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STUDY OF BONE MARROW EXAMINATION IN HEMATOLOGICAL DISORDERS IN WESTERN RAJASTHAN

Pathology

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ABSTRACT

Background: Bone marrow is an organ with architecture and components intact in their natural spatial context. the haematological disorders are quite common in population and bone marrow examination is simple and easy approach to diagnose haematological disorders. **Methods:** The present study was conducted in Department of Pathology, Dr. S. N. Medical College, Jodhpur to establish the diagnostic value of bone marrow biopsy along with bone marrow aspiration study. **Results:** This prospective study comprised study of bone marrow aspiration and biopsy of 97 patients with suspected haematological disorders. Megaloblastic anaemia was the most common haematological disorder (20.61%) followed by iron deficiency anaemia (16.49%) and Aplastic anaemia (8.24%). In present study peripheral blood smear showed pancytopenia in 100% cases of aplastic anaemia **Conclusion:** The present study concluded Simple haematological investigations like haemoglobin level, total and differential leukocyte count, platelet count and peripheral blood smear examination can indicate an underlying haematological disorder

KEYWORDS

Haematological Disorders, Bone Marrow Biopsy, Bone Marrow Aspiration

INTRODUCTION

Sir William Harvey described blood as "the fountain of life and the primary seat of the soul. The marrow of our bones is the seedbed of our blood"[1]. Careful assessment of the blood elements is often the first & crucial step in assessment of hematological function and diagnosis. The adult hematopoietic system includes tissues and organs ,which are involved in the proliferation, maturation and destruction of hematopoietic cells. These organs and tissues include the bone marrow, thymus, spleen and lymph nodes. Bone marrow is the site of myeloid, erythroid, and megakaryocytic as well as lymphoid cell development[2].

Bone marrow is an organ with architecture and components intact in their natural spatial context. There is complex structural and hormonal interrelation between the cellular and tissue elements of the marrow. A disturbance of each of the principal blood element is reflected much earlier and changes are more conspicuous in the marrow than in peripheral blood.

Examination of the marrow is critically important in the study and management of wide variety of hematological disorders.

Bone marrow examination usually involves two separate but interrelated specimens[3].

1. Cytological preparation of bone marrow cells obtain by aspiration of the marrow and a smear of the cells.
2. Specimen is a needle biopsy of bone and associated marrow.

Several sites may be used for bone marrow aspiration and bone marrow biopsy[4]. At birth hematopoietic marrow was found in all the bones of the body. However in adults haematopoiesis is limited to the axial skeleton and proximal portion of the extremities[5]. Thus younger children may have marrow examination from the anterior medial tebial area whereas adult marrow may be sampled from the sternum at the second intercostals either anterior or posterior iliac crest area. The posterior superior iliac spine is usually the preferred site[6]. Biopsy from the iliac spine may be technically difficult in subjects who are obese[7] and immobile and puncture of sternum may be necessary. Bone marrow biopsy was first performed by Pianese in 1903, since then, needle biopsy and aspiration have gained importance over open surgical procedures. The introduction of bone marrow biopsy needle by Jamshidi and Swaim(1971)[8] has made the procedure simpler and less painful with better processing modes and improved light microscopic techniques. The evaluation has been made easier, thus widening the scope of biopsy. Bone marrow biopsy is essential for diagnosis of inadequate marrow aspirate, bone marrow fibrosis, Granulomatous lesions, myeloproliferative disorders, myelo dysplastic syndromes, aplastic anaemia, metastatic tumour and plasma cell dyscrasias. It also serves as most reliable method for assessing marrow cellularity following administration of antineoplastic drugs and in assessing status of engraftment following bone marrow transplantation.

The present study carried out at the Department of Pathology, Dr. S. N. Medical College, Jodhpur to establish the diagnostic value of bone marrow biopsy along with bone marrow aspiration study.

AIMS & OBJECTIVES

1. To evaluate the prevalence of various haematological disorders in Jodhpur(Rajatshan)
2. To compare the findings of peripheral blood film , bone marrow aspiration smears and bone marrow trephine biopsy sections with relevant stains.

MATERIALS AND METHOD

This was carried out in department of pathology, Dr. Sampurnanand Medical College Jodhpur and associated group of hospitals.

Sample size: All the bone marrow aspirates and biopsy received during the study period. Total 97 patients were examined.

Study design: Prospective study

Methods of collection of data:

In this prospective study patient admitted with suspected haematological disorder interrogated in detail for history and thorough physical examination. Haematological investigations like haemoglobin estimation, total and differential leukocyte count, platelet count was done on each patient. Peripheral blood film examination for cell morphology was performed after staining with Giemsa stain.

Bone Marrow Aspiration-

Bone marrow aspiration and biopsy taken from posterior superior iliac spine under proper sterile condition with jamshidi needle. The patient placed in lateral dequbitus position. The skin at the site of biopsy shaved if found necessary and cleaned with betadine solution. The skin, subcutaneous tissue and periostium in the area of biopsy anaesthetised with a local anaesthetic like 2-5 ml of 2% lignocaine using a 24 gauge needle. The marrow aspiration needle with an obturator inserted through the skin, subcutaneous tissue and bone cortex with a slight rotating motion. After the entrance of needle in to the bone marrow cavity the needle obturator removed and needle attached to 10-20 ml syringe. Aspiration of marrow achieved by rapid suctioning with a syringe so that 2-3 ml of bloody fluid achieved. Smears were fixed by immersing in a jar of methanol for 15-20 minutes. After fixing, smears were stained with Giemsa stain which allow excellent morphologic detail and allow differential counts to be performed. Some of the unstained smears retained for special staining if indicated.

Bone marrow smears than examined under low power for –
1. Assessment of cellularity
2. Presence of megakaryocytes
3. Presence of any clumps of abnormal cells

The smears than examined under high power –
- To find out any maturation abnormality
- To perform deferential count.
- To find out any morphological abnormality.

Prussian blue staining done on the smear and iron content of the marrow assessed.

Bone Marrow Biopsy-
The bone marrow biopsy performed following the aspiration using the same skin incision. The same needle (jamshidi needle) reused that was used for the bone marrow aspiration. Jamshidi needle has a tapering distal end to reduce the crush artefact. The biopsy site repositioned away from the area where the aspiration was performed. Bone marrow biopsy specimen fixed in 10% formalin and subjected to decalcification if needed. The biopsy than processed in routine histological processor and embeded in paraffin. Thin section (3-4micrometer) cut and subjected for hematoxylin and eosin staining. Reticulin stain also done when indicated.

Steps for Standard Haematoxylin and Eosin staining for paraffin sections :
1. Dewax sections, hydrate through graded alcohols to water.
2. Remove fixation pigments, if necessary.
3. Stain in Harris Haematoxylin for 10 – 12 minutes.
4. Wash in running tap water until sections blue (for 5 minutes or less).
5. Differentiate in 1% acid alcohol for 5 – 10 sec.
6. Wash well in tap water until sections are again blue (for 5 minutes or less).
7. Stain with 1% Eosin for 1 minute.
8. Wash in running tap water for 1 – 5 minutes.
9. Dehydrate through alcohols, clear and mount.

RESULTS :
Nucleus – Blue, Black.
Cytoplasm – varying shades of pink.
Muscle fibers – Deep pink red.
Red blood cells – Orange, Red.
Fibrin – deep pink.

RESULTS-
The present study was conducted in Department of Pathology, Dr. S. N. Medical College, Jodhpur to establish the diagnostic value of bone marrow biopsy along with bone marrow aspiration study.

Distribution Of Hematological Disorders In Studied Group
Table no. 1 showed frequency of various haematological disorders in studied group. Total 97cases included in study. Megaloblastic anaemia was the most common haematological disorder (20.61%) followed by iron deficiency anaemia (16.49%) and Aplastic anaemia (8.24%). In haematological malignancies Acute myeloid leukemia (10.30%) was most common followed by Acute lymphoblastic leukemia (7.21%), Lymphoproliferative disorders (6.18%), and Multiple myeloma (5.15%). Bone marrow findings were inconclusive in (4.12%) patients, normal study in (2.06%) patients.

Myeloproliferative neoplasms were seen in(9.27%), myelodysplastic syndrome (2.06%) and drug induced suppression of bone marrow in (2.06%) cases. Immune thrombocytopenic purpura seen in(5.15%) cases.

Table:1- Distribution Of Hematological Disorders In Studied Group

Disorders	Number of cases	Percentage
Megaloblastic Anaemia	20	20.61%
Iron defiency anaemia	16	16.49%
Aplastic Anaemia	08	08.24%
Acute Myeloid Leukaemia	10	10.30%
Acute lymphoblastic Leukaemia	07	7.21%
Lymphoproliferative Disorders	06	06.18%
Myeloproliferative Disorders	09	09.27%
Myelodysplastic Syndrome	02	02.06%
Multiple Myeloma	05	05.15%
Platelet Disorders	05	05.15%
Others	09	9.27%
Total	97	100

2. Bone Marrow Findings Of Myeloproliferative Neoplasms –
Table no.2 Shows bone marrow aspiration findings in myeloproliferative neoplasms. In CML myelopoiesis was hyperplastic, megakaryopoiesis was hyperplastic but dysmega karyopoietic with M:E ratio of 20:1.In Myelofibrosis erythropoisis and myelopoiesis were normal with increased fibrosis. In case of PV all lineages were hyperplastic. In essential thrombocythemia megakaryopoiesis was hyperplastic with M:E ratio of 4:1.

Table No:2 Bone Marrow Findings Of Myeloproliferative Neoplasms

Type of disorder	Bone marrow finding				
	Erythropoiesis	Myelopoiesis	Megakaryopoiesis	M:E ratio	Fibrosis
CML	Decreased	Hyperplastic	Hyperplastic, Dysmegakaryopoiesis	20:1	-
Myelofibrosis	Normal	Normal	Megakaryocyte proliferation	3:1	Increased
PV	Hyperplastic	Hyperplastic	Hyperplastic	4:1	Slightly increased
ET	Normal	Mild hyperplastic	Marked hyperplastic	4:1	-

Table No. 3 Bone Marrow Findings In Various Hematological Disorders

Type of disorder	Bone Marrow				
	Cellularity	Erythropoiesis	Myelopoiesis	Megakaryopo-iesis	Tumour cells
Megaloblastic anaemia	Hypercellular	Megaloblastic	Ineffective, giant metamyelocytes	Ineffective	-
IDA	Hypercellular	Micronormoblastic	Normal	Normal	-
AA	Hypocellular	Normoblastic, suppressed	Suppressed	Suppressed	-
AML	Hypercellular	Normoblastic	Hyperplastic	Normal	-
ALL	Hypercellular	-	Blast cells	-	-
MPN	Hypercellular	-	Hyperplastic	-	-
MM	Hypercellular	Nomal	Normal	Normal	-
MDS	hypercellular	Megaloblastic	Hyperplasia	Dysmegakaryopoiesis	-

DISCUSSION
The present study showed that Megaloblastic anaemia was the most common haematological disorder (20.61%) followed by iron deficiency anaemia (16.49%) and aplastic anaemia (08.24%). In haematological malignancies acute myeloid leukaemia more common (10.30%) followed by acute lymphoblastic leukaemia (7.21%). Myeloproliferative neoplasm were seen in 9.27% cases out of them chronic myeloid leukaemia was most common (4.13%) followed by Myelofibrosis (3.09%). Multiple myeloma was seen in 5.15% cases. This is concordant with Ciqudasa, Calasanze, Garcia Delquado[9].

In present study peripheral blood smear showed pancytopenia in 100% cases of aplastic anaemia. Pancytopenia was common finding in patients of myelodysplastic syndrome (both cases showed pancytopenia) and Megaloblastic anaemia (60%). This finding was consistent with study done by Khandoori et al[10] and Ng S C et al[11]. They found pancytopenia in 62% and 64% cases of Megaloblastic anaemia respectively. In a study done by Chan et al 85 pancytopenia found in 23% cases of Megaloblastic anaemia. Pancytopenia was least common

finding in acute myeloid leukaemia, acute lymphoblastic leukaemia, Polycythemia Vera and multiple myeloma. This is accordance with Kishore Khodke et al[12].

Present study showed that pancytopenia was a common denominator in various haematological disorders. Megaloblastic anaemia (34.28%) was most common causes of pancytopenia followed by Aplastic anaemia (22.85%). This finding was consistent with many studies like Gamal et al[13], Kishre Khodke et al[12], Tilak et al[14], Mobina et al[15].

Bone marrow aspiration alone is diagnostic in (89.73%) cases. This is well correlated with the study done by Nanda et al[16]. In the remaining cases the trephine biopsy was necessary for making a diagnosis. Nanda et al[16] also found that bone marrow sufficient to make diagnosis in(88.6%) cases. Lukas Graf et al[17] also studied the comparative efficacy of bone marrow aspiration and biopsy and found that aspiration alone were sufficient to make diagnosis in (80.5%) cases. Bone marrow biopsy was essential to make diagnosis in cases of Myelofibrosis and was found more informative in some cases of myelodysplastic syndrome, lymphoproliferative disorders and Aplastic anaemia. Bone marrow aspiration smears were sufficient to make diagnosis in cases of Megaloblastic anaemia iron deficiency anaemia ,acute leukemias and multiple myeloma. This is consistent with study done by Varma et al[18].Similarly Sabharwal et al[19] . comparatively evaluated the bone marrow smears, Imprints and biopsy sections and found that aspiration and biopsy usually complement each other with aspirated smears being primarily useful for cytological diagnosis and biopsy sections mainly for histological diagnosis as cellularity, fibrosis and architectural patterns. Imprint preparations obtained from biopsy can be useful in patients with malignancy but they were found to be of limited value except in cases of dry taps.

SUMMARY AND CONCLUSION

The present study was conducted at Department of pathology, Dr. S. N. Medical College and associated group of hospitals, Jodhpur. This prospective study comprised study of bone marrow aspiration and biopsy of 97 patients with suspected haematological disorders.

The present study concluded that haematological disorders are quite common in population and bone marrow examination is simple and easy approach to diagnose haematological disorders. Simple haematological investigations like haemoglobin level, total and differential leukocyte count,platelet count and peripheral blood smear examination can indicate an underlying haematological disorder. Bone marrow aspiration smear and trephine biopsy section examination is very useful to diagnose a haematological disorder. Bone marrow aspiration and trephine biopsy is a safe and minimally invasive procedure and require simple histopathological diagnostic facilities. Bone marrow aspiration smears provide better cellular details and informative in Megaloblastic anaemia ,iron deficiency anaemia. Bone marrow trephine biopsy sections are more informative than aspiration smears in dry taps and especially in cases of Myelofibrosis and Aplastic anaemia. Bone marrow aspiration and trephine biopsy were found to be complementary to each other and superiority of one method over the other depend on the specific disease process.

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