Group of Hospitals, Gwalior, Madhya Pradesh, India. The study was undertaken after obtaining approval from the Institutional Ethics Committee, and all procedures were performed in accordance with institutional ethical guidelines.

Study Population: Patients presenting with severe anaemia who were advised bone marrow aspiration for diagnostic evaluation during the study period were included. Patients of all age groups and both sexes who fulfilled the eligibility criteria and provided informed consent were enrolled consecutively.

Inclusion Criteria

- Patients with severe anaemia requiring bone marrow examination.
- Patients who provided written informed consent.
- Patients with complete clinical and laboratory records.

Exclusion Criteria

- Patients unwilling to participate.
- Patients with inadequate or diluted bone marrow aspirate samples.
- Patients with incomplete clinical or laboratory data.

Clinical and Laboratory Evaluation

Detailed demographic and clinical information, including age, sex, presenting complaints, and relevant medical history, was recorded. All patients underwent complete blood count analysis using an automated hematology analyzer. Peripheral blood smears were examined after Leishman staining for assessment of red blood cell morphology, leukocyte abnormalities, and platelet morphology.

Bone Marrow Aspiration and Iron Staining

Bone marrow aspiration was performed under aseptic precautions, primarily from the posterior superior iliac spine. Aspirated marrow smears were stained with Leishman stain for cytomorphological evaluation. Additional smears were stained using Perl's Prussian Blue stain to assess bone marrow iron stores.

Bone marrow cellularity, erythroid and myeloid maturation, megakaryocyte morphology, plasma cells, lymphoid cells, and abnormal infiltrates were evaluated according to standard hematopathological criteria.

Assessment of Bone Marrow Iron Stores

Iron stores were graded using Gale's grading system based on Perl's Prussian Blue staining. The marrow iron content was categorized from Grade 0 (absence of stainable iron) to Grade 6 (marked iron overload). Iron grades were correlated with the final hematological diagnosis established by bone marrow examination.

Statistical Analysis

The collected data were entered into Microsoft Excel and analyzed using appropriate statistical software. Continuous variables were expressed as mean ± standard deviation, whereas categorical variables were presented as frequencies and percentages. Associations between bone marrow iron stores and hematological diagnoses were assessed using appropriate statistical tests. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 100 patients with severe anaemia who underwent bone marrow aspiration during the study period were included in the analysis. The mean age of the study population was 11.61 ± 9.29 years, with patients ranging from infancy to adulthood. Females constituted 55% of the study population, while males accounted for 45%, giving a female-to-male ratio of approximately 1.2:1.

The mean hemoglobin concentration at presentation was 6.03 ± 1.32 g/dL, confirming severe anaemia in all enrolled patients. Generalized weakness, pallor, fever, and bleeding manifestations were among the common presenting complaints.

Bone marrow examination demonstrated a wide spectrum of hematological disorders. Megaloblastic anaemia was the most frequent diagnosis, accounting for 35% of cases, followed by mixed nutritional deficiency anaemia (21%). Acute lymphoblastic leukemia (8%) was the most common malignant disorder identified. Other diagnoses included iron deficiency anaemia, aplastic anaemia, acute myeloid leukemia, immune thrombocytopenic purpura,

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myelodysplastic syndrome, and other less common hematological disorders.

Evaluation of bone marrow iron stores using Perl's Prussian blue stain demonstrated distinct iron storage patterns among different diagnostic categories. Patients with iron deficiency anaemia showed absent or markedly reduced stainable iron, whereas patients with megaloblastic anaemia generally exhibited normal to increased marrow iron stores. Mixed nutritional deficiency anaemia demonstrated variable iron grades depending on the relative contribution of iron deficiency. Cases of anaemia of chronic disease and myelodysplastic syndrome typically showed preserved or increased marrow iron stores.

Bone marrow aspiration established the diagnosis in the majority of patients and proved particularly valuable in differentiating nutritional anaemias from marrow infiltrative disorders and hematological malignancies. The addition of Perl's Prussian blue staining significantly improved the diagnostic accuracy for assessment of body iron stores and facilitated appropriate therapeutic planning.

Table 1: Age wise distribution of Study Participants

S.no.	Age	Frequency	Percentage (%)
	Mean age=11.61, SD=9.29		
1	1 to 5 years	14	14
2	6 to 10 years	36	36
3	11 to 15 years	42	42
4	>15 years	8	8
	Total	100	100

Table 2: Sex wise distribution of Study Participants

S. No.	Sex	Frequency	Percentage (%)
1.	Male	45	45
2.	Female	55	55
	Total	100	100

Table 3: Anaemia according to Hb(gm/dl) of study participants

S. No.		Frequency	Percentage(%)
	Mean Hb= 6.03 gm/dl, SD=1.32		
1.	Severe Anaemia (6.5 to 7.9 gm/dl)	57	57
2.	Life threatening Anaemia (<6.5 gm/dl)	43	43
	Total	100	100

Table 4: Total leukocyte count (TLC) of Study participants

S. No.	TLC (Per cumm)	Frequency	Percentage(%)
1	<4000	40	40
2	4000-11000	36	36
3	>11000	24	24
	Total	100	100

Table 5: Platelet count of Study participants

S. No.	Platelet count	Frequency	Percentage (%)
1	Extremely low (<50000/cumm)	33	33
2	Low (50000 to 150000/cumm)	49	49
3	Normal (150000-400000/cumm)	18	18
4	High (>400000/cumm)	0	0
	Total	100	100

Table 6: Diagnosis for the cause/type of Anaemia

S. No.	Diagnosis/Cause	Frequency	Percentage(%)
1	ALL	8	8
2	AML	5	5
3	AOCD	4	4
4	Aplastic/Hypoplastic Anaemia	6	6
5	CLL	1	1
6	CML	2	2
7	Hemolytic	7	7
8	Iron deficiency Anaemia	6	6
9	MDS	5	5
10	Megaloblastic Anaemia	35	35
11	Mixed Nutritional Deficiency Anaemia	21	21
	Total	100	100

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Table 7: Peripheral blood smear findings of the study participants

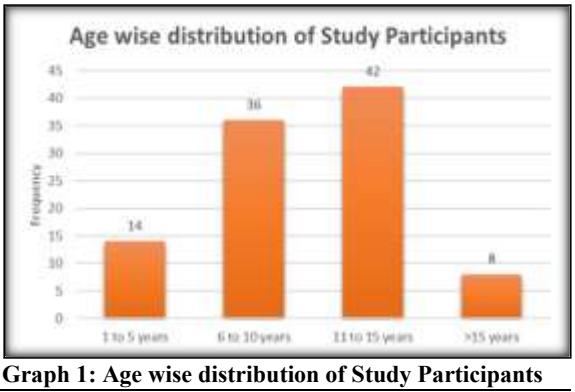
		Frequency	Percentage (%)
Size of RBC	Microcytic	28	28
	Normocytic	27	27
	Macrocytic	45	45
Nucleated RBC	Present	41	41
	Absent	59	59
Hypersegmented Neutrophils	Present	34	34
	Absent	66	66
Pancytopenia	Present	9	9
	Absent	91	91
Shape of RBC	Normal (Biconcave)	59	59
	Spherocyte	7	7
	Ovalocyte	34	34
Tear drop cells and Helmet Cells	Present	7	7
	Absent	93	93

Table 8: Bone Marrow findings of the study participants

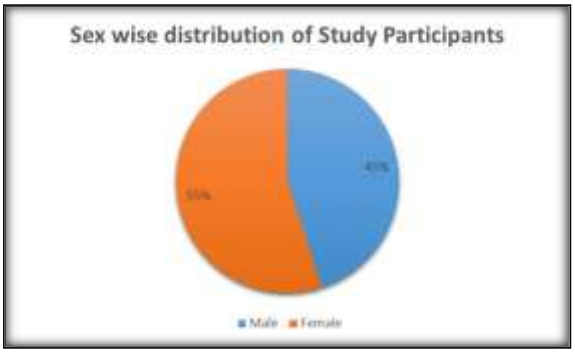
Bone Marrow Aspiration Findings	Frequency	Percentage (%)
Hypocellular BM	6	6
Hypercellular BM	29	29
Dyserythropoeisis	39	39
Micronormoblast	24	24
Normoblasts	49	49
Megaloblastic change	54	54
Erythroid hyperplasia	58	58
Marrow replaced by Myeloid series cells	6	6
Marrow replaced by Lymphoid series cells	8	8
Marrow replaced by fat	5	5
Metamyelocyte	27	27
Dysplastic changes with hypogranular megakaryocyte	5	5
Lymphoid mast cell, Plasma cell	6	6

Table 9: Findings of Perl's stain

Perl's stain grading	Frequency	Percentage(%)
0	14	14
1+	11	11
2+	20	20
3+	39	39
4+	15	15
5+	1	1
Total	100	100



Graph 1: Age wise distribution of Study Participants



Graph 2: Pie Chart showing Sex wise distribution of Study Participants

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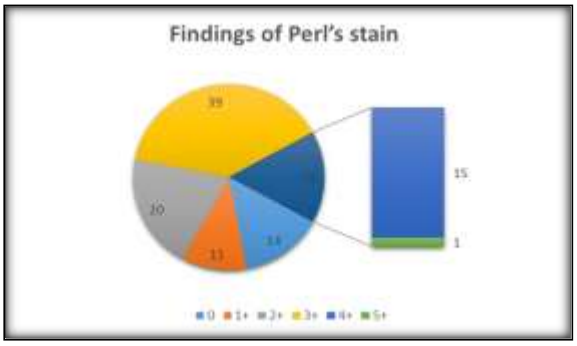

Graph 3: Pie chart showing findings of Perl's Stain

DISCUSSION

The present study evaluated bone marrow iron stores in 100 patients with severe anaemia using bone marrow aspiration and Perl's Prussian blue staining. Bone marrow examination remains an indispensable diagnostic tool for patients with unexplained or severe anaemia because it provides comprehensive information regarding marrow morphology, hematopoiesis, and iron stores.[6] In the present study, the majority of patients were young, with a slight female predominance. The higher proportion of female patients may be attributed to increased nutritional deficiencies, menstrual blood loss among adolescents and adults, and greater physiological iron requirements. Similar demographic observations have been reported in previous studies conducted in developing countries. Bone marrow iron assessment using Perl's Prussian blue stain demonstrated distinct patterns across different hematological disorders.[7] Patients with iron deficiency anaemia consistently exhibited absent or markedly depleted marrow iron stores, confirming the diagnostic value of marrow iron grading. In contrast, patients with megaloblastic anaemia generally had normal or increased iron stores because ineffective erythropoiesis limits iron utilization despite adequate or increased body iron reserves.[8]

Patients with mixed nutritional deficiency anaemia showed variable iron grades depending on the relative contribution of iron deficiency and megaloblastic change. Bone marrow aspiration also proved valuable in identifying malignant and non-nutritional disorders, including acute leukemias, aplastic anaemia, and myelodysplastic syndromes. In these conditions, marrow morphology provided definitive diagnostic information that could not be obtained reliably from peripheral blood examination alone.[9] The procedure therefore plays an important role in the diagnostic work-up of severe anaemia, particularly when routine laboratory investigations are inconclusive. The findings of the present study are consistent with previous reports demonstrating that bone marrow iron assessment remains the reference standard for evaluating body iron stores. Although biochemical markers such as serum ferritin are widely available, their interpretation may be affected by inflammation, chronic infection, liver disease, and malignancy.[10]

Direct assessment of marrow iron by Perl's staining therefore continues to have an important role in selected clinical situations.

The strengths of this study include prospective data collection, standardized bone marrow examination, and systematic evaluation of iron stores in all enrolled patients.[11] However, certain limitations should be acknowledged. The study was conducted at a single tertiary care center with a relatively modest sample size, which may limit the generalizability of the findings.[12]

In addition, serum ferritin and other iron profile parameters were not available for correlation in all patients. Multicenter studies with larger sample sizes and comprehensive biochemical evaluation would further strengthen the evidence regarding the diagnostic utility of bone marrow iron assessment.[13]

Overall, the study highlights the continuing importance of bone marrow aspiration with Perl's Prussian blue staining in the evaluation of severe anaemia. The procedure remains highly effective for differentiating iron deficiency from other causes of anaemia and for diagnosing underlying hematological disorders that require disease-specific management.[14,15]

CONCLUSION

Bone marrow aspiration combined with Perl's Prussian blue staining is a reliable and effective diagnostic modality for evaluating severe anaemia. It facilitates accurate assessment of marrow iron stores and enables differentiation between iron deficiency anaemia, megaloblastic anaemia, mixed nutritional deficiency anaemia, anaemia of chronic disease, and hematological malignancies. In the present study, megaloblastic anaemia was the most common cause of severe anaemia, followed by mixed nutritional deficiency anaemia. Bone marrow examination proved particularly valuable in establishing definitive diagnoses when peripheral blood findings were inconclusive.

Despite advances in biochemical assessment of iron status, bone marrow iron grading remains the reference standard in selected clinical situations. Early and accurate diagnosis through bone marrow examination allows timely initiation of appropriate therapy, thereby improving patient outcomes. Larger multicentric studies incorporating biochemical iron parameters are recommended to further validate these findings.

Ethics Approval

The study was approved by the Institutional Ethics Committee of G.R. Medical College and J.A. Group of Hospitals, Gwalior. Written informed consent was obtained from all participants or their legal guardians before enrolment.

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