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Original Research Paper

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# A STUDY OF BONE MARROW ASPIRATION IN EVALUATION OF DIFFERENT HAEMATOLOGICAL AND NONHAEMATOLOGICAL DISORDERS IN A TERTIARY CARE CENTER OF SOUTH BIHAR

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## ABSTRACT

**Background:** Bone marrow examination is one of the important investigations to diagnose various haematological and nonhaematological disorders. Bone marrow examination includes both bone marrow aspiration and bone marrow biopsy. However, bone marrow aspiration studies are sufficient to diagnose different anemias, acute leukemias, leukemia relapse and remission, parasitic infections and storage disorders.

**Aim :** primary objective of this study was to know the spectrum of various haematological disorders in our center and also to evaluate efficacy of bone marrow aspiration in diagnosis of various diseases.

**Methods :** This study was conducted in the Department of pathology in Vardhman Institute of Medical Sciences, Pawapuri, Nalanda for a period of 2 years from January 2018 to December 2019. Bone marrow aspiration was done from posterior iliac crest under local anaesthesia. Slides were stained with Leishman stain for morphological examination. Slides were studied by two of the pathologists.

**Results :** A total of 180 cases were included in this study. Male to female ratio in our study was 1.25:1. The age range of cases was from 4-83 years and the mean age was 42.8 years. Anemia was the most common haematological disorder in our study accounting for 33.33% of cases followed by hypolplastic anemia (7.8%) acute leukaemia (7.8%) and multiple myeloma (12.2%). Total 14 (7.8%) marrows were normal. 6 Showed visceral Leishmaniasis and one patient each showed Plasmodium vivax and granulomatous infection. Erythroid hyperplasia was seen in 13 patients. Lymphoma infiltrate was seen in 2 patients and metastatic deposits of adenocarcinoma were seen in three cases. 3 cases of myelodysplastic syndrome were also seen.

**Conclusions :** Bone marrow aspiration cytology is a mildly invasive technique which can diagnose many hematological and non-hematologic diseases that can be confirmed by more advanced investigations viz. serological, biochemical or molecular. Findings in this study are consistent with previous studies.

**KEYWORDS :** Anemia, Bone marrow aspiration, Leukemia, Megaloblastic, Myeloma

## INTRODUCTION :

Haematological disorders are common in clinical practice. Almost all age groups are affected by a wide spectrum of haematological disorders ranging from different types of anemia and infective conditions to different haematol ymphoid malignancies. Bone marrow examination is performed after initial investigations to exclude or confirm various hematological and non-hematological disorders.[1] Bone marrow aspiration alone is sufficient to diagnose nutritional anemias, most of the acute leukaemias and immune thrombocytopenia.[2] It is also particularly useful in unexplained cytopenias and sub-leukaemic leukaemia.[2] The aspirates are generally studied in conjunction with clinical findings, peripheral blood smear and biopsy. Systematic and properly performed bone marrow study gives significant information about clinically suspected disease process or into unsuspected diseases.[3,4] Trephine biopsy study is more informative in selected haematological disorders like hypoplastic/aplastic anemia, various conditions of dry tap, lymphoma infiltration/staging, suspected metastatic diseases. Bone marrow biopsy gives wider area of marrow for study and gives information about spatial arrangement of haematopoietic cells.[5] Bone marrow aspiration (BMA) is a quick technique for marrow evaluation but has certain limitations like dry tap, patchy marrow involvement etc in various disorders. Bone marrow aspirate is also used for immunophenotyping, cytogenetic and molecular genetic analysis. [2,5,6] Bone marrow examination is an invasive procedure, however, it is safe even in thrombocytopenic patients with little or no risk of bleeding.[7] Common indications for bone marrow aspiration are refractory anemia, cytopenias, haematolymphoid malignancies, infections, storage disorders and granulomatous conditions.

## MATERIAL AND METHODS :

This study was conducted in the Department of Pathology in Vardhman Institute of Medical Sciences, Pawapuri, Nalanda for a period of 2 years extending from January 2018 to December 2019. Total 180 cases examined were included in the study. Approval from ethical committee of the college was obtained. All patients were examined meticulously and all relevant investigations like complete blood count, peripheral smears, reticulocyte count, biochemical tests and relevant radiological findings were noted. Bone marrow aspiration was done from posterior iliac crest under full aseptic conditions using salah needle. 20 ml dispovan was used to obtain aspirate which was immediately spread on labelled clean glass slides. Early spread is essential to prevent clotting that makes smear preparation difficult. Dried slides were stained using Leishman stain. Slides were examined for morphological details by two of the pathologists.

### Inclusion Criteria-

all patients with clinical and haemato logical indications of bone marrow examination referred from various clinical departments were included in the study.

### Exclusion Criteria-

patients who were not willing to undergo bone marrow examination after proper explanation and those with infection at local site were excluded from study. Cases with 20% or more blasts in peripheral blood were also excluded.

## RESULTS :

In the present study, males outnumbered females with a ratio of 1.25 :1. Youngest patient was 4 year old male child with features of pancytopenia and maximum age was 83 years - a male patient with refractory anemia and bone pain that proved to be a case of multiple myeloma. Age range was 4 to 83 years with mean age 42.80. Majority of the patients belonged to age group 20-50 years (54% of total patients) table1 describes age distribution of patients.

**Table1 : Age distribution of patients**

| Age group | Number of patients | Percentage |
|---|---|---|

| | | |
|---|---|---|
| 10 -20 | 19 | 10.56 |
| 20 -30 | 29 | 16.10 |
| 30- 40 | 51 | 28.33 |
| 40 -50 | 18 | 10.00 |
| 50 -60 | 21 | 11.67 |
| 60 -70 | 24 | 13.33 |
| 70 -80 | 12 | 6.67 |
| >80 | 06 | 3.33 |

Common clinical indications for bone marrow examination were as given in table 2.

**Table2: Clinical Indications**

| Clinical Manifestations | Number of patients | Percentage |
|---|---|---|
| Anemia | 60 | 33.3 |
| Pancytopenia | 51 | 28.3 |
| Fever of unknown origin | 22 | 12.2 |
| Bone pain / pathological fracture | 22 | 12.2 |
| Bleeding | 13 | 7.22 |
| Organomegaly | 12 | 6.67 |

**Table3: Bone Marrow Findings/Diagnosis**

| Clinical Manifestations | Number of patients(N=180) | Percentage |
|---|---|---|
| Megaloblastic anemia | 28 | 15.6 |
| Iron deficiency anemia | 17 | 9.45 |
| Dual deficiency anemia | 15 | 8.33 |
| Hypoplastic anemia | 14 | 7.80 |
| Acute leukemia | 14 | 7.80 |
| Erythroid hyperplasia | 13 | 7.22 |
| Myelodysplasia | 03 | 1.67 |
| Chronic myeloid leukemia | 02 | 1.11 |
| Polycythemia rubra vera | 02 | 1.11 |
| ITP | 07 | 3.89 |
| Plasma cell dyscrasia | 22 | 12.2 |
| Lymphoma infiltrate | 02 | 1.11 |
| Metastatic carcinoma | 03 | 1.67 |
| Visceral Leishmaniasis | 06 | 3.33 |
| Granulomatous disease | 01 | 0.55 |
| Malaria | 01 | 0.55 |
| Reactive plasmacytosis | 02 | 1.11 |
| Reactive marrow | 09 | 5.00 |
| Dry tap | 05 | 2.78 |
| Normal marrow | 14 | 7.80 |

**Table 4: Pancytopenia**

| Diagnosis | Number of patients(N=51) | Percentage |
|---|---|---|
| Megaloblastic anemia | 21 | 41.18 |
| Hypoplastic/aplastic anemia | 13 | 25.48 |
| Acute leukemia | 08 | 15.70 |
| Visceral Leishmaniasis | 06 | 11.80 |
| Lymphoma infiltrate | 01 | 1.96 |
| Metastatic carcinoma | 02 | 3.92 |

**Table 5: -Pyrexia of unknown origin**

| Diagnosis | Number of patients(N=22) | Percentage |
|---|---|---|
| Acute leukemia | 04 | 18.2 |
| Hypoplastic/aplastic anemia | 08 | 36.4 |
| Visceral Leishmaniasis | 04 | 18.2 |
| Granulomatous disease/TB | 01 | 4.55 |
| Lymphoma infiltrate | 01 | 4.55 |
| HIV | 01 | 4.55 |
| Unknown | 03 | 13.6 |

A total of 180 cases were diagnosed on BMA cytology with Anemia as the most common haematological disorder accounting for 33.33% of all cases followed by acute leukaemia and hypoplastic anemia, each comprising of 7.8% of total patients. Among anemias, megaloblastic anemia was most common followed by dual deficieny anemia. Megaloblastic anemia presents with typical clinical features as fatigue, exercise intolerance, paleness etc and in some cases with mild jaundice. Out of 28 cases of megaloblastic anemia, 21 patients had serum Vit.B12 markedly below the normal range and all these patients responded well to parenteral Vitamin B12. Hypoplastic marrow was seen in 14 cases (7.8%). All these cases showed hypocellular marrow particles comprising predominantly of lymphocytes, stromal cells, plasma cells, mast cells and adipocytosis. Majority of these patients clinically presented with prolonged fever and bleeding in some of these patients. CBC in majority of these patients revealed pancytopenia with severe anemia and thrombocytopenia of variable degree. In suspected cases of hypoplastic or aplastic anemia, bone marrow biopsy gives more accurate information due to larger area and cellularity for evaluation.

Erythroid hyperplasia: Erythroid hyperplasia was reported in 13 cases of total bone marrow aspirates. Out of 13 erythroid hyperplasia, 6 were normoblastic, 2 were predominantly micronormoblastic and remaining 5 showed both micronormoblastic and megaloblastic proliferation.

In present study total 9 cases demonstrated features of reactive marrow. Reactive cellular marrow are characterized by hyperplasia of one or more cell lineages or in few cases hypoplasia or aplasia. Reactive marrow is generally associated with inflammatory conditions or intrinsic marrow insult. Reactive plasmacytosis was seen in two cases. Plasma cells were 32% and 48% of all nucleated cells including some binucleated forms in aspirate smears. Both the cases of reactive plasmacytosis were HIV positive.

Total 6 cases of Visceral Leishmaniasis (Fig.1A & 1B) were diagnosed in our study. Bone marrow aspirate demonstrated many intracellular and extracellular amastigote forms of Leishmania donovani. All these patients showed mild to moderate fever, weakness, splenomegaly and pancytopenia in peripheral blood examination. One of the female patients of visceral leshmaniasis had multiple nodular cutaneous lesions for more than 1 year. These nodular lesions on aspiration showed few scattered amastigote forms of Leishmania donovani. Two of these patients with visceral Leishmaniasis had high grade fever for many months and on further evaluation, both the patients were seropositive for HIV. Parasitic load in retropositive patients was much higher than HIV negative patients. One patient was diagnosed with Plasmodium vivax infection showing multiple trophozoites and schizonts in the marrow aspirates. One of the patients with features of pancytopenia in peripheral blood examination revealed many granulomas and necrosis that were in favour of tuberculosis. This patient responded well to antitubercular therapy.

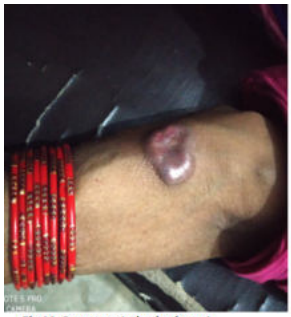

Fig.1A, Cutaneous lesion in visceral Leishmaniasis

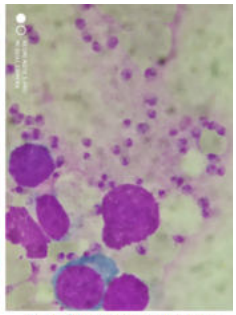

Fig.1B: BMA, Leishman stain 1000X , intracellular LD bodies

Among haematolymphoid malignancies, plasma cell dyscrasia / multiple myeloma was most common(22 cases)

followed by acute leukemia (14 cases) and chronic leukemias (CML 02, Polycythemia 02, Lymphoma infiltrate 2 cases). Majority of the patients of multiple myeloma presented with back pain, pathological fracture and some with features of chronic renal failure . Two of the patients presented with soft tissue lesion in lower back and bone marrow examination revealed sheets of plasma cells including many atypical plasmablasts, binucleated and multinucleated plasma cells (Fig.2A & B). Serum protein electrophoresis in 12 of the myeloma patients were positive for distinct M peak in gamma region. Total 14 cases [7.8%] were diagnosed as acute leukemia (Fig.3A). Majority of these cases presented with prolonged fever, bleeding from various sites, petechiae and anemia of variable degree. Except three cases of subleukemic leukemia, majority were negative for blasts in peripheral blood. We excluded cases of acute leukemia diagnosed on peripheral blood examination showing blasts 20% or more. Two cases of chronic myeloid leukemia (Fig.3B) were examined to rule out accelerated / blast crisis phase. Bone marrow examination in three of the patients with bone pain and pathological fracture revealed metastatic deposits of adenocarcinoma. Two of the patients with pancytopenia showed atypical lymphoid cells constituting more than 40 % of total nucleated cells. These patients had multiple enlarged intraabdominal lymphnodes which on FNAC revealed monomorphic atypical lymphoid cells as predominant population. Total three cases of myelodysplasia were seen involving all the three cell lineages

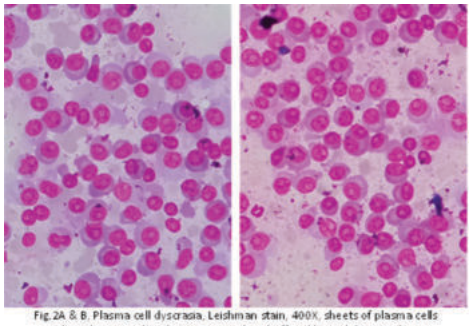

Fig.2A & B. Plasma cell dyscrasia, Leishman stain, 400X, sheets of plasma cells with nuclear pseudoinclusion, perinuclear hoff and basophilic cytoplasm.

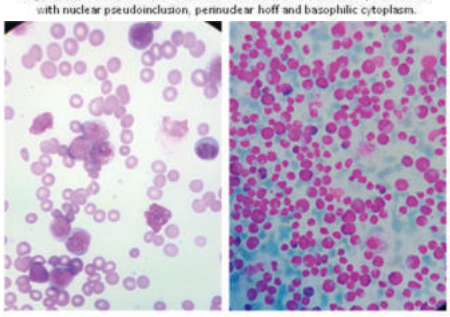

Fig.3A case of acute leukemia with many blasts in peripheral blood.400X, Fig.3B. a case of chronic myeloid leukemia in chronic phase.

Pancytopeia was seen in 51 cases (Table4). Megaloblastic anemia(21) was the most common cause of pancytopenia followed by hypoplastic anemia(13), acute leukemia (08) and Leishmanisis (06). Metastatic deposits of adenocarcinoma (Fig:4) in bone marrow aspirates were seen in three patients while lymphoma infiltration was seen in two of the patients.

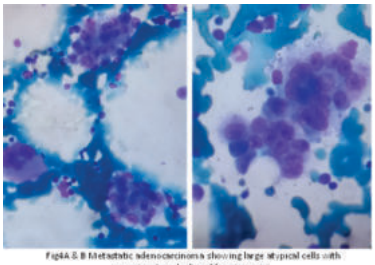

Fig4A & B Metastatic adenocarcinoma showing large atypical cells with prominent nucleoli and forming acini.

Evaluation of pyrexia of unknown origin (Table5) showed hypoplastic anemia in 8 cases, acute leukemia 6 cases and visceral leismaniasis in two cases. One of the patients with reactive plasmacytosis was HIV positive. Normal marrow was observed in 14 of the patients and dry tap was noted in 5 of the patients.

## DISCUSSION :

Bone-marrow is one of the most common sites of hematopoiesis. Bone-marrow aspirate examination is one of the important investigations to diagnose, confirm and stage hematological disorders. Evaluation of different cytopenias, thrombocytosis, leukocytosis, anemias, and iron status largely depends on bone marrow aspirate examination. Diagnosis of different nonhematological disorders like storage disorders and systemic infections needs bone marrow examination. It is a minimally invasive outdoor procedure.

We observed male predominance with a male female ratio of 1.25 : 1. Age range of the study subjects was 4 to 83 years and mean age was 42.8 years. Niazi et al[8] and Adewoyin[9] et al reported male predominance. Niazi et al[8] reported age range of 1-75 years and according to Gayathri et al[10] age range was 2-80 years.

Anemia was most common finding in our study with Vitamin B12/Folate deficiency anemia being the most common type of anemia which is consistent with studies by Gayathri et al and Ranabhat et al.[10,11] Niazi et al[8] and Jha et al[12] in their study found megaloblastic anemia as second most common type of anemia. In present study Iron deficiency anemia was the second most common type of anemia (9.45% of all cases). Iron deficiency anemia reported in different studies ranges between 8 -14%.[13,14] Total 8.33% showed features of dual deficiency anemia which was consistent with other previous studies.

Total 13 cases (7.22% of total cases) of hypoplastic anemia were noted in our study. Diagnosis of hypoplastic marrow was based on BMA findings alone. All these cases were characterized by the presence of hypocellular particles and reduced cellularity in trail and predominant cells comprising of mature lymphocytes, plasma cells, stromal cells and increased mast cells. All the three lineages were markedly depressed. In suspected cases of hypolastic or aplastic anemia, bone marrow biopsy gives more accurate and confirmatory information due to larger area and cellularity for evaluation. In comparison to present study, various previous studies have shown hypoplastic anemia ranging from 14 to 29%. [10,12,13]

Idiopathic thrombocytopenic purpura was seen in 7 (3.89%) cases. All these patients have normal myloid and erythroid maturation and increased number of megakaryocytes in the bone marrow aspirate.

Pancytopenia was one of the most common indications for bone marrow aspiration. Total 51 Pancytopenia was one of the most common indications for bone marrow aspiration. Total 51 cases with pancytopenia were evaluated. Vitamin B12/Folate deficiency anemia was the most common cause of pancytopenia in our study (41.2% of total pancytopenia cases) which is consistent with studies by Gayathri et al and Tilak et al.[10,15] Other important causes of pancytopenia includes hypoplastic anemia (25.5%), acute leukemia (15.7%) and visceral Leishmaniasis (11.8%). Khodke et al[13] while evaluating pancytopenia observed 14% cases of pancytopenia due to visceral Leishmaniasis.

Erythroid hyperplasia: Erythroid hyperplasia was reported in 13 cases [7.22%] of total bone marrow aspirates. Out of 13 erythroid hyperplasia, 6 were normoblastic, 2 were

predominantly micronormoblastic and remaining 5 showed both micronormoblastic and megaloblastic proliferation. Pudasaini S et al[16] reported erythroid hyperplasia as the most common finding in bone marrow aspirates.

In present study total 9 cases demonstrated features of reactive marrow. Reactive cellular marrow are characterized by hyperplasia of one or more cell lineages or in few cases hypoplasia or aplasia. Reactive marrow is generally associated with inflammatory conditions or intrinsic marrow insult. Reactive plasmacytosis was seen in two cases out of which one was HIV positive.

Acute leukemia, hypoplastic anemia, visceral Leishmaniasis, lymphoma infiltrates were among common causes of pyrexia of unknown origin in our study.

Haematolymphoid malignancies constitute 23.33% of total cases (42 out of 180 cases) and is second only to anemia.. Among haematolymphoid malignancies, multiple myeloma was the most common primary malignancy comprising of 12.2% (22) of total cases. 12 of these patients had distinct M peak in serum protein electrophoresis while 4 cases showed monoclonal proliferation in immunofixation. Adewoyin et al[9] in their study reported 7.9% cases of malignant plasmacytosis. Total 14 cases of acute leukemia were diagnosed out of which 7 were morphologically resembling acute myeloid leukemia (Based on blast morphology, Auer rods, granules), 5 lymphoblastic leukemia and 2 undifferentiated type. Ranabhat S et al[11] in their study reported haematolymphoid malignancies as the second most common diagnosis. Pudasaini et al[16] reported 12.3 pecent cases of haematolymphoid malignancies. Some of the cases of acute leukemia presented with total leukocyte count within normal range, haemoglobin and platelets marginally decreased. Such cases need proper evaluation with bone marrow studies and ancillary tests. High degree of clinical suspicion and proper haematological studies including bone marrow examination help diagnose such cases. 2 cases each of chronic myeloid leukemia, polycythemia rubra vera and lymphoma infiltrate were also seen in our study. Total three cases(1.67%) of myelodysplastic syndrome were included in present study. Various previous studies have reported MDS ranging from 2% to 7.9%.[1,10,12,13]

Total 3 cases in our studied were positive for adenocarcinoma cells infiltrating bone marrow. All the three cases were metastatic adenocarcinoma showing tumour cells forming glandular pattern. Adewoyin AS et al[9] reported marrow carcinomatosis in 7.9% cases while Ghartimagar et al[17] in their study reported metastasis in 6% of total cases. Total 6 cases of visceral Leishmaniasis were seen in this study. All patients of visceral Leishmaniasis had pancytopenia, fever on and off and splenomegaly. Bone marrow examination revealed many intracellular and extracellular amastigote forms of Leishmania – aflagellate organism. Normal marrow study was seen in 7.8% of the cases while Atla et al.[18] reported 3.8% cases and Pudasaini et al.[16] reported 10.5% cases as normal marrow .

## CONCLUSION :

Bone marrow aspiration examination is an essential, mildly invasive technique to diagnose various haematological and nonhaematological diseases. Bone marrow aspration studies need simultaneous knowledge of clinical findings, CBC, peripheral blood examination, reticulocyte count and other ancillary test whenever needed. In certain disorders like hypoplastic anemia and various condtions of dry tap, we need to do bone marrow biopsy. Timely proper evaluation and diagnosis is essential for management of different leukemias and other haematolymphoid disorders.

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**Conflict of interest:** None declared

**Ethical approval:** The study was approved by the Institutional Ethics Committee

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