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BONE MARROW EXAMINATION IN PANCYTOPENIA: A STUDY OF SIX YEARS

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ABSTRACT: INTRODUCTION: Pancytopenia is reduction in all the three hematopoietic cell lines as seen in the peripheral blood. As hematopoietic cells are produced in the marrow, its examination forms an important tool in assessing the etiology of pancytopenia. **AIMS:** The aim of this study was to identify the etiology of pancytopenia using bone marrow examination and to correlate it with iron stores in the marrow. **MATERIAL AND METHODS:** This retrospective study was carried out over a period of six years in the department of pathology at Kamineni Academy of Medical Sciences and Research Centre from August 2008 to July 2014. In this period, a total of 765 bone marrow examinations were done, out of which 172 (22%) fulfilled the criteria for pancytopenia. Clinical details, complete blood picture and bone marrow aspiration were done in all the cases. In some cases, trephine biopsy was also done. The biochemical investigations of serum B 12, folic acid and the serum iron profile were available in few cases. **RESULTS:** In the 172 cases studied, the most common etiology for pancytopenia on marrow examination was that of megaloblastic anemia (38.95 %), followed by erythroid hyperplasia (19.18 %) and dimorphic anemia (14.53 %). Trephine biopsies helped in the diagnosis of aplastic anemias. The iron stores were found to be increased in cases of megaloblastic anemias (61.19 %). There were seven patients on antituberculous treatment, four showed megaloblastic anemia and two had hypoplastic marrows. **CONCLUSIONS:** Megaloblastic anemia was the commonest cause of pancytopenia in our study. The iron stores are usually high in cases of megaloblastic anemia. Patients on antituberculous drugs should be monitored. The biochemical investigations and the marrow examination should always be performed prior to instituting the treatment.
KEYWORDS: Pancytopenia, bone marrow examination, megaloblastic anemia, iron stores.

INTRODUCTION: Pancytopenia is defined as a reduction in all the three types of cellular components in peripheral blood below the normal reference range.[1] The presenting symptoms are usually attributable to the anemia, thrombocytopenia and/or leucopenia which can become a serious threat during the subsequent course of the disorder.[2] Various hemopoietic and non-hemopoietic conditions can manifest with features of pancytopenia.

The underlying mechanisms are decrease in hematopoietic cell production, marrow replacement by abnormal cells, suppression of marrow growth and differentiation, ineffective hematopoiesis resulting in cell death.[3,4] Bone marrow examination is extremely useful in evaluation of pancytopenia.[5] Few similar studies have been published in India.[6,7] This study was carried out to find the common causes of pancytopenia, its clinical presentation and also to correlate it with the iron stores in the marrow in order to help the management of patients with pancytopenia.

MATERIALS AND METHODS: The present study was a retrospective and prospective one carried out in the department of pathology at Kamineni Academy of Medical Sciences and Research Centre over a period of six years, from August 2008 to July 2014. The inclusion criteria for pancytopenia were

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Hemoglobin (Hb) < 10gm/dl, Total leucocyte count (TLC) <4000/cmm, Platelet count< 150000/cmm.[3,8] After explaining the procedure and obtaining proper consent from the patient, bone marrow aspiration (BMA) was performed from the iliac crest/sternum under local anesthesia, using Salah's needle. In addition, trephine biopsy was performed in some of the cases using Jamshidi needle. Peripheral smears were also made at the time of marrow sampling.

The clinical details, hematological parameters and biochemical investigations were noted from the case sheets wherever they were available. The peripheral smears, marrow aspirates and imprints were stained by Leishman stain. Perls' stain was done on aspiration slides to demonstrate the iron stores. The trephine biopsies were formalin fixed, decalcified, processed and stained by routine hematoxylin and eosin. Serum vitamin B12, folate and ferritin estimation were done using electrochemiluminescence assays. Serum iron and total iron binding capacity (TIBC) were done by ferrozyme colorimetric assay and lactate dehydrogenase (LDH) was done by enzymatic method.

RESULTS: The total number of bone marrow examinations performed over six years for various indications was 765. In 172 cases (22 %), the indication was pancytopenia. There were 87 males and 85 females, with male to female ratio of 1.02:1. The mean age was 34 years with a range of 1 to 80 years. Maximum patients were in the third decade of life (Table 1). Detailed clinical history was noted in all cases. Majority of patients presented with complaints of generalized weakness, fever, shortness of breath and yellowish discoloration of eyes. Pallor was a universal finding in all the cases, followed by jaundice. Very few patients had features of thrombocytopenia with purpuric rash. None of the cases of pancytopenia due to megaloblastic anemia had features relating to cord degeneration.

A detailed peripheral smear examination was done in all patients. Anisopoikilocytosis was the predominant finding in megaloblastic anemia (MA), dimorphic anemia (DA), iron deficiency anemia (IDA) and erythroid hyperplasia (EH). Other findings in MA were hyper segmented neutrophils and few cases showed relative lymphocyte prominence. Bone marrow aspiration (BMA) was of diagnostic value in patients with pancytopenia.

Majority of the cases showed increased cellularity with altered myeloid-erythroid ratio. MA had typical megaloblasts with sieved chromatin and asynchronic nuclear-cytoplasmic maturation (Figure 1). Myeloid series showed giant band forms and giant metamyelocytes. In microcytic anemia, micronormoblasts were noted. Hypoplasia of marrow was noted in hypocellular marrow and atypical cells were noted in non-Hodgkin lymphoma (NHL) and acute leukemias (AL). BMA findings are shown in Table 2. Trephine biopsies were available in 25 cases (14.53 %) (Table 3).

Perls' stain was done using standard method in 135 cases. Iron stores were graded into 0 to 6 categories[9] as follows:

  0 - Absent iron stores.
  1 - Small iron particles in reticulum cells visible in oil objective.
  2 - Small, sparse iron particles in reticulum cells.
  3 - Numerous small particles in reticulum cells.
  4 - Larger particles with tendency to aggregate into clumps.
  5- Dense, large clumps.
  6- Very large clumps and extracellular iron.

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1 - Reduced iron stores.

2 – Normal.

3 - Slightly increased.

4 - Moderately increased.

5 and 6 - Markedly increased.

The distribution of iron stores in bone marrow aspirates is shown in Table 4.The marrow iron stores were absent in iron deficiency anemia as expected and were variable in dimorphic anemia. They were increased in 41 cases (61.19 %) of megaloblastic anemia (Figure 2).

Biochemical investigations like serum lactate dehydrogenase (LDH), serum iron, total iron binding capacity (TIBC), serum ferritin, B12 and folate levels were done only in few cases due to financial constraints. The serum B12 and folate were reduced in many cases of megaloblastic anemia and so were iron parameters in iron deficiency anemia. Also in some cases, these parameters were elevated in the serum where patients had already received supplementation at outside clinics and where biochemical investigations were performed few days after initiating treatment.

Three cases of megaloblastic anemia and two cases of dimorphic anemia also had hypothyroidism. An interesting observation was that there were four cases in the 60-80 years age group who presented with history of fall due to weakness and routine investigations showed pancytopenia. On marrow aspiration, all four cases showed megaloblastic anemia. Another interesting finding was that there were seven patients of pulmonary and/or extra pulmonary tuberculosis on treatment. Four of them had megaloblastic anemia, two cases showed hypoplastic marrow on aspiration and granulomas in trephine biopsy (Figure 3) and one case showed reactive marrow on aspiration.

DISCUSSION: Pancytopenia is a serious hematologic problem, which makes the patient prone to anemic manifestations, infection and bleeding tendency. Bone marrow examination for the evaluation of pancytopenia is a frequently requested investigation. In the present study, 172 cases (22 %) of the total BMA cases were done for evaluation of pancytopenia. In a study conducted by Jha et al[4] the frequency of bone marrow examination for evaluation of pancytopenia was 15.74 %. There are limited number of studies as to the frequency of causes of pancytopenia.[5]

Some studies are reported from the Indian subcontinent regarding the variation in the frequency of various diagnostic entities causing pancytopenia. This variation is attributed to differences in methodology and stringency of diagnostic criteria, geographic area, period of observation, genetic differences and varying exposure to myelotoxic agents. The incidence of MA varies from 0.8 % to 72 %.[3,7,10,11] Our incidence of MA was 38. 95 % and this correlated well with the studies done by Kumar[8] et al, who showed an incidence of 37% and Khodke[3] et al, who observed an incidence of 44 % of MA among pancytopenia.

Tilak[6] et al in 1999 showed 68 % incidence and Khunger[7] et al in 2002 observed incidence of 72 %. All the above studies done in India stress the importance of megaloblastic anemia (MA) as the major cause of pancytopenia. It is a rapidly correctable disorder and should be promptly notified to the treating clinician. The increased incidence of MA correlates with the high prevalence of nutritional anemias in our country. In our series, 60 patients (90 %) of megaloblastic anemia were strict vegetarians which could have led to dietary deficiency of vitamin B12.

In our study seven patients were on antituberculous treatment and showed pancytopenia.

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Antituberculous drugs are known to reduce serum folate level thereby causing megaloblastic anemia and patients of tuberculosis can even have marrow aplasia or hypoplasia.[12,13]

The bone marrow findings in cases of pancytopenia were those of megaloblastic anemia (MA), followed by EH, DA and IDA. These findings strongly point out the prevalence of nutritional anemias in India. The incidence of marrow hypoplasia in our study was 5 %, whereas aplastic anemia incidence varies from 10 to 52.7 % among pancytopenia patients.[14] The marrow iron stores were absent in iron deficiency anemia as was expected and were variable in dimorphic anemia.

They were increased in 41 cases (61.19 %) of megaloblastic anemia which could be due to the ineffective erythropoiesis and also many patients gave history of long term use, over 3-6months of iron supplements, some taken by patients themselves as over the counter syrups and some prescribed by the local practitioners. Also some of the referral patients were already transfused with packed cell units which might have altered the peripheral smear findings and also the serum biochemical parameters to some extent.

CONCLUSION: The physical examination and peripheral blood picture play an important role in planning investigations in patients of pancytopenia. Megaloblastic anemia, dimorphic anemia and iron deficiency anemia were the major findings in bone marrow in pancytopenia. Patients on antituberculous treatment should be monitored to detect megaloblastic anemia and marrow hypoplasia. The biochemical investigations and the marrow examination have to be performed prior to instituting the treatment so as to establish the correct diagnosis.

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Age in years	Frequency	Percentage
1-10	3	1.74
11-20	32	18.60
21- 30	40	23.25
31-40	32	18.60
41-50	28	16.27
51-60	13	7.55
61-70	16	9.30
71-80	9	5.23
Total	**172**	**100**
Table 1: Age Distribution in Cases of Pancytopenia		

Marrow findings	Number of cases	Percentage	Male: Female
MA	67	38.95	45:22
EH	33	19.18	12:21
DA	25	14.53	8:17
IDA	6	3.48	0:6
RM	10	5.81	7:3
HM	14	8.13	7:7
NHL	7	4.06	3:4
AL	5	2.90	2:3
Myeloma	1	0.58	0:1
MDS	4	2.32	2:2
Total	**172**	**100**	**87:85**
Table 2: Bone marrow Findings in cases of Pancytopenia with Gender Distribution			

(MA-Megaloblastic anemia, EH- Erythroid hyperplasia, DA-Dimorphic anemia, IDA- Iron deficiency anemia, RM-Reactive marrow, HM-Hypocellular marrow, NHL-non-Hodgkins lymphoma, AL-Acute leukemia, MDS- Myelodysplastic syndrome)

Findings on Trephine	Number of cases	Percentage
Aplastic anemia	7	28.0
NHL	7	28.0
Acute leukemia	5	20.0
MDS	1	4.0
Granulomas	2	8.0
Reactive marrow	3	12.0
Total	**25**	**100**
Table 3: Findings on trephine biopsy		

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Grade	MA	EH	DA	IDA	RM	HM	Total
Absent - 0	02	05	13	06	00	3	29
Reduced-1	5	6	2	00	2	2	17
Normal-2	16	8	9	00	3	2	38
Increased(3,4)	35	03	02	00	1	3	44
Markedly increased(5,6)	06	01	00	00	00	00	07
Total	**64**	**23**	**26**	**06**	**6**	**10**	**135**
Table 4: Iron stores distribution in Pancytopenia (135 cases) – Perls' stain							

(MA-Megalobalstic anemia, EH- Erythroid hyperplasia, DA-Dimorphic anemia, IDA- Iron deficiency anemia, RM-Reactive marrow, HM-Hypocellular marrow)
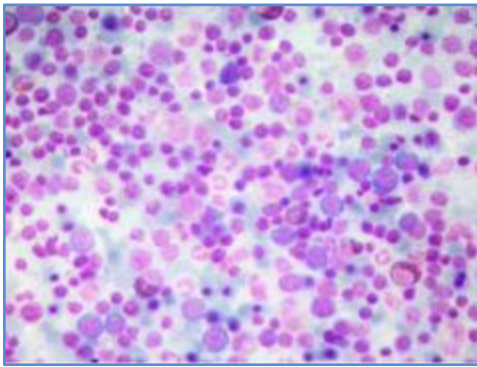

Fig. 1: Marrow aspirate showing erythroid hyperplasia and megaloblasts. (Leishman stain 100X)
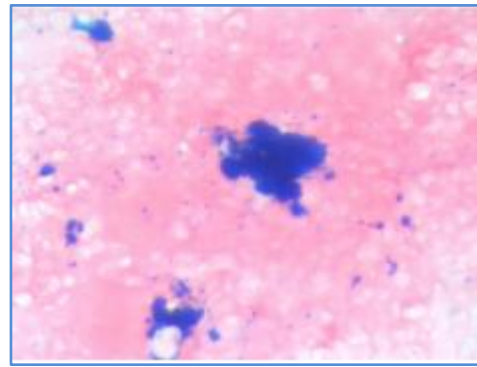

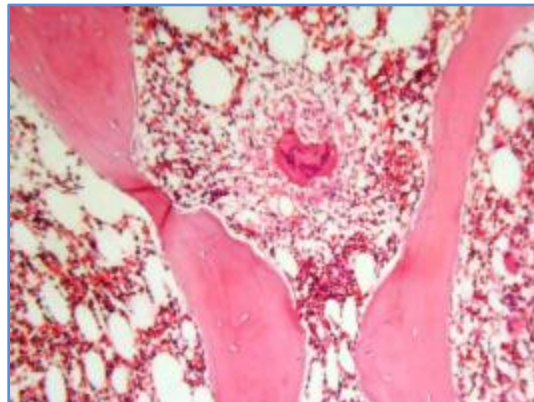**Fig. 2: Marrow aspirate showing iron stores, grade 5 (Leishman stain 100X)**

Fig. 3: Trephine biopsy showing epithelioid cell granulomas. (Hematoxylin and eosin, 400X)

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