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Original Research Article

Study of Bone marrow aspiration and biopsies in Pancytopenia – A study on 48 patients

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Abstract: Pancytopenia is a relatively common entity. However, it has received inadequate attention in the Indian subcontinent. A study of pancytopenia using easily available diagnostic techniques is therefore relevant. Bone marrow aspiration (BMA) and biopsy is an important diagnostic tool in hematology to evaluate various causes of pancytopenia. The present study was conducted on 48 patients for a period of 2 years from June 2011 to May 2013. Complete evaluation of clinical findings, haematological indices and bone marrow examination was carried out. Megalo blastic anaemia (41.66%) was the most common cause of pancytopenia, followed by mixed nutritional anaemia (29.16%), Multiple myeloma and metastatic deposits contributed the least with 01 case each (2.09%). Macrocytic anaemia was the predominant finding observed when studying peripheral smears, and a few cases of normocytic or microcytic anaemia were observed. Hypercellular marrow was seen in 56.25% of cases, whereas hypocellular marrow was present in 6.25% of cases. Female patients were 29 and male were 19 in our study. Pancytopenia is an important clinico haematological entity encountered in our day-to-day clinical practice. The possible underlying aetiologies range from transient viral marrow suppression to life-threatening malignant neoplasm. The aetiological diagnosis is essential for the clinical management and prognosis of the patient.
Keywords: Pancytopenia, bone marrow, aspiration, biopsy, megalo blastic anemia

INTRODUCTION:

Bone marrow is the largest and most widely distributed organ in the body. It is the principal site for blood cell formation. In the normal adult its daily production and export of blood cells amounts to about 2.5billion red cells, 2.5 billion platelets and 1 billion granulocytes per kilogram of body weight [1]. Cytopenia is a disorder in which production of one or more blood cell types ceases or is greatly reduced [2]. Pancytopenia is a disorder in which all three major formed elements (red blood cells, white blood cells and platelets) are decreased than normal [3].

It is not a disease entity but a triad of findings that may result from a number of diseases processes-primarily or secondarily involving the bone marrow. The presenting symptoms are usually attributable to anemia, leucopenia or thrombocytopenia [4]. The underlying mechanisms of pancytopenia are decrease in hematopoietic cell production, marrow replacement by abnormal cells, suppression of marrow growth and differentiation, ineffective hematopoiesis with cell death, defective cell formation, antibody mediated sequestration or destruction of cells in a hypertrophied and active reticuloendothelial system [5]. Pancytopenia is a serious hematological problem, the underlying cause of which is diagnosed by bone marrow aspiration and biopsy. Bone marrow examination is extremely helpful in evaluation of pancytopenia [6]. Various factors encompassing geographic distribution and genetic disturbances may cause variation in the incidence of disorders causing pancytopenia [7-9].

MATERIALS & METHODS:

The present was conducted for a period of 2 years from June 2011 to May 2013, 48 patients were examined. Inclusion criteria was, Hemoglobin <13.5gm/dl in males, <11.5gm/dl in females**.** Total leucocyte count <4000cells/cumm and Platelet count <1, 50,000/cumm. Patients on chemotherapy were

excluded. Clinical history and examination of all identified cases of pancytopenia were done as per proforma. 2ml of anticoagulated blood was collected for complete hemogram. Bone marrow aspiration was done in all patients to identify the etiology. An informed consent was obtained. A Salah's needle was used to aspirate material from the Posterior iliac crest in adults or tibial tuberosity in children, who were less than two years of age. Local infiltration of anaesthesia was used after administering a test dose. Sterile precautions were observed. Aspirated smears were stained with Leishman or Giemsa and Perl's stain to demonstrate hemosiderin in bone marrow macrophages and erythroblasts and examined. Biopsy done when required and when aspiration revealed a dry tap, along with aspiration in the same setting. Following aspiration, needle was removed, now Jemshedi needle introduced by rotating movements. The stillete is removed and the needle is further advanced. This process captures the marrow core sample within the needle. The needle was then withdrawn in the reverse rotating direction. The specimen was fixed in 10%formalin overnight and decalcified with 10% Formic acid for 72hrs. Then it was processed similar to histopathological sample and H&E sections were studied. Special stains like PAS, reticulin were done whenever necessary.

RESULTS:

Forty eight patients with a hematological diagnosis of pancytopenia were studied during the period of June 2011 to May 2013. All clinical details and other findings were examined. In the present study Megalo blastic anemia was the commonest cause constituting 41.66% followed by Mixed nutritional anemia (29.16%), Hypersplenism (14.59%), Aplastic Anemia (6.25%), Leukemia (4.16%), Multiple myeloma (2.09%), Metastasis (2.09%) [Table 1].

Table 1: Showing the Aetiology for Pancytopenia

SL.NO	AETIOLOGY	NO. OF CASES	PERCENTAGE (%)
1	Megalo blastic Anemia	20	**41.66**
2	Mixed Nutritional Anemia	14	29.16
3	Hypersplenism	7	14.59
4	Aplastic Anemia	3	6.25
5	Leukemia	2	4.16
6	Multiple Myeloma	1	2.09
7	Metastasis	1	**2.09**
	TOTAL	**48**	100

Pancytopenia showed its highest incidence in age group of 21-30 years and its occurrence was less frequent in the age group of 1-10 years. The incidence of pancytopenia showed female preponderance [Table 2].

Table 2: Showing age and sex distribution

SL.NO	AGE (YEARS)	MALE	FEMALE	TOTAL NO OF CASES	PERCENTAGE(%)
1	01 - 10	1	0	1	**2.08**
2	11 - 20	3	7	10	20.83
3	21 - 30	5	8	13	**27.08**
4	31 - 40	4	6	10	20.84
5	41 - 50	3	2	5	10.41
6	50 - 60	1	2	3	6.26
7	61 - 70	2	4	6	12.5
	TOTAL	**19**	**29**	**48**	**100**

The commonest mode of presentation was fever which was about 29.16% followed by generalized weakness which constituted to 25% .Other causes were splenomegaly (20.84%), shortness of breath (18.75%) and Hepatomegaly (6.25%) [Table 3]

Table 3: Showing the clinical findings

SL.NO	CLINICAL FEATURES	NO. OF CASES	PERCENTAGE (%)
1	Fever	14	**29.16**
2	Generalized Weakness	12	25
3	Splenomegaly	10	20.84
4	SOB	9	18.75
5	Hepatomegaly	3	**6.25**
	TOTAL	**48**	**100**

Most of the patients had hemoglobin percentage between 5.1-7gm% in 16 cases accounting for (33.33%) followed by 7.1-10gm% in 14 cases (29.16%). Leukocyte count was in the range of 2100-3000cells/cumm in 23 cases (47.92%) and 11 cases had count between 1100-2000cells/cumm accounting for (22.91%). Platelet count was between 76,000-1, 00,000cells/cumm in 19 cases (39.58%) and 4 cases had count between 4,000-25,000cells/cumm (8.34%). Reticulocyte count in the range of 1.1-2% in 24 cases accounting for (50%). Maximum number showed Hypercellular marrow in 27 cases for (56.25%) [Table 4].

Table 4: Showing cellularity of the marrow

SL.NO	CELLULARITY	NO OF CASES	PERCENTAGE (%)
1	Hypercellular	27	**56.25**
2	Hypocellular	3	**6.25**
3	Normo Cellular	18	37.5
	TOTAL	**48**	**100**

DISCUSSION:

Pancytopenia is a serious haematological problem, which makes the patient prone to anaemic manifestations, infections and bleeding tendency. Underlying it are many diseases, which are diagnosed by means of bone marrow aspiration and trephine biopsy. In the present study Megalo blastic Anemia was the commonest cause of pancytopenia (41.66%) and the second commonest cause was Mixed Nutritional Anemia (29.16%).This was comparable with three other Indian studies. In the study by Sudha Horakereppa *et al.;* [10] in 2013 on 58 cases revealed Megalo blastic Anemia as the commonest cause (39.6%) and the second commonest cause was Mixed Nutritional Anemia (24.1%) which was comparable with our study.

In megalo blastic anaemia, peripheral smear may show pancytopenia. Ovalomacrocytes, usually with considerable anisopoikilocytosis, are the main features. Mean corpuscular volume (MCV) is more than 100 fl. In others MCV may be normal due to excess fragmentation of red cells. Polychromatophilic cells are reduced. Reticulocyte count may be less than 1%. The leucocyte count is reduced due to reduction of both neutrophils and lymphocytes. Hyper segmented neutrophils are usually seen. A minimum of five percent of five lobed or a single six lobed neutrophil is considered significant. Thrombocytes are reduced in number, but this is rarely enough to cause hemorrhagic complications. Together, macrocytosis and hyper segmented neutrophils strongly suggest megalo blastic haematopoiesis. [Figure 1]

In the present study macro valocytes with considerable degree of anisopoikilocytosis, hyper segmented neutrophils were seen in most of the cases, dimorphic blood picture was seen in 6cases. In the study by Kishore khodke[5] et al, 20/22 cases showed anisocytosis, 10/22 cases showed dimorphic blood picture, 20/22 cases showed hyper segmented neutrophils. In the study by Tilak *et al.;* [11] 51/53 cases showed anisocytosis, 45 / 53 cases showed hyper segmented neutrophils. Nutritional anemia as a common etiological factor causing pancytopenia is well recognized and established. The nutritional deficiency of either B12 or Folate results in megloblastic anemia. Other being mixed deficiency anemia (microcytic and macrocytic). Most of the patients had dimorphic picture on peripheral smear .Bone marrow was hypercellular and showed erythroid hyperplasia with both megalo blastic and micro normo blastic maturation in most of the cases [Figure 2].

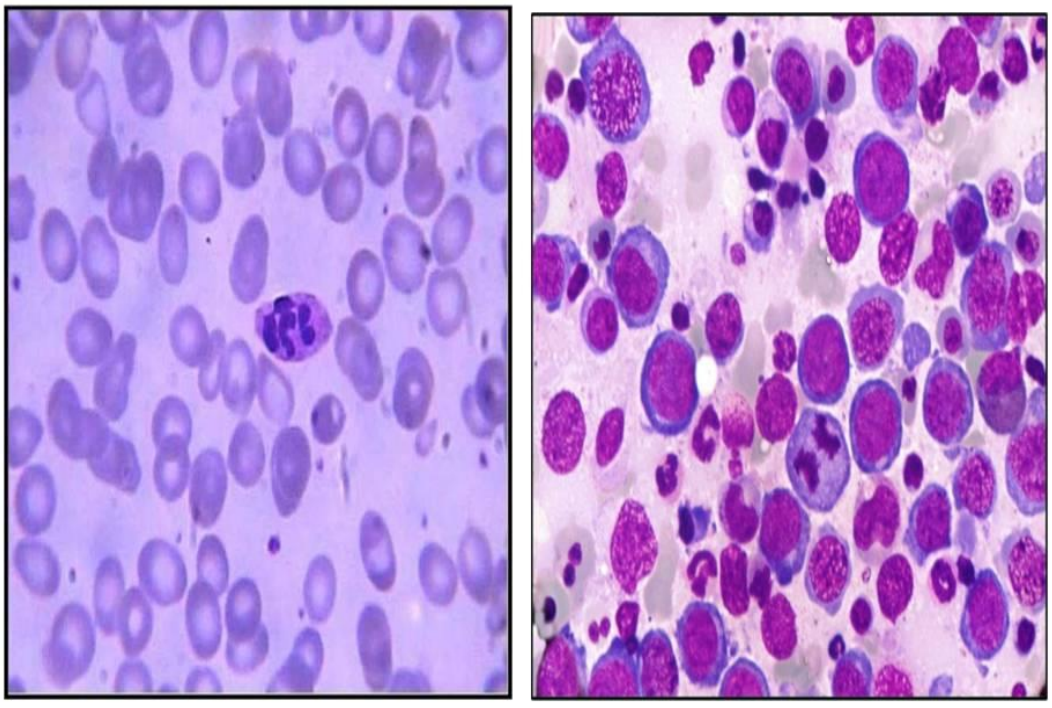
In the present study Mixed nutritional anemia was the second commonest cause of pancytopenia in 14cases accounting for 29.16%.This was comparable

with other studies like Sudha Horakereppa *et al.;* [10]. In the study by Shazia Menon [11] mixed deficiency was seen in 20cases (8.69%), Mobina *et al.;* [6] in their study of 392 cases of pancytopenia found 11.2% of cases with mixed deficiency anemia. Falzur Rahim *et al.;* [12] in their study reported 4.71% of cases with mixed deficiency as a cause of pancytopenia.

Bone marrow is usually hypercellular with predominantly megalo blastic erythropoiesis. Giant band forms meta myelocytes and giant megakaryocytes are also seen. The bone marrow was hypercellular with reduction of fat cells in most of the patients (90%). Two patients (10%) had normo cellular marrow. Erythroid hyperplasia with megalo blastic maturation was seen in all the patients. Hypersplenism is known to cause pancytopenia by sequestration of blood cells. In cases of Aplastic anemia, bone marrow revealed a hypocellular and the aspirate was mostly composed of fat cells in all patients [Figure 3]. Bone marrow trephine biopsy revealed replacement of marrow by fat cells.

Bone marrow in cases diagnosed as leukemia was hypercellular in 50% and normo cellular in 50% cases. One case was diagnosed as AML-M2; other case was diagnosedAML-M3. Patients with multiple myeloma can develop pancytopenia due to replacement of bone marrow by immune proliferating cells [Figure 4]. In the present study multiple myeloma was the cause of pancytopenia in 1 case (2.09%). Patient presented with normocytic hypochromic anemia with markedly increased ESR. Bone marrow aspirate was hypercellular with infiltration of plasma cells with many binucleate forms [Figure 5]. Tilak *et al.;* [13] reported one case of pancytopenia due to multiple myeloma in their study of 77 cases. Jha *et al.;* [14] in their study of 148 cases reported one case of plasma cell myeloma. Kishore khodke *et al.;* [5] reported 2 cases of multiple myeloma in their study of 50 cases of pancytopenia.

Bone marrow aspiration and biopsy revealed metastatic deposits with tumor cells arranged in acinar pattern. Patient had high PSA levels [Figure 6]. On evaluation patient had prostatic adenocarcinoma which metastasized to bone marrow. In the study by Jha *et al.;* [14] metastatic neuroblastoma was found in 1case (0.67%).



a b

Fig 1: Peripheral smear showing hyper segmented neutrophils & macro ovalocytes (Figure a, Leishman, 100x). BMA showing Megalo blasts & Mitotic figures (Figure b, Giemsa, 100x).

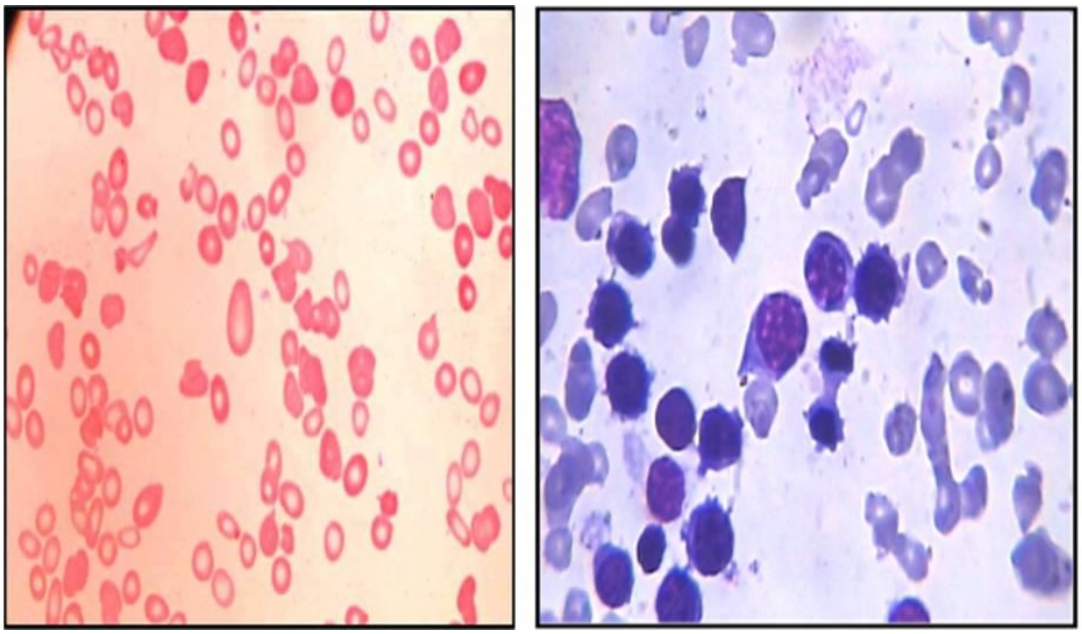


Fig 2: Peripheral smear showing dimorphic picture with macrocytes and microcytes (Figure a, Leishman, 100x). BMA – Showing Megalo blasts and micro normo blasts (Figure b, Leishman, 40x).

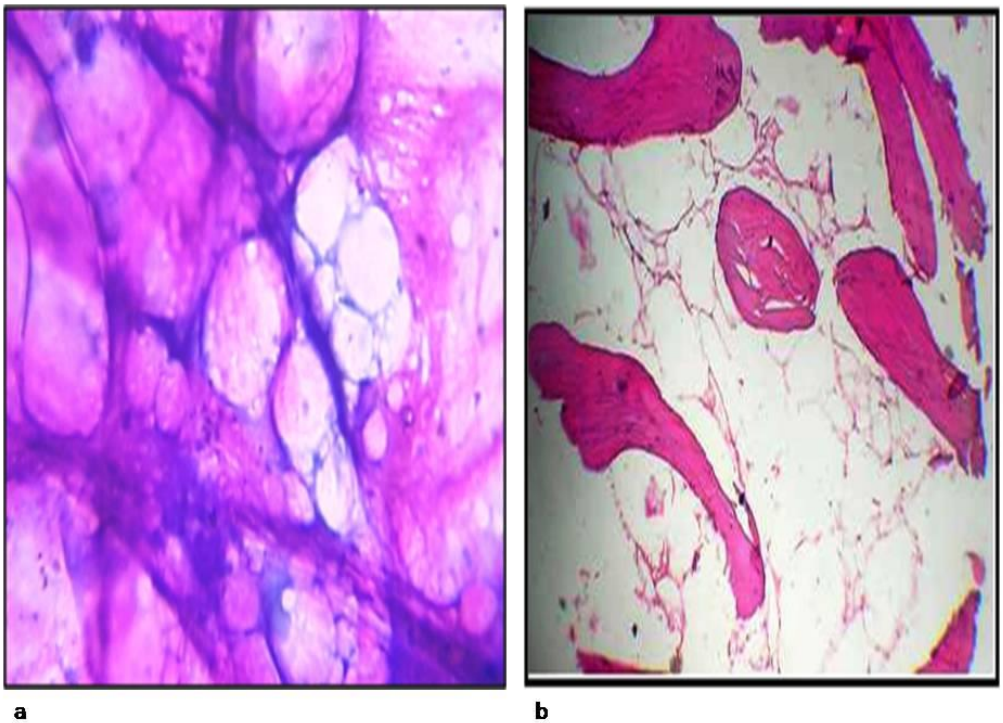
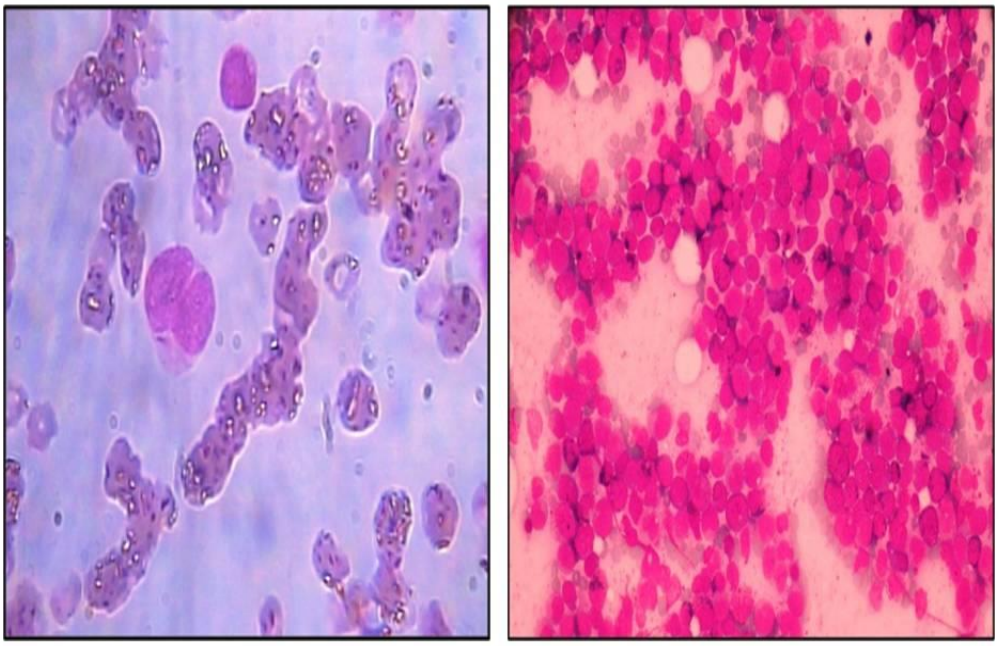
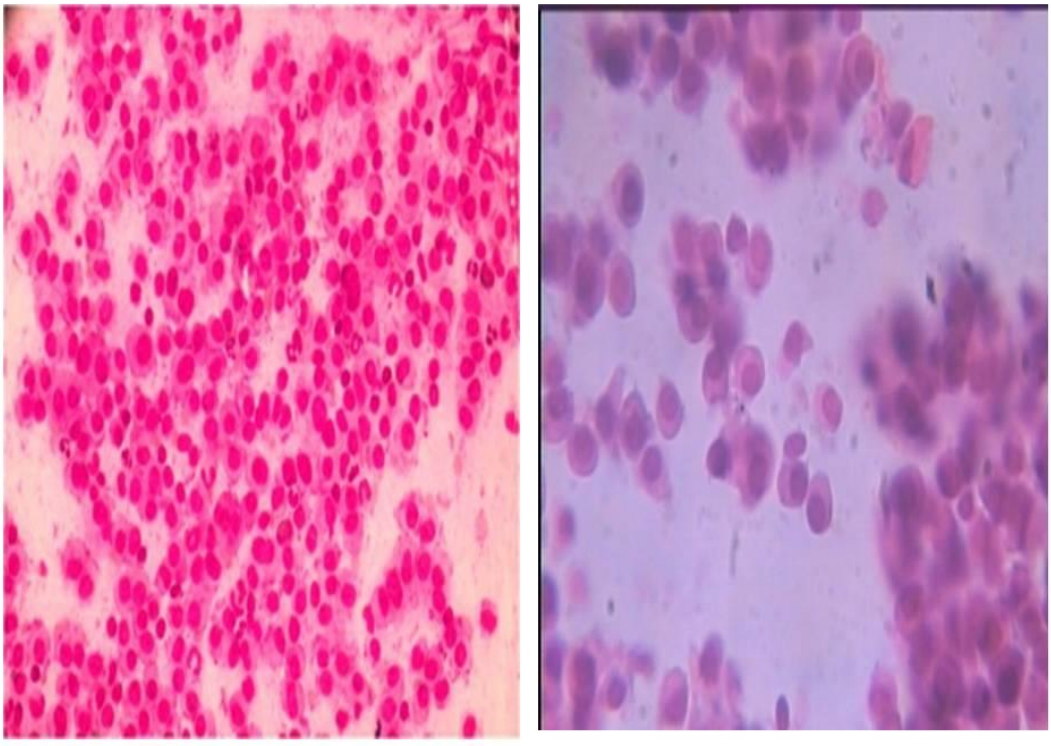


Fig 3: BMA showing increase in fat cells (Figure a, Leishman, 10x). BM biopsy showing increase in fat cells (Figure b, H & E, 10x).



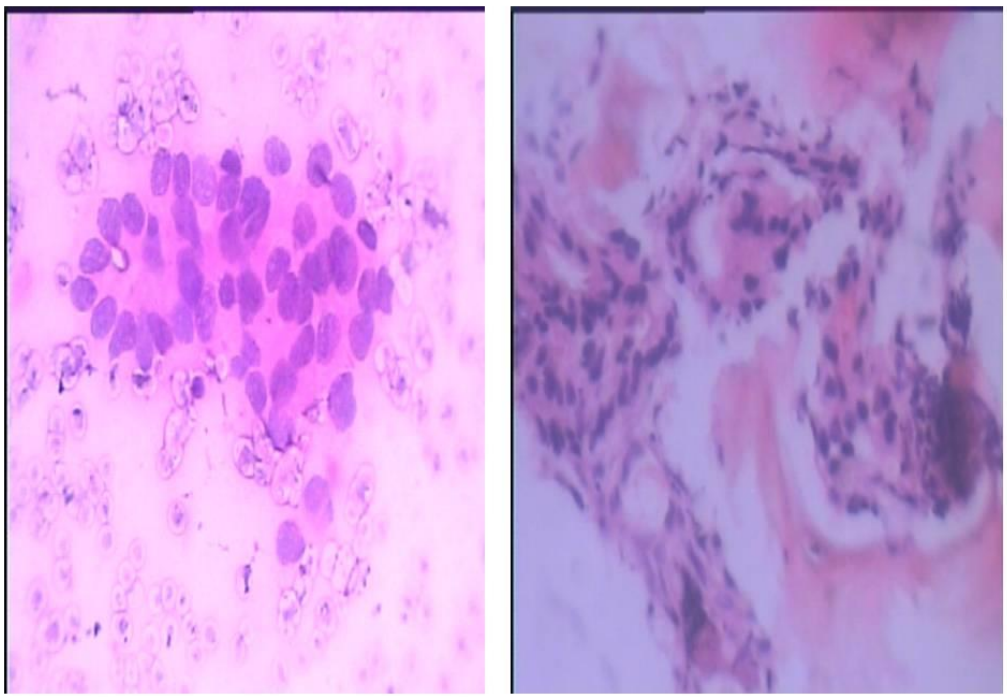
a b

Fig 4: Peripheral smear showing blast with Auer rods (Figure a, Leishman, x40). BMA showing sheets of Myelo blats (Figure b, Leishman, x40).



a b

Fig 5: BMA showing sheets of plasma cells with binucleate forms (Figure a, Leishman, 40x). BM Biopsy – Showing sheets of plasma cells (Figure b, H & E, 40x).



a b

Fig 6: BMA showing tumor cells arranged in acinar pattern (Figure a, Leishman, 40x). BM biopsy showing bony trabeculae with intervening tumor cells arranged in acinar pattern (Figure b, H & E, 40x)

CONCLUSION:

Pancytopenia is an important Clinicohaematological entity encountered in our day-to-day clinical practice. The possible underlying aetiologies range from transient viral marrow suppression to left-threatening malignant neoplasm. The aetiological diagnosis is essential for the clinical management and prognosis of the patient. Evaluation of peripheral blood film reveals the most probable cause of anemia, presence of immature myeloid cells may suggest marrow infiltration of hematologic disorder. Bone marrow examination – aspiration and biopsy is an important diagnostic tool in hematology to evaluate various causes of pancytopenia.

Bone marrow examination is accurate, reproducible, rapidly available information at an economical cost and with minimal discomfort to the patient. Bone marrow aspiration is sufficient in making diagnosis of megalo blastic anemia, mixed nutritional anemia and initial diagnosis of leukemia. Megalo blastic anaemia was commonest cause of pancytopenia in the present study; this reflects the higher prevalence of nutritional anaemia in Indian subjects. The other common causes were hypersplenism and aplastic anemia. Less common etiologies like multiple myeloma, metastatic deposits in bone marrow were identified in this study. A comprehensive clinical and haematological study of patients with pancytopenia will usually help in identification of underlying cause. However, in view of wide array of etiologies, pancytopenia continues to be a challenge for hematologists.

REFERENCES:

1. Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Siberstein LE. Hematology. Basic Principles and practice.3rded, USA, Churchill Livingstone; 2005.
2. Cytopenias- Anaemia, leucopenia, neutropenia, thrombocytopenia. www.oncologychannel.com /- 46K- 6/24/2007.
3. Ishtiaq O, Baqai HZ, Anwer F, Hussai N. Patterns of pancytopenia in a general medical ward and a proposed diagnostic approach.www.ayubmed.edu.pk / JAMC/PAST/16-1/osama.htm-206K-6/24/2007.
4. Guinan EC, Shimamura A. Acquired and inherited aplastic anemia syndromes In: Greer JP, Foerster J, Lukens JN, Rodgers GM, Paraskevas F, Glader B Edts, Wintrobe's Clinical Hematology,11th edn, Philadelphia

:Lippincott Williams and Wilkins 2004: 1397-1419.

5. Khodke K, Marwah S, Buxi G, Yadav RB, Chaturvedi NK. Bone marrow examination in cases of pancytopenia. Journal, Indian Academy of Clinical Medicine. 2001Jan-June. 2001; 2:1-2.
6. Mobina Ahsan Dodhy, Nusrat Bokhari, Abbas Hayat. Aetiology of Pancytopenia, A five-year experience Ann Pak Inst Med Sci 2005 Apr-Jun; 1(2):92-5.
7. International agranulocytosis and aplastic anaemia study. Incidence of aplastic anaemia, the relevance of diagnostic criteria. Blood 1987; 70: 1718-21.
8. Wintrobe MM Clinical Haematology. 8ed. Philadelphia; Lea and Febigerth 1981: 699-915.
9. Keisu M, Öst Å. Diagnoses in patients with severe pancytopenia suspected of having aplastic anemia. European journal of haematology. 1990 Jul 1; 45(1):11-4.
10. Thakkar BB, Bhavsar UN, Trivedi NJ, Agnihotri AS. A study of pancytopenia in adult patients more than 12 years of age in North West region of saurashtra. Nat J Med Res. 2013; 3(1):48-52.
11. Memon S, Shaikh S, Nizamani MA. Etiological spectrum of pancytopenia based on bone marrow examination in children. J Coll Physicians Surg Pak. 2008 Mar 1; 18(3):163-7.
12. Hussain M, Khattak TA, Bano Q. Spectrum of hematological disorders in children observed in 424 consecutive bone marrow aspirations/biopsies. Pak J Med Sci October-December. 2005; 21(4):433-6.
13. Tilak V, Jain R. Pancytopenia--a clinico-hematologic analysis of 77 cases. Indian journal of pathology & microbiology. 1999 Oct; 42(4):399-404.
14. Jha A, Sayami G, Adhikari RC, Panta AD, Jha R. Bone marrow examination in cases of pancytopenia. JNMA J Nepal Med Assoc. 2008;47(169):12-7.