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Spectrum of Haematological Disorders Observed on Bone Marrow Aspiration in a Tertiary Care Hospital

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Abstract

Background- Bone marrow aspiration plays a major role in the diagnosis of various haematological disorders. Bone marrow aspiration cytology is a proven method for the evaluation of haematological conditions, malignancies, storage disorders and some chronic systemic conditions. This study was carried out to study etiological spectrum of haematological disorders on bone marrow aspiration examination.

Materials and methods- The present observational study was carried out on eighty five patients in department of Pathology, Dr. SCGMC, Nanded over the period of one and half year. Bone marrow aspirate was carried out in 85 cases after screening through routine haematological investigations and examined.

Results- Out of 85 cases, Dimorphic anaemia was most common finding followed by Megaloblastic anaemia. Among haematological malignancies Chronic Myeloid Leukaemia was most common finding and Acute Lymphoblastic Leukaemia was second most common. Other cases were of Iron deficiency anemia Acute Myeloid Leukaemia, Hypersplenism, Aplastic anemia, Myelofibrosis, Multiple myeloma Polycythemia Vera, Liver Cirrhosis and Eosinophilia.

Conclusion- The present study concludes that detailed primary haematological investigations along with bone marrow aspiration are helpful for understanding the disease process. Bone marrow aspiration examination is relatively safe procedure. It is an important step to arrive at the confirmatory diagnosis of broad-spectrum haematological disorders. In a developing nation like ours it can be an easy and effective procedure in order to combat the nutritional anaemia.

Keywords: Anaemia, Bone marrow aspiration, Haematological disorders, Safe procedure

Introduction

Sir William Harvey described blood as "The fountain of life and primary seat of the soul. The marrow of our bones is the seedbed of our blood."[1]

Anaemia is common worldwide but prevalence of anaemia is disproportionately high in developing countries due to poverty, inadequate diet and poor access to health resources. Anaemia is not a diagnosis, but a manifestation of an underlying disorder. Thus, even mild asymptomatic anaemia should be investigated to diagnose and treat the primary cause.[2]

Haematological disorders in any age group usually presents with anaemia. Various haematological disorders where bone marrow examination is

necessary are unexplained cytopenias, acute blastic leukaemia, lymphoproliferative disorders (such as lymphomas, hairy cell leukaemia and multiple myeloma) and in various myeloproliferative disorders.

Without bone marrow examination the diagnosis is not confirmatory. Bone marrow examination also gives explanation for unexplained cytopenias, leukaemias and bleeding disorders.[3] It gives more complete picture of the reaction of haematological tissue to anaemia.[4] It is the most frequent and safe invasive procedure, well tolerated by patient, done to arrive at a final diagnosis of haematological disorders within a short span of time. There is very little or no risk of bleeding and can be safely done in case of severe thrombocytopenia.

Marrow aspirate can be primarily utilized for cytological assessment. It allows for studies of the marrow's overall cellularity, detection of focal lesions and extent of infiltration by various pathological entities.[5] BMA specimens are also relevant for additional investigations including molecular studies, cytogenetic, cytochemistry, flow cytometry/Immunophenotyping, microbiological studies and others.[6, 7]

It is only through the clinical, haematological and bone marrow examination findings that proper evaluation and management of patients with haematological disorders can be made.

The present study was conducted to study etiological spectrum of haematological disorders on bone marrow aspiration examination, to know the age incidence of haematological disorders and their male to female ratio.

MATERIALS AND METHODS

The present observational study was carried out in department of Pathology, Dr. SCGMC, Nanded. The study subjects were enrolled using convenient sampling method through screening patients admitted from the clinical departments.

Inclusion criteria:

All cases of suspected haematological disorders admitted at tertiary care centre.

Exclusion criteria:

Already diagnosed cases of haematological disorders,

Patients on anticoagulant medications or with serious haemorrhagic disorders,

Pregnant women.

Clinical history recording and examination of all the identified cases were done. These patients were subjected to routine haematological investigations like complete blood count, peripheral smear study. Whole procedure was explained and written consent was taken either from patient or relative before starting the procedure. For bone marrow aspiration a needle with strong, wide base, short bevelled with stillete and adjustable guard was used. Sites of aspiration were sternum and posterior iliac crest. The procedure was performed in a sterile manner. Local anaesthesia was infiltrated & the needle was introduced in the bony cavity & fitting syringe was attached. Strong but brief suction was applied to with draw 0.3 ml of bone marrow tissue. The aspirated material was immediately placed on a glass slide & smears were prepared. Smears were air dried and then fixed with methanol. The smears were stained by field's stain and leishman's stain. Special stains like Perl's Prussian blue, Periodic Acid Schiff, and Myeloperoxidase were used as per requirements. Bone marrow examination was done of all smears and final reports were prepared.

RESULTS

In the present study nutritional anaemia contributed highest number of cases. Out of nutritional anaemia Dimorphic anaemia was the most common disorder i.e. 34 cases (40%) followed by 11 (12.96%) cases of Megaloblastic anaemia and 8 (9.42%) cases of Iron deficiency anaemia. Chronic Myeloid Leukaemia was the commonest malignant haematological disorder i.e. 9 (10.59%) cases. Other haematological malignancies were 6 (7.07%) cases of Acute Lymphoblastic Leukaemia, 4 (4.70%) cases of Acute Myeloid Leukaemia, 2 (2.35%) cases of Multiple Myeloma and Myelofibrosis while 1 (1.17%) was of Polycythemia Vera. Other cases diagnosed were of Hypersplenism 4 (4.70%) cases, 2 (2.35%) cases of Aplastic anaemia whereas 1 (1.17%) case of Liver Cirrhosis and Eosinophilia each. The etiological spectrum is shown in Table 1.

Out of 85 cases, age group 23-32 years had maximum number of cases i.e. 24 (28.24%). Mean

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(±SD) age was 33.56 (± 17.24) years. The number of cases in various age groups is shown in Table 2.

Out of 85 cases, 45 (52.94%) cases were males and 40 (47.06%) cases were females showing slight male preponderance. Male to Female ratio was 1.12: 1.

In present study, maximum number of cases, [49 (57.65%)] had Anisopoikilocytosis, 42 (49.41%) cases had Dimorphic blood picture. Other blood pictures are shown in Table 3.

In this study most of the cases had hypercellular marrow i.e. 78 (91.77%) cases while 3 (3.53%) cases had hypocellular and normocellular marrow. Dry tap was seen in one case.

Among 34 cases of dimorphic anaemia, bone marrow aspirate from 33 (97.06%) cases showed hypercellularity while 1 (2.94%) case showed normocellularity. On assessment of bone marrow iron store by Perl's Prussian blue reaction was done in 34 cases of dimorphic anaemia out of which ,15 cases (44.12%) showed 2+ grade, 8 cases (23.53%) grade 1+, 5 cases (14.71%) grade zero, 4 cases (11.76%) grade 3+, and 2 cases (5.88%) grade 4+ of iron store.

Bone marrow aspirate from all cases of megaloblastic anaemia showed hypercellularity On iron study, 6 cases (54.55%) showed 3+ grade, 4 cases (36.36%) grade 4+, 1 case (9.09%) grade 1+ of iron store.

Bone marrow aspirate from all cases of iron deficiency anaemia showed hypercellularity. On iron study, 6 cases (75%) showed zero grade and 2 cases (25%) grade 1+ of iron store.

Bone marrow aspirate from all cases of aplastic anaemia showed increase fat spaces and decreased cellularity. All 3 lineages were markedly decreased with relative increase in lymphocytes and plasma cells.

In one case of eosinophilia, the bone marrow aspirate was hypercellular. Further investigations done were sputum cytology and bronchoalveolar lavage cytology examination. Both showed presence of septate fungal hyphae with acute angle branching.

Bone marrow aspirate was normal in cellularity and maturation in a case of liver cirrhosis. Ultrasonography showed evidence of cirrhosis.

Bone marrow aspirate from all cases of chronic myeloid leukaemia showed hypercellularity. Myeloid series showed all stages of maturation with basophilia. 8 (88.89%) cases were in chronic phase while 1 (11.11%) case was found to be in blast crisis with blast count of 43%. One case had further investigation with Chromosome Analysis (FISH) which showed BCR-ABL1 positivity and Ph Chromosome was present in 80% of interphase cells.

Bone marrow aspirate from all cases of Acute Lymphoblastic leukaemia showed hypercellularity. Out of 6 cases of Acute Lymphoblastic Leukaemia, 4 (66.67%) cases were of ALL-L1 and 2 (33.33%) cases were of ALL-L2. All 4 cases of ALL–L1 showed PAS positive blasts on Bone marrow aspirate. 1 case of ALL had bone marrow biopsy with immunophenotyping at higher centre and was diagnosed as ALL-L2 with CD3 and CD7 positivity.

Bone marrow aspirate from all cases of Acute Myeloid leukaemia showed hypercellularity. Out of 4 cases of Acute Myeloid Leukaemia, 3 (75%) cases were of AML-M1 and 1 (25%) case was of AML-M3. All 4 cases of AML showed MPO positive blasts on Bone marrow aspirate.

In multiple myeloma cases, Bone marrow aspirate was hypercellular showing decreased erythropoiesis, granulopoiesis and megakaryopoiesis along with altered Myeloid to Erythroid ratio with more than 40% of plasma cells.

In 2 cases of myelofibrosis, attempted bone marrow aspirations yielded dry tap. Bone Marrow biopsy in both the cases showed marked marrow fibrosis along with dysmegakaryopoiesis and diffusely positive reticulin stain.

In Polycythemia Vera case, the bone marrow aspirate was hypercellular with marked erythroid hyperplasia showing micronormoblastic maturation and slight increase of granulocytes, granulocyte precursors and megakaryocytes were normal in number and morphology.

Other bone marrow aspirate findings are shown in Table No. 4

DISCUSSION

The spectrum of haematological disorders both in children and adults is very wide. Bone marrow examination is a useful test in reaching the final diagnosis. Bone marrow aspiration is one of the most common and safe procedure done routinely in

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medical practices in arriving at an etiological diagnosis. Rarely infection, excessive bleeding or embolism has been reported.

In the present study, haematological disorders were divided into two categories: A) Non-malignant haematological disorders B) Malignant haematological disorders. Out of total 85 cases, 61 (71.77%) cases were found with non-malignant haematological disorders and 24 (28.23%) cases were with malignant haematological disorders.

Dimorphic anaemia (40%) was the most common haematological disorder followed by Megaloblastic anaemia (12.96%). Among malignancies, chronic myeloid leukaemia (10.59%) was most common followed by acute lymphoblastic leukaemia (7.07%).

The most common haematological disorder, reported from various studies throughout the world has been Aplastic anaemia, while in most of the sub continental studies megaloblastic anaemia was found to be either the commonest or the second most common haematological disorder.

The high prevalence of dimorphic maturation in present study is explained by the occurrence of nutritional deficiencies in rural areas. Nutritional deficiencies of both iron and folate commonly occur together. Iron deficiency occurring concurrently with megaloblastic anaemia has been reported in many areas. In some instances, the megaloblastosis may be intermediate in degree but may become more marked after administration of iron, and some cases of severe dimorphic anaemia do not respond initially to iron therapy, probably owing to concomitant severe folate and occasionally Vitamin B12 deficiency.

Thiyagarajan P et al. (2015)[8] found Dimorphic anaemia (38.4) as most common haematological disorder followed by megaloblastic anaemia (33.4%). Merla J et al. (2017)[9] found Dimorphic anaemia (26.59%) as most common followed by iron deficiency anaemia (16.92%). Kulkarni N et al. (2017)[10] found Dimorphic anaemia (36.23%) as most common etiology followed by megaloblastic anaemia (34.78%).

The age of the patients ranged from 3 to 80 years. Most common age group in present study was 23-32 years which is comparable with the study by Shastry SM et al. (2012)[11]

Haematological disorders were observed more in males (51.77%) than females (48.23%) with Male to female ratio of 1.12:1. Age and sex distribution was consistent with other studies of haematological disorders.

Among dimorphic anaemia cases, bone marrow aspiration study showed usually hypercellularity with erythroid hyperplasia with micronormoblastic and megaloblastic maturation. Giant band form, giant metamyelocytes were also seen. In study by R Athar et al (2014)[12], bone marrow studied in 58 cases of dimorphic anaemia which showed hypercellularity with micronormoblastic and megaloblastic change and trilineage dyspoiesis. In the study by Deepthi A et al. (2017)[13] bone marrow examinations showed normocellular marrow (32.36%) and hypercellular marrow (67.74%). Erythroid series showed hyperplasia with megaloblastic and micronormoblastic maturation. All of these cases also showed giant metamyelocytes.

Incidence of megaloblastic anaemia was 12.96% in present study. Pudasaini et al (2012)[14] reported 12.3% incidence of megaloblastic anaemia. Parajuli S et al (2014)[15] reported 12 % incidence of megaloblastic anaemia. Majority of the studies in India, stress the importance of megaloblastic anaemia being the one of the major cause of pancytopenia. Although bone marrow aspiration studies are uncommon in suspected cases of megaloblastic anemia, if the diagnosis does not appear straightforward or if the patient requires urgent treatment and haematological assays are not available, bone marrow aspiration is indicated. In the present study bone marrow aspirate from all the cases showed erythroid hyperplasia with megaloblastic maturation. Myelopoiesis showed giant metamyelocytes and giant band forms. In the study by Chandra K et al. (2014)[16] bone marrows in cases of megaloblastic anaemia was hypercellular (100%) with characteristic megaloblastic erythropoiesis with giant metamyelocytes and band forms. In a study by Gandhi P et al. (2016)[17], bone marrow aspirates were hypercellular (67.91%) and showed megaloblastosis.

Incidence of Iron Deficiency anaemia in present study was 9.42% which correlated with the studies done by Gore CR et al. (2018)[18] in which incidence were 5.7%. Parajuli S et al. (2014)[15] reported 10.66 % incidence of Iron deficiency anaemia. The bone

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marrow was hypercellular with reduction of fat cells in all patients (100%). Erythroid hyperplasia with micronormoblastic maturation was seen in all the cases. Myeloid and megakaryocytes series showed normal maturation and morphology. The bone marrow study by Javalgi et al (2013)[19] showed hypercellularity with altered M: E ratio and increased erythropoiesis showing micronormoblasts in most of the cases. Myelopoiesis was normal and there was slight increase in megakaryocytes.

In the present study, Hypersplenism was one of the etiology of haematological disorders in 4 (4.70 %) cases. Kumar R et al. (2001)[20] reported an incidence of hypersplenism in 19 (11.45%) cases

In present study, aplastic anaemia accounted for 2.35% of the total cases of haematological disorders. Merla J et al. (2017)[9] reported 1.51% cases of aplastic anaemia. Gore CR et al. (2018)[18] found 1 (0.7%) case of aplastic anaemia in their study. Bone marrow aspirate was hypocellular. In a study by Gandhi P et al. (2016)[17], bone marrow was hypocellular in 100% of aplastic anaemia cases.

In present study Cirrhosis of Liver was found in 1 (1.17%) case. Patel F et al. (2017)[21] reported cirrhosis of liver as cause of pancytopenia in 8 % of cases.

In the present study there was one case (1.17%) of eosinophilia as one of the etiology Nigam R et al. (2014)[22] reported 5 (1.81%) cases with excess eosinophils. Ranabhat S et al (2017)[23] and Bashir N et al (2018)[24] reported 1.3% and 0.6% cases of eosinophilia respectively.

Among haematological malignancies most common malignancy encountered was Chronic myeloid leukaemia followed by Acute lymphoblastic leukaemia. Patel J et al (2014)[25] reported 15% incidence of haematological malignancies from which most common was chronic myeloid leukaemia (5.33%). Parajuli S et al (2014)[15] reported 22.72% incidence of haematological malignancies in which most common was Acute leukaemia (12%) followed by Chronic myeloid leukaemia (6.66%).

Variations in the studies may be due to differences in the studied age groups & population in different areas, their genetic mutations and exposure to different myelotoxic agents.

In Chronic myeloid leukaemia, most cases can be diagnosed on the basis of peripheral blood findings combined with detection of the Ph chromosome and/or BCR-ABL1 by cytogenetic and molecular genetic techniques. However, bone marrow aspiration is essential to ensure sufficient material for a complete karyotype and for morphological evaluation to confirm the phase of disease. Kibria SG et al (2010)[3] found 7.34% incidence of Chronic myeloid leukaemia. In study by Patel J et al (2014)[25], chronic myeloid leukaemia (5.33%) was the most common malignancy. Anjum MU et al (2014)[26] found 10.25% incidence of chronic myeloid leukaemia in their study.

In present study, we found 7.07% incidence of acute lymphoblastic leukaemia. Kibria SG et al (2010)[3] found 9.04% incidence of Acute Lymphoblastic leukaemia in their study while it was 7.95% in study by Parajuli S et al (2014)[15].

In present study, incidence of AML was 4.70%. Shastry SM et al (2012)[11] found 3.63% cases of AML. Parajuli S et al (2014)[15] reported 5.68% incidence of AML. Bashir N et al (2018)[24] found 6% incidence of AML.

In present study myelofibrosis was found as one of the etiological factor in 2.35 % cases. Kumar R et al. (2001)[20] found myelofibrosis as cause of pancytopenia in their study in 1.2% of cases. Parajuli S et al (2014)[15] found 2.27% incidence of myelofibrosis.

In present study, Multiple myeloma was seen in 2.35% of cases. Khodke K et al. (2001)[27] and Parajuli S et al (2014)[15] reported 2 cases of multiple myeloma in their study.

In the present study there was one case (1.17%) of Polycythemia vera, Nigam R et al. (2014)[22] and Bashir N et al (2018)[24] in their study found 1 case and 4 cases of polycythemia vera respectively.

CONCLUSION

Dimorphic anaemia was commonest non-malignant cause of anaemia in the present study. Most other studies have reported megaloblastic and aplastic anaemia as the commonest cause. The spectrum of haematological disorders both in children and adults is very wide ranging from nutritional anaemia to haematological malignancies.

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It is relatively different in the developing world than the developed countries. Haematological disorder in any age group usually presents with anaemia. Most of the time the diagnosis can be arrived at by detail clinical examination and few simple investigations. However, Bone marrow aspiration plays a very important role not only in determining the cause of disease but also help in establishing a definitive diagnosis. The present study concludes that bone marrow aspiration is helpful for understanding the disease process and is relatively safe procedure. It is an important step to arrive at the confirmatory diagnosis of broad-spectrum haematological disorders. In a developing nation like ours it can be an effective procedure in order to combat the nutritional anaemia. So, that the morbidly and mortality associated with haematological disorders can be aptly managed.

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FIGURES

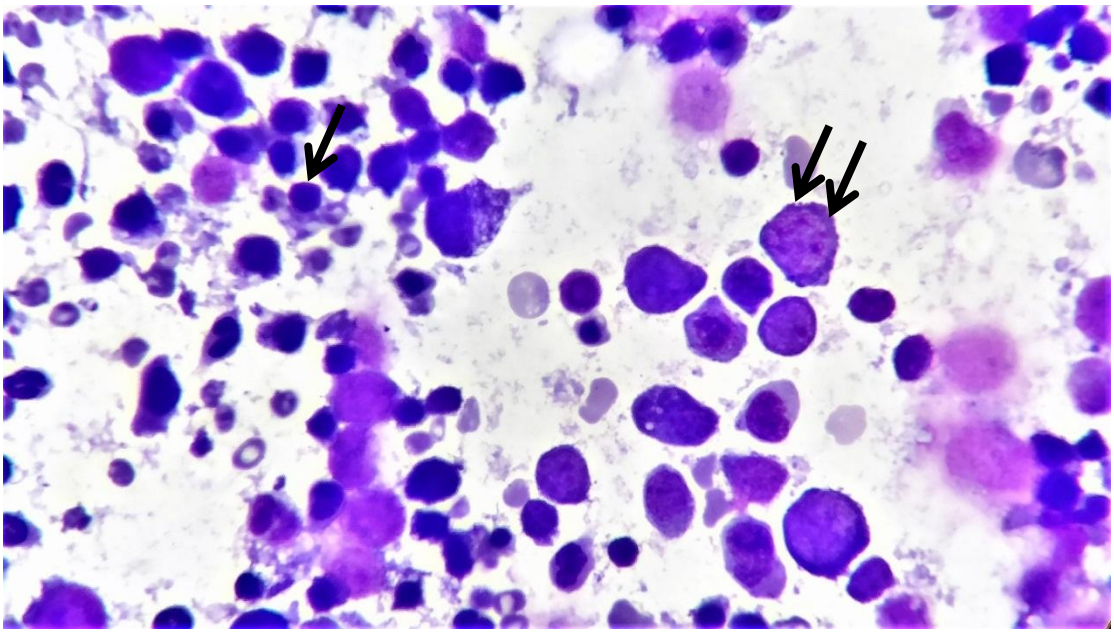

Figure 1: Bone marrow aspirate showing micronormoblastic (single arrow) and megaloblastic maturation (double arrow) - Dimorphic anaemia (Leishman's stain- 100x).

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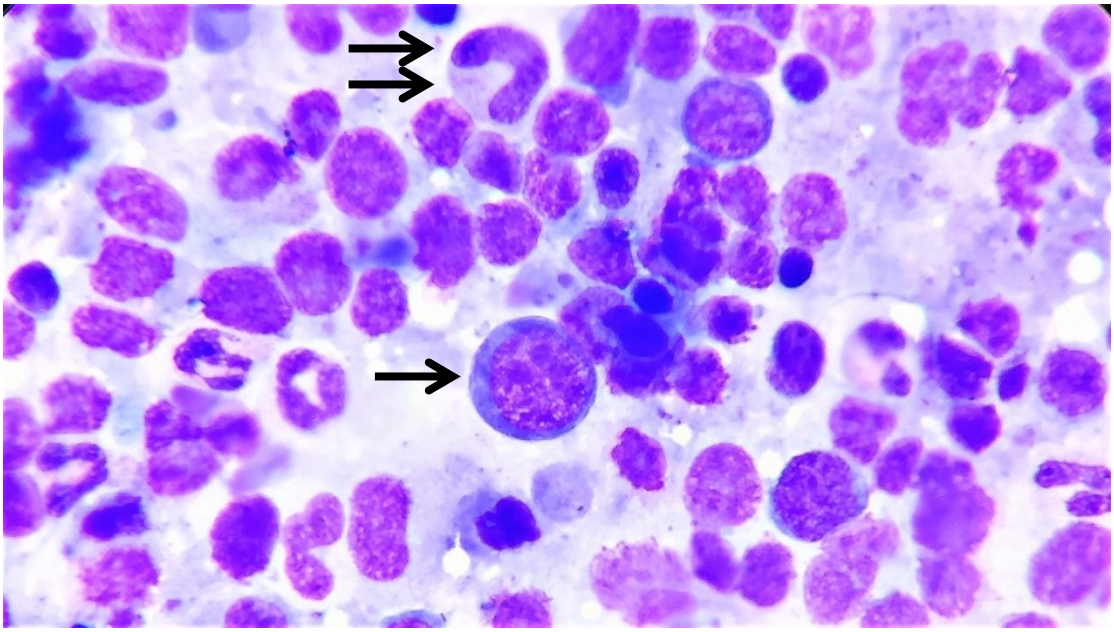

Figure 2: Bone marrow aspiration smear showing megaloblastic maturation (single arrow) with royal blue cytoplasm and sieve like chromatin along with Giant Band form (double arrow) - Megaloblastic anaemia. (Leishman's Stain, 100x)

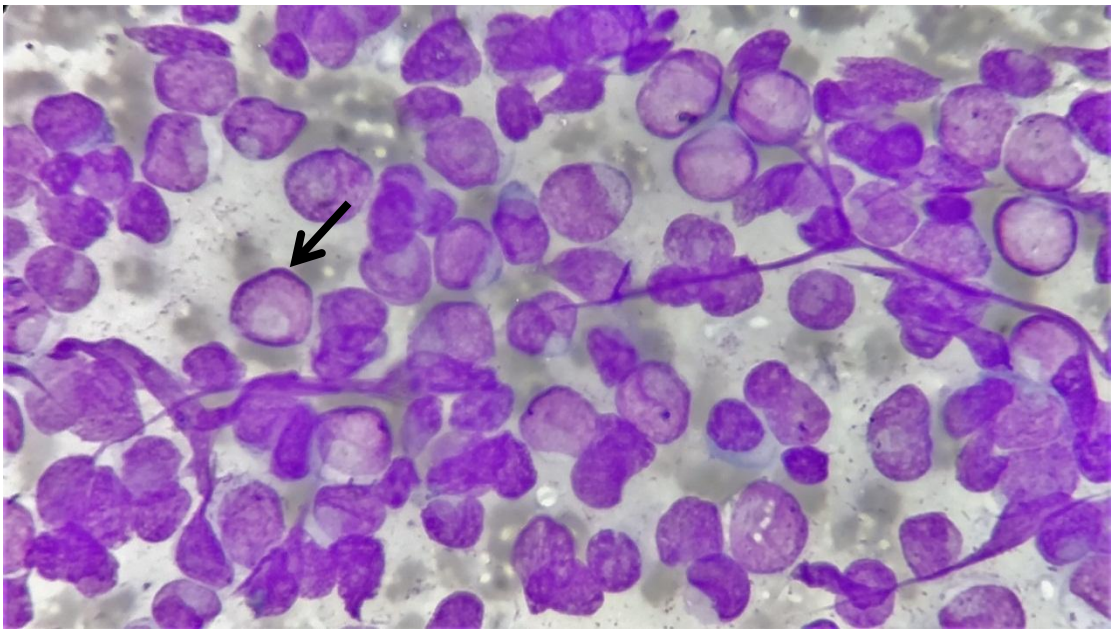

Figure 3: Bone marrow aspiration smear showing hyperplastic bone marrow with blast cells (arrow) population more than 90% having condensed chromatin with scanty cytoplasm and 1-2 prominent nucleoli – AML - M1. (Leishman's stain, 100x).

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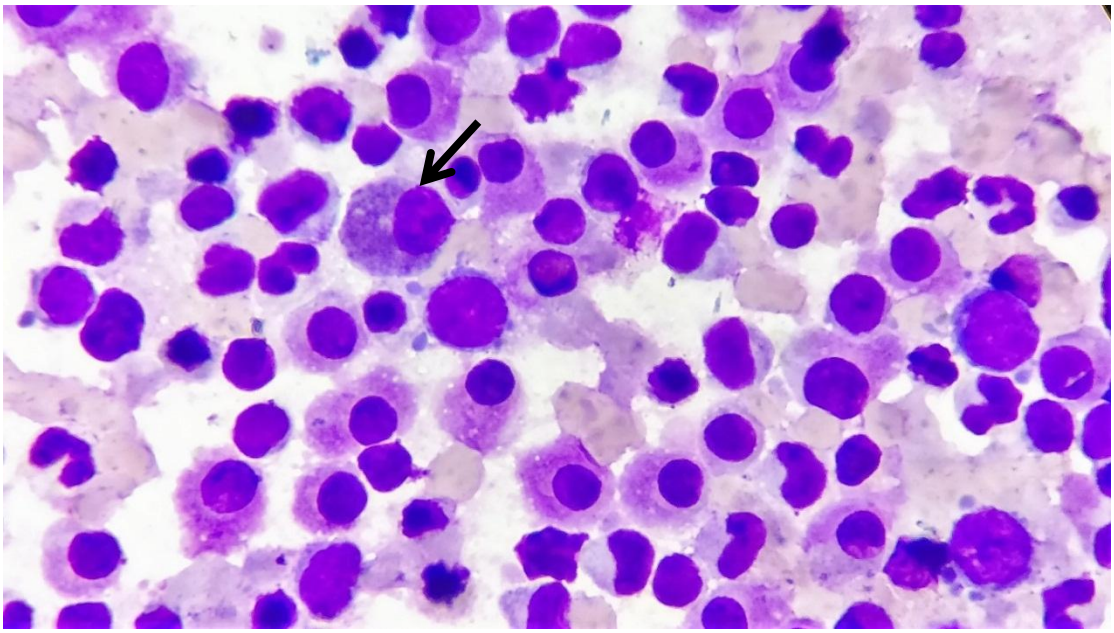

Figure 4: Bone marrow aspiration smear showing increased number of plasma cells (arrow) - Multiple Myeloma. (Leishman's stain, 100x).

TABLES

Category	Etiology	No. of cases	Percentage
Non-malignant haematological disorders	Dimorphic anaemia	34	40%
	Megaloblastic anaemia	11	12.96%
	Iron deficiency anaemia	08	9.42%
	Hypersplenism	04	4.70%
	Aplastic anaemia	02	2.35%
	Liver cirrhosis	01	1.17%
	Eosinophilia	01	1.17%
Malignant haematological disorders	Chronic myeloid leukaemia	09	10.59%
	Acute lymphoblastic leukaemia	06	7.07%
	Acute myeloid leukaemia	04	4.70%
	Myelofibrosis	02	2.35%
	Multiple myeloma	02	2.35%

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	Polycythemia vera	01	1.17%
Total		**85**	**100%**

Table 1: Etiological spectrum of Haematological disorders.

Age(years)	No. of cases	Percentage
3-12	8	9.41%
13-22	16	18.82%
23-32	24	28.24%
33-42	15	17.65%
43-52	9	10.59%
53-62	7	8.24%
63-72	4	4.70%
73-82	2	2.35%
Total	85	100%

Table 2: Age wise distribution of cases

Blood Picture	Cases	Percentage
Anisopoikilocytosis	49	57.65%
Dimorphic	42	49.41%
Microcytic Hypochromic	24	28.23%
Macrocytic Hypochromic	11	12.94%
Normocytic Hypochromic	8	9.41%

Table 3: Peripheral blood picture in cases of haematological disorders

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Type of disorder	Cellularity	M:E ratio	Erythroid series	Myeloid series	Megakaryocyte series
Dimorphic anaemia	Hypercellular	Decreased	Erythroid hyperplasia with micronormoblastic and megaloblastic maturation	Normal progressive maturation with giant metamyelocytes and band form	Normal in number and morphology
Megaloblastic anaemia	Hypercellular	Decreased	Erythroid hyperplasia with megaloblastic maturation	Normal progressive maturation with giant metamyelocytes and giant band form	Normal to increase in number, increase in size
Iron deficiency anaemia	Hypercellular	Decreased	Erythroid hyperplasia with micronormoblastic maturation	Normal in maturation and morphology	Normal in maturation and morphology
Aplastic anaemia	Hypocellular	Altered/ Decreased	Supressed	Supressed	Supressed
Hyper-splenism	Hypercellular	2:1	Increased with normoblastic maturation	Increased, normal in maturation	Increased, normal in maturation
Liver Cirhossis	Normocellular	Normal	Normal	Normal	Normal
Eosinophilia	Hypercellular	Altered	Normal	Hyperplastic with increased eosinophilic precursors	Hyperplastic with immature hypolobated megakaryocytes
Chronic myeloid leukaemia	Hypercellular	Increased	Supressed	Hyperplastic with basophilia	Hyperplastic, Dysmegakaryo-poiesis
Acute lymphoblastic leukaemia	Hypercellular	Increased	Supressed	Hyperplastic	Suppressed
Acute myeloid leukaemia	Hypercellular	Increased	Supressed	Hyperplastic, blast cells	Normal

Table 4: Bone marrow aspirate findings in haematological disorders

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