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Original Research Article

Study of Bone Marrow Aspiration for a Period of Two Years

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Abstract

Introduction: Bone marrow study is a common procedure. It is highly informative procedure, though invasive, it is safe when performed with proper precautions. It is used routinely in medical institutions for the diagnosis and management of hematological disorders which often pose diagnostic challenge to physician and the knowledge of accurate etiologies is crucial in their management.

Aim of the study: To study the morphology and spectrum of bone marrow in hematological diseases, in cases of Pancytopenias to determine the etiology and also to study the bone marrow morphology in isolated splenomegaly cases presenting as haematological disorder.

Materials and Methods: This was a prospective study done over a period of two years. Peripheral blood smear examination and bone marrow aspirations were performed in 176 cases. The patient demographics, bone marrow findings and clinical findings were correlated.

Results: There were total 176 patients. Majority of the cases were reported in the age group range of 21-30 years and the male to female ratio was 1.1:1. Disorders of RBC were most common with predominance of megaloblastic anaemia. Disorders of WBC were less with cases of Acute leukaemia. There were 2 cases of Idiopathic thrombocytopenic purpura and 10 cases of Plasma cell dyscrasia. Normal marrow study was seen in 17 cases.

Conclusion: Bone marrow aspiration is a safe, easy, cost-effective procedure. Bone marrow aspiration shows better cellular details when compared to bone marrow biopsy. The latter is required for confirmation and prognostication of certain hematological diseases. The results and accuracy are highly dependent on the quality of samples and smears. Diagnosis is strengthened by correlating the marrow findings with peripheral smear, other relevant biochemical investigations and clinical features.

Keywords: Bone marrow study; Acute leukemias; ITP; Megaloblastic anemia.

Introduction

Bone marrow aspiration cytology is a frequent done procedure. It is a highly informative procedure, though invasive, it is safe when performed with proper precautions. It is routinely used in medical institutions for the diagnosis and management of hematological disorders. Hematological disorders are common and have variable clinical presentation. They often pose diagnostic challenge to physician and the knowledge of accurate etiologies is crucial in their management.

Bone marrow examination usually involves two separate, but interrelated, samples. The first is a cytologic preparation of bone marrow cells obtained by aspiration of the marrow and a smear of the cells, allowing excellent visualization of cell morphology and enumeration of the marrow cellular elements.[1]

The second sample is of a needle biopsy from bone marrow, which allows optimal evaluation of bone marrow cellularity, architecture of the hematopoietic cell elements in relation to the normal constituents of bone marrow i.e., bony trabeculae, vascular channels and adipose tissue. The study gives valuable information regarding disease processes involving hematopoietic cell elements(including leukaemias), presence of infections/parasitic diseases, fibrosis/sclerosis and presence of any infiltrative pathology.

Apart from cytomorphological assessment, bone marrow aspiration studies are useful for the culture of microorganisms, immunophenotyping, cytogenetics, molecular genetics and other specialised investigations like, deoxyuridine suppression test, culture of colony forming units and for ultrastructural examination.[1]

In addition to hematological disorders, thediagnosis and management of many non-hematological diseases depend on examination of the bone marrow in order to institute appropriate therapy and assess cure or recurrence at a later date. For example the evaluation of primary bone marrow tumors, and for staging for bone marrow involvement by metastatic tumors.

The advantage in bone marrow aspiration in comparison to a biopsy is earlier availability of specimens for interpretation and a better possibility to assess the morphology of individual cells. When both the procedures are performed simultaneously, they are complementary to each other and there is more material to study the morphology and the pattern of distribution of the cells.[2]

The present study emphasizes the significance of bone marrow evaluation to achieve optimum standards in patient care and management by providing proper diagnosis.

Aims and Objectives

To study the morphology and spectrum of bone marrow in hematological diseases, in cases of Pancytopenias to determine the etiology and also to study the bone marrow morphology in isolated splenomegaly cases presenting as haematological disorder.

Materials and Methods

Permission was taken from the Institutional ethics committee. Informed consent was taken from all patients included in the study.

This was a prospective study done over a period of two years i.e. from June 2012 to June 2014. This study was carried out in the department of Pathology at MediCiti Institute of Medical Sciences (MIMS), Ghanpur, Medchal, Telangana.

Patients referred from depatment of General Medicine for bone marrow aspiration studies were included.

Inclusion criteria:

Age 11 to 80 years.

Both genders.

Cases which were referred from general medicine department for bone marrow aspiration.

Exclusion criteria: Age below 11 years and above 80 years.

Peripheral blood smear examination and bone marrow aspirations were performed in all the cases. Other relevant investigations like ultrasound abdomen, blood culture, serum vitamin B12, folate etc. were also done as indicated by the clinicians' request.

Bone marrow smear examination: Bone marrow cells were counted in an area where the cells were well dispersed with good cytological detail, and where there was the least number of smudged (lysed) cells. At least 500 cells were counted in at least two smears when a precise percentage of an abnormal cell type was required for diagnosis and disease. At least 300 cells were counted if the nucleated differential cell count was not essential to the diagnosis.

Following aspects were examined on routine Leishman stain:

Estimation of cellularity.

Assessment of erythropoiesis.

Assessment of myelopoiesis.

Assessment of megakaryocytes.

Assessment of other cells likeplasma cells, monocytes, lymphocytes and mast cells.

Interpretion for iron stores.

Any parasites, any other cells (like osteoblasts).

Grading of iron stores on bone aspiration

Only intracellular iron in reticuloendothelial cells was taken into account. Extracellular iron if present was ignored.

Table 1: Grading of Bone marrow iron stores.

Grade	Interpretation
Grade 0: No hemosiderin granules.	Grade 0: Iron deficiency.
Grade 1: Fine granules in every 3–4 HPF.	Grade 1–2: Normal stores.
Grade 2: Heavier granules in every 2–3 HPF.	Grade 3–4: Increased iron stores.
Grade 3: Granules in every HPF in one/more cells.	–
Grade 4: Massive deposits with clumps and heavy granules.	–

The data was statistically analyzed for various parameters like age incidence, clinical features and underlying etiology as to the causes of hematological diseases.

Observations and Results

The present study was a prospective study done in MediCiti Institute of Medical Sciences over a period of two years.

A total of 176 bone marrow aspirations (BMA) were performed and all these cases were studied in correlation with peripheral blood smear findings and relevant clinical features.

Table 2: Distribution of cases based on findings determined by BMA.

Bone Marrow Findings		No. of Cases	Percent (%)
Disorders of Red blood cells *n* = 63 (35.7%)			
	Megaloblastic anaemia	33	18.7
	Erythroid hyperplasia with depleted iron stores	24	13.6
	Aplastic anaemia	4	2.2
	Hypoplastic marrow	2	1.1
Myelodysplastic Syndrome		1	0.5
Disorders of white blood cells *n* = 4 (2.2%)			
	Acute myeloid leukaemia	3	1.7
	Myelofibrosis	1	0.5
Disorders of platelets *n* = 2 (1.1%)			
	Idiopathic thrombocytopenic purpura	2	1.1
Disorders of plasma cells *n* = 10 (5.6%)			
	Reactive plasmacytosis	9	5.1
	Multiple myeloma	1	0.5
Normoblastic marrow		17	9.6
Total		176	100

Disorders of RBC-63 cases (Megaloblastic anaemia 33 cases.

Erythroid hyperplasia with depleted iron stores 24 cases and Aplastic anaemia 6 cases).

Myelodysplastic syndrome– 1 case.

Disorders of WBC– 4 cases (Acute leukaemias– 3 cases, Myelofibrosis– 1 case).

Disorders of Platelets– 2 cases (Idiopathic thrombocytopenic purpura– 2 cases).

Plasma cell dyscrasias– 10 cases (Reactive plasmacytosis 9 cases and Multiple myeloma– 1 case).

Normal study 17 cases (Normoblastic marrow– 17 cases).

Age and gender: Majority of the cases were reported in the age group range of 21 – 30 years (14 cases) with male to female ratio 1.1:1. Under 11–20 years age group 7 cases, in 41–50 years 5 cases, 4 cases in 31 – 40 years were reported. Least number of cases (3) were reported in 51 – 70 years age group.

In the present study there was a slight male preponderance with 52 (53.6%) male patients, compared to females 45 (46.4%). Male to female ratio was 1.1:1.

In the present study, a majority of 58 patients presented with pallor and least number of patients presented with lymphadenopathy and hepatomegaly.

Clinical findings: A majority of 25 patients (75.7%) amongst 33, presented with symptoms of anaemia like pallor and fatigue. Others presented with fever (33.3%), jaundice (24.2%), hepatosplenomegaly (12.12%) and splenomegaly (12.12%).

Peripheral smear findings: Hematologic findings included macrocytic anaemia, pancytopenia and dimorphic picture. Macro-ovalocytes/hypochromasia was seen along with hypersegmented neutrophils in a majority of 25 cases.

Bone marrow morphology: In the present study, bone marrow was hypercellular with predominantly megaloblastic erythropoiesis. Giant metamyelocytes were evident in 10 cases. Megakaryocytes were normal or decreased and of normal morphology.

Ate and vitamin B12: They were estimated in all cases and the levels were altered in only 18 (54.5%) out of 33 cases which reflects the higher prevalence of nutritional deficiency. In other cases the folate and vitamin B12 levels were normal indicating other etiology for Megaloblastic anaemia like chronic alcoholism, hypothyroidism and liver disease.

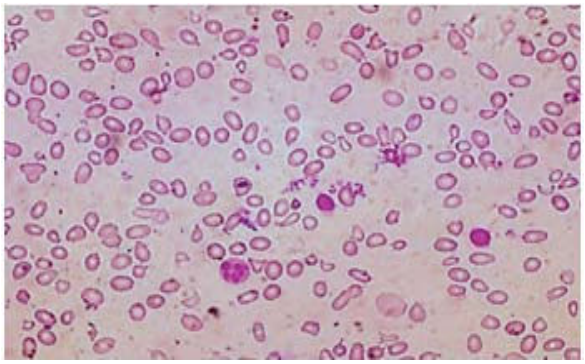

Fig. 1: Megaloblastic anemia–Peripheral smear showing macroovalocytes and hypersegmented neutrophils (Leishman stain, 100X).

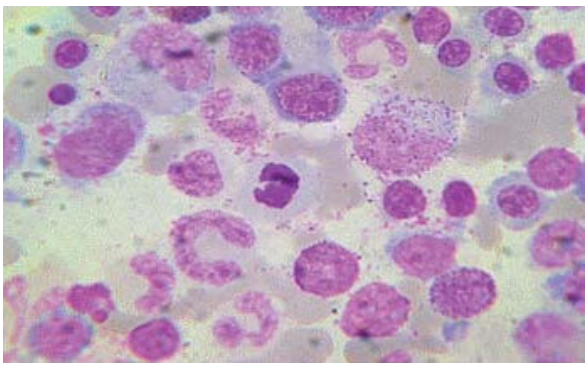

Fig. 2: Megaloblastic anemia–Bone marrow aspirate showinf giant metamyelocytes and megaloblasts (Leishman stain, 100X).

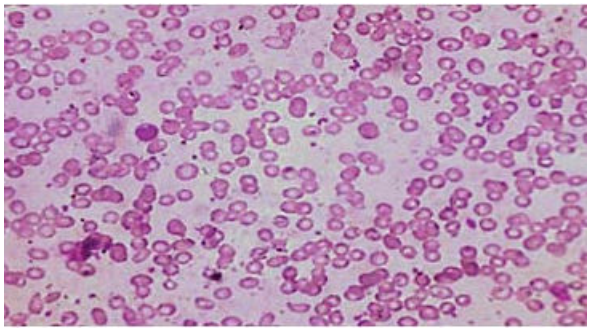

Fig. 3: Peripheral smear showing microcytic hypochromic RBC (Leishman stain, 40X).

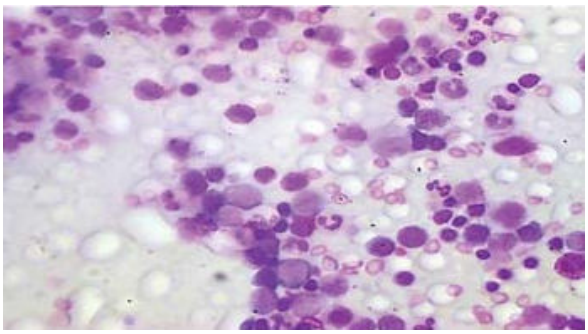

Fig. 4: Bone marrow aspirate showing erythroid hyperplasia (Leishman stain, 40X).

Discussion

The various diseases reported on bone marrow aspiration were:

Bone marrow findings: Similar incidence with Megaloblastic anaemia was reported in Damulak and Damen et al[3] study as 33% while it was reported to be second common finding in Pudasaini S et al[4] (12.3%) study which showed Erythroid hyperplasia as the commonest finding. Pudasaini S et al[4] showed correlation with respect to incidence of Myelodysplastic syndrome, and Multiple myeloma with incidence being least and 3.5% each.

Table 3: Comparative studies for Bone marrow morphology.

Study	Pudaisaini S et al[4]	Damulak and Damen et al[3]	Present Study
Total no. of cases; *n* (%)	57 (100%)	97 (100%)	97 (100%)
Megaloblastic Anaemia	7 (12.3%)	32 (33%)	33 (34%)
Erythroid Hyperplasia	18 (21%)	–	24 (24.7%)
Hypoplastic Marrow	3 (5.3%)	–	2 (2%)
Aplastic anemia	–	–	4 (4.1%)
Idiopathic Thrombo Cytopenia	6 (10.5%)	2 (2.4%)	2 (2%)
Acute Leukaemia	7 (12.3%)	27 (28.6%)	3 (3.0%)
Multiple Myeloma	1 (3.5%)	4 (4.1%)	1 (1%)
Others	15 (28.1%)	31 (31.9%)	28 (28.5%)

Age and gender incidence: In the present study, the age of the patients ranged from 11 to 80 years. In a similar study done by Egesie et al 5 the age of patients ranged from 3 to 80 years. A study done by Kibria et al 6 showed the age of patients ranging from 3.5 to 80 years.

The present study showed slight male preponderance with males being 52 (53.6%) and females accounted for 45 (46.4%) cases. Male to female ratio was 1.1:1. It correlated with other similar studies including that of Egesei et al[5] (1.5:1) and Niazi et al 7 (1.7:1) where both the studies showed male preponderance.

Table 4: Comparative studies for age and gender incidence.

Study	Age (in years)	Male : Female
Egesie et al[5]	3 – 80	1.5:1
Kibria et al[6]	3.5 – 80	1:0.59
Niazi et al[7]	1 – 75	1.7:1
Present study	11 – 80	1.1:1

Comparative Studies of Haematological Diseases

Megaloblastic anaemia: The present study shows an incidence of 33 cases (34%) and the diagnosis of Megaloblastic anaemia is made in correlation with biochemical and other parameters. Its incidence

correlated with similar studies by Dapus and Damen et al 3 with incidence of 33%, Kibria et al[6] with an incidence of 24.2% and with Metikurke et al[8] who reported an incidence of 39.65%.

Erythroid hyperplasia with depleted iron stores: In the present study, a total of 24 (24.7%) cases were diagnosed as erythroid hyperplasia with depleted iron stores. It was in concordance with Metikurke et al[8] study which had an incidence of 24.13%.

Bone marrow morphology: Bone marrow showed erythroid hyperplasia in all the cases, while additional normoblasts were seen in 5 cases. Perl's staining revealed depleted iron stores in all the cases. Pudasaini et al[4] showed erythroid hyperplasia as commonest finding in bone marrow studies accounting for 12 cases and 2 cases out of 12 showed micronormoblasts. In a similar study by Egesei et al[5] combined megaloblastic and iron deficiency anaemia (nutritional deficiency anaemia) was diagnosed in 34 (18.4%) cases.

Aplastic Anemia

Age and gender: In the present study 4 (4.1%) cases were diagnosed as aplastic anaemia of which 2 cases were in the age group range 21 – 30 years and one each in age ranging from 11 – 20 years, 51 – 60 years; and showed equal gender distribution. The incidence was in concordance with Metikurke et al[8] study showing 7 (12.06%) cases, while Jha et al[9] reported an increased incidence of 29.5%.

Hypoplastic marrow: Two patients (2%) were diagnosed with hypoplastic marrow. Pudasaini et al[4] reported 3 (5.3%) such cases.

Myelodysplastic syndrome (MDS): In the present study, only one case (1%) was reported with Myelodysplastic syndrome in a female aged 71 years. In a similar study,Pudasaini et al[4] also reported an incidence of 3.5%.

Bone marrow morphology: Hypercellular marrow showing megaloblastic maturation with dysplastic myeloid series showing pseudo-Pelger Huet cells were seen. Megakaryocytes were normal in morphology. Perl's stain on aspirate smears revealed grade–3 (increased iron stores). Refractory anaemia with unilineage dysplasia was the final diagnosis. Pudasaini et al 4 revealed that patients with MDS showed increased erythroid series of cells with megaloblastic changes and dyserythropoiesis and was correlating with the present study.

Acute myeloid leukaemia (AML): In the present study, 3 (3%) cases were reported to be Acute Myeloid Leukaemia M2 (according to FAB group classification). Its incidence correlated well with Pathak et al[9] who reported incidence of 4 (3.9%) cases. Pudasaini et al[4] reported 7 cases out of which 6 (10.5%) cases were that of AML. The commonest type was AML M3 (3 cases) followed by AML M2 (2 cases) and AML M3 (1 case). Metikurke et al[8] reported one case of AML with incidence of 1.7%.

Myelofibrosis: Further work up with bone marrow biopsy revealed decreased marrow cellularity, stroma showed increased reticulin fibers with intersection (Grade–2 Myelofibrosis). Pre-fibrotic and early stage primary myelofibrosis was the final diagnosis. Parajuli Set al[10] reported 2 cases with 2.2% incidence.

Idiopathic thrombocytopenic purpura (ITP): Two cases (2%) were reported as ITP in the age group of 31 – 40 years and both were females. Similar studies by Pudasaini et al[4] showed an incidence of 6 (10.5%) cases and Kibria et al[6] reported an incidence of 6.21%.

Reactive plasmacytosis: In the present study, a total of 9 (9%) cases were reported. In a similar study by Pathak R et al[11] they reported an incidence of 2 (1.9%) cases.

Multiple myeloma: In the present study only one case (1%) was diagnosed with multiple myeloma. Patient was a 38 year old female. Pudasaini et al[3] showed an incidence 2 (3.5%) cases, Metikurke et al[8] had an incidence of 3 (5.1%) cases. Kibria et al[6] and Jha A et al[9] reported an incidence of 9.04% and 0.94% respectively.

Normoblastic marrow: In the present study, 17 (18.1%) cases were reported to have normoblastic marrow. Of these, 11 were males and 6 were female patients. Pudasaini S et al[4] reported 6 (10.5%) cases as normal marrow. Indications for bone marrow aspiration were fever (more than 3 weeks duration) and anaemia associated with chronic kidney disease, congestive cardiac failure, endocrine disorders like hyperthyroidism, hypothyroidism, diabetes, chronic obstructive pulmonary disease, sepsis, acute kidney disease, cirrhosis and lymphadenopathy.

Isolated splenomegaly in haematological diseases: In the present study, 15 cases (15.4%) were reported to have isolated splenomegaly. Swaroop et al 19 studied 317 cases for 8 years, and concluded that hematological diseases had significant positive associations with massive splenomegaly, lymphadenopathy and blood cytosis i.e. erythrocytosis, leucocytosis or thrombocytosis.

Table 5: Comparative studies for Pancytopenia.

Study	Country/Year	No. of Cases	Most Common Cause (%)	2nd Most Common Cause (%)
Khodke et al[12]	India/2000	50	Megaloblastic anaemia (44)	Aplastic anaemia (14)
Kumar et al[13]	India/2001	166	Aplastic anaemia (29.51)	Megaloblastic anaemia (22.28)
Khunger et al[14]	India/2002	100	Megaloblastic anaemia (72)	Aplastic anaemia (14)
Savage et al[15]	Zimbabawe/1999	134	Megaloblastic anaemia	Aplastic anaemia
IAAS Group[16]	Israel and Europe/1987	319	Hypoplastic anaemia (52.7)	Myelodysplastic syndrome (4.5)
Keisu and Ost[17]	Israel and Europe/1990	100	Neoplastic disease, radiation (32)	Hypoplastic anaemia (19)
Pine and Walter[18]	United States/2010	64	Infections	–
Present study	India	97	Megaloblastic anaemia (43.7)	Aplastic anaemia (25)

Conclusion

Bone marrow aspiration is a safe and easy procedure with relatively less patient discomfort. It is cost-effective and does not require sophisticated equipment. It is easy to perform aspiration under local anaesthesia in co-operative patients. Bone marrow aspiration is one of the affordable diagnostic investigation in anaemias of chronic diseases and fever with associated haematological changes. In leukaemias, it is useful for diagnosis and staging. Bone marrow aspiration shows better cellular details when compared to bone marrow biopsy. Bone marrow biopsy is further required for confirmation and prognostication of Aplastic Anaemia, Myelofibrosis, Myelodyplastic syndrome. The results and accuracy are highly dependent on the quality of samples and smears. Appropriate training and experience is essential to consistently achieve optimal material for diagnosis. The advantages in correct diagnosis of a case by bone marrow aspiration (when required biopsy) in conjunction with the clinical, haematological study, far outweigh the minor disadvantages. Diagnosis is strenghtened by correlating the bone marrow findings with peripheral smear findings, other relevant investigations (like serum B12 and folate values) and presenting clinical features.

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