Original Research Article

Pancytopenia associated megaloblastic anemia: a clinico-hematological study in a tertiary care hospital

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Abstract

Background: Pancytopenia is a common and important clinical and haematological problem. It is a striking feature of many serious and life-threatening illnesses, ranging from simple drug-induced bone marrow hypoplasia, megaloblastic anemia to fatal bone marrow aplasias and leukemias. Thus, identification of the correct cause will help in implementing appropriate therapy and determine the better management and prognosis. **Objectives:** To study the clinical presentations and evaluate the hematological parameters including bone marrow aspiration in pancytopenia associated megaloblastic anemia cases. **Materials and Methods:** It was a prospective study, in which 75 cases of megaloblastic anemia associated with pancytopenia were evaluated clinically, along with hematological parameters and bone marrow aspiration in Department of Pathology, CMCH, Bhopal, over a period of one and half year. **Results:** Among 150 cases of pancytopenia studied, the commonest cause for pancytopenia was megaloblastic anemia (50%), followed by hperslenism (18%), and aplastic anemia (11%). Most of the patients presented with generalized weakness and fever. The commonest physical finding was pallor, followed by splenomegaly. Macrocytic normochromic red cells morphology was observed in 44(59%) patients of megaloblastic anaemia, followed by dimorphic blood picture in 20(27%) cases. The commonest marrow finding was hypercellularity with megaloblastic erythropoiesis. **Conclusion:** The present study concludes that in pancytopenia cases megaloblastic anaemia is a common and important clinical and haematological problem. Detailed primary hematological investigations along with bone marrow aspiration in pancytopenic patients are helpful for understanding of disease process and to diagnose or to rule out other causes of pancytopenia. These are also helpful in planning further investigations and management because many of them are completely curable while others are manageable.

Keywords: Megaloblastic anemia, Pancytopenia, Bone marrow aspiration

Introduction

Pancytopenia is an important clinico-haematological entity encountered in our day to day clinical practice. It is a disorder in which three major formed elements of blood (red blood cells, white blood cells and platelets) are decreased in number [1].

The causes of pancytopenia can be due to decrease in hematopoietic cell production in the marrow resulting from infections, toxins, malignant cell infiltration, chemotherapies and radiation [2].

Ineffective hematopoiesis with cell death in the marrow; formation of defective cells which are rapidly removed from circulation; sequestration and/or destruction of cells by the action of antibodies or trapping of normal cells in a hypertrophied and over-reactive reticulo-endothelial system may result in pancytopenia. Patients usually present with complaints related to anemia, leukopenia and thrombocytopenia, which if not diagnosed at an early stage, may be fatal. [3] Pancytopenia is a temporary or permanent pathologic finding in the peripheral blood that is a result of a disease, not a disease.

The causes of pancytopenia are bone marrow-originated causes (aplastic anemia, myelodysplastic syndrome, etc), bone marrow infiltration (myelofibrosis, acute leukemia, multiple myeloma, metastatic carcinoma, hairy cellleukemia), splenomegaly (congestive splenomegaly, hematological malignancies splenic infiltration, storage diseases, primary splenic pancytopenia), paroxysmal nocturnal hemoglobinuria, tuberculosis, brucellosis, Q fever, Legionnaires' disease,

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fungal infection and septicemia, and other rea-sons (sarcoidosis, systemic lupus erythematosus, anorexia nervosa, alcoholism, vitamin B12, folic acid deficiency, coagulopathy) [4]. In published articles from different countries, megaloblastic anemia was the most reported cause of pancytopenia, followed by aplastic anemia and other hematological diseases, respectively [5]. Careful assessment of the blood elements is often the first step in assessment of hematologic function and diagnosis of disease. Physical findings and peripheral blood picture provide valuable information towards the work up of pancytopenic patients and help in planning investigations on bone marrow samples [6].

Bone marrow evaluation is an invaluable diagnostic procedure which may confirm the diagnosis of suspected cytopenia, from the clinical features and peripheral blood examination. It may occasionally give a previously unsuspected diagnosis [7]. A detailed history, physical examination, and review of blood film remains fundamental to the diagnosis [8]. The present study is intended to evaluate the various cases of pancytopenia associated with megaloblasicanemia in patients admitted to Chirayu Medical College and Hospitals and study the clinical and haematological findings with bone marrow aspiration.

Materials and Methods

The present study was conducted in the Department of Pathology, Chirayu Medical college and Hospitals, Bhopal, over a period of one and half year. All the OPD and indoor patients admitted were included in the study.

Inclusion criteria were as follows:

Presence of two or all three of the following:

1. Hemoglobin< 11.5 gm/dl in females, and < 13.5gm/ dl in males.
2. Total leukocyte count(TLC) < 4000/ul
3. Platelet count < 100000/ul

Exclusion criteria were as follows:

1. Patients who have already been diagnosed with pancytopenia.
2. Patients who do not give consent for bone marrow aspiration and biopsy.
3. Patients who have recently received blood transfusions.
4. Patients who were on chemotherapy/radiotherapy.

Methodology- The study was performed in patients attending outdoor and indoor departments of Chirayu medical college and hospital, Bhopal (CMCH) during the period of 1 June 2016 to 31 December 2017.

Informed consent was obtained from each of the patient fulfilling the inclusion criteria prior to their enrolment in the study. The patients were interviewed for relevant history including treatment history, history of drug intake, radiation exposure and examined for important physical findings such as pallor, icterus, hepatomegaly, splenomegaly, lymphadenopathy and ascites.

Sample Collection: Under strict aseptic precaution 3 ml of blood was collected by vene puncture. The sample was put in an ethylenediamine tetra-acetic acid (EDTA) coated vial and, at the same time, a dropof blood was used to prepare peripheral blood smear on a glass slide.Complete hemogram was performed with collected EDTA anticoagulant blood on automated cell counter analyser (BC5380, 5-part differential cell counter). Blood and bone marrow smears were stained with various stains as per requirement**.**

Stains

1. Leishman's stain
2. Perl's Prussian blue stain
3. Myeloperoxidase stain.
4. Giemsa stain

Results

The present study evaluated 150 patients with pancytopeniaof which 75 cases of pancytopenia associated megaloblastic anemia were taken for furthervaluation who fulfilled the inclusion criteria and consented to enrollment in the study. In our study we performed bone marrow aspirate examination in all cases which presented in the peripheral blood film with pancytopenia (i.e. leucopenia, anemia and thrombocytopenia) Our study has also included the correlation with physical findings like ascitis, bleeding, icterus, lymphadenopathy, hepatomegaly, edema, pallor and splenomegaly. We also correlated with the sign and symptoms (complaints) of the patient presented in CMCH. Major complains in the cases of pancytopenia were abdominal distention, abdominal pain, back pain, bleeding, breathlessness, fever, hemoptysis, hematemasis, hematuria, joint pain, loose motion, swelling, weakness and vomiting.

Complete hemogram and peripheral smear examination and bone marrow aspiration was performed in all patients. Megaloblasticanaemia was observed in 75 (50%) patients. Hypersplenism was seen in 27(18%) cases. Aplastic anaemia was detected in 17(11%) and acute leukaemia was detected in 13(9%). The other etiology of pancytopenia included myelodysplastic syndrome which was seen in 15(10%) and nutritional anaemia see in 3(2%) patients. Megaloblastic

anaemia was themost common etiology observed in pancytopenic patients and hypersplenism was the second common cause of paneytopenia in our study. Etiological distribution of pancytopenia cases is shown in (Table 1)

Table-1: Etiological distribution of pancytopenia.

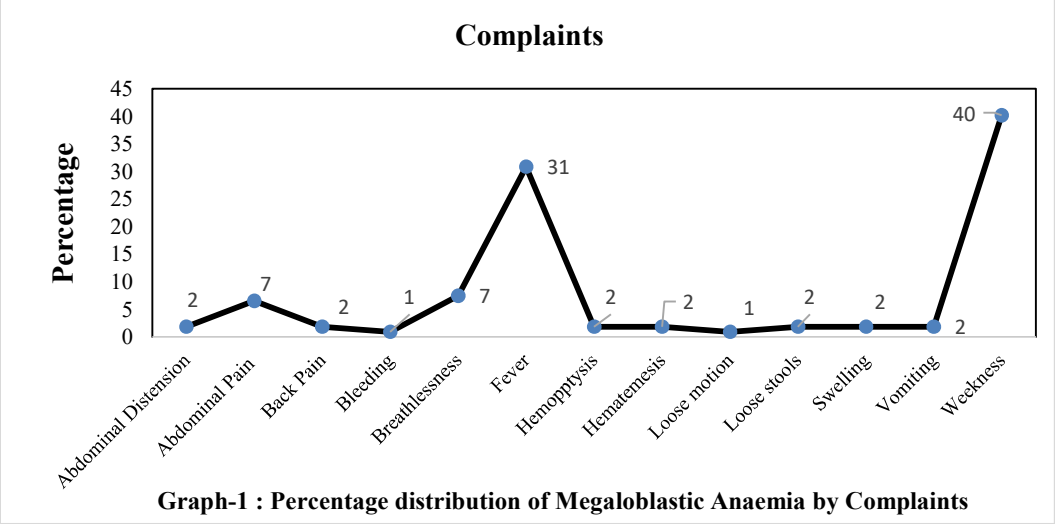
Etiology	Number of Patients	Percentage
Megaloblastic Anaemia (MA)	75	50
Hypersplenism (HS)	27	18
Aplastic Anaemia (AA)	17	11
Acute Leukaemia (AL)	13	9
Myelodysplastic Syndrome (MDS)	15	10
Nutritional Anaemia (NA)	3	2
Total	**150**	**100**

Pancytopenia with Megaloblastic Anaemia- In the present study, megaloblastic anaemia was seen to occur in the age group ranging from 01-70 years with maximum number of cases 17(23%) were 21-30 years of age group (Table 2). There was male preponderance with male to female ratio being 1.5:1.

Table-2: Age and sex wise distribution in patients with megaloblastic anemia

Age groups in year	No of Patients	Percentage (%)	Sex (No of Patients)	
			Male	Female
1-10	1	1	1	0
11-20	14	19	9	5
21-30	17	23	10	7
31-40	9	12	6	3
41-50	9	12	5	4
51-60	12	16	7	5
61-70	10	13	6	4
Above 70	3	4	2	1
Total	**75**	**100**	**46**	**29**

Presenting complaints in patients with megaloblastic anemia, fever was observed in (31%) cases. Breathlessness was seen in 8(7%) cases, bleeding manifestations were present in 1(1%) patients and abdominal pain in 8(7%) patients. Other common symptoms included swelling, and loose motion, each were present in 2(3%) patients. Abdominal distension and vomiting each were present in 4(4 %) patient. Fever was the commonest symptom observed in pancytopenic patients (Graph 1).



Graph-1 : Percentage distribution of Megaloblastic Anaemia by Complaints

Physical findings in patients with megaloblastic anemia: On examination, pallor was detected in 55(49%) patients of megaloblastic anaemia. Splenomegaly was observed in 30(27%) cases. Odema, and icterus were seen in 9(8%) patients respectively (Table 3)

Table-3: Physical examination wise distribution of Megaloblastic Anaemia cases in the study

Physical examination	No. of Patients	Percentage
Ascitis	**1**	1
Bleeding	1	1
Icterus	4	4
Liver	17	15
Oedema	5	4
Pallor	55	49
Spleen	30	27
Total	**113**	**100**

4. Hemoglobin- Hemoglobin value varied from 1-3 gm/dl in 7(9%) cases, 3.1-5 gm/dl in 19(25%), and 5.1-7 gm/dl in 31(41%). 18(24%) patients had values in the range of7.1-10 gm/dl. (Table 4)

Total Leukocyte Count- The total leukocyte count ranged from 100->4100 cells/cumm. 28(37%) patients had a leukocyte count in the range of 1100-3000 cells/cumm. 13(17%) patients had values ranging from 3100-4000 cells/cumm. (Table 4)

Platelet count- The platelet count varied from 0->1,00,000 cells/cumm. 24(32%) patients had a platelet count in the range of 50100-75,000 cells/cumm and 21(28%) patients had values in the range of 25,100-50,100 cells/cumm. Range of Platelet Count wise distribution of Megaloblastic Anaemia samples in the study conducted is shown in (Table 4).

Table-4: Range of Hemoglobin, Leukocyte count and Platelet count in patients with megaloblastic anemia

Parameter	Range	No of cases	Percentage (%)
Hemoglobin (gms/dl)	1-3	7	9
	3.1- 5	19	25
	5.1-7	31	41
	7.1-10	18	25
	TOTAL	75	100
Total leukocyte count (cells/ cu mm)	100-1000	3	4
	1100-2000	28	37
	2100-3000	28	37
	3100-4000	13	17
	4000- Above	3	4
	TOTAL	75	100
Platelet count (cells/cu mm)	10000-25000	20	27
	25100-50000	21	28
	50100-75000	24	32
	75100-100000	9	12
	100000- above	1	1
	TOTAL	75	100

Red blood cell morphology- Macrocytic normochromic red cells morphology were observed in 44(59%) patients of megaloblastic anaemia, followed by dimorphic blood picture in 20 (27%) cases. 6 (8%) cases showed normocytic normochromic red cell morphology. (Table 5)

Table-5: Red Blood Cell Morphology wise distribution of Megaloblastic cases

Red Blood Cells	Total	Percentage
Bicytopenia, Dimophic	2	3
Dimorphic	20	27
Macrocytic-hypochromic	2	3
Macrocytic-Normochromic	44	59
Microcytic-hypochromic	1	1
Normocytic-Normochromic	6	8
Grand Total	**75**	**100**

Peripheral smear findings- Macro-ovalocytosis with a considerable degree of anisopoikilocytosis were the main features. Mean corpuscular volume was more than 100. Dimorphic blood picture was seen in many patients. Hyper segmented neutrophils were seen in most of the patients. Basophilic stippling and cabot rings were present. Platelets were reduced in number in most of the cases.

Bone marrow- In the present study, most of the megaloblastic anaemia bone marrow smears showed hypercellular marrow in 52(69%) patients, whereas 14(19%) and 9(12%) patients exhibited normocellular and hypocellular marrow respectively (Table 6).

Table-6: Bone marrow cellularity in patients with megaloblastic anemia

Bone Marrow Cellularity	No. of Patients	Percentage
Hypercellular	52	69
Hypocellular	9	12
Normocellular	14	19
Total	**75**	**100**

Discussion

The present study analyzed the clinico-haematological and etiological profile of 75 patients of pancytopenia associated megaloblastic anemia cases. Age, gender distribution, clinical features and the haematological findings including complete hemogram, peripheral smear and bone marrow examination findings were studied.

The age of the patients in this study ranged from 2 to more than 70 years. The highest incidence was observed in the age group of 11-50years (66%). Similar findings were observed in many other studies [3,5,6,9,10].

In the present study, we observed a male to female ratio 1.5:1 which correlated with the male preponderance noted in other studies from the Indian subcontinent. The most common presenting complaint in our study was weakness (40%), fever (31%) followed by breathlessness (7%). Other symptoms noted in the present study included bleeding manifestations, pain abdomen, swelling, bone pain, abdominal distension, vomiting and loose motion. Pallor (49%) was the most common physical finding observed followed by splenomegaly (27%) in present study. These findings were comparable to other studies reported from the Indian subcontinent [3,5,6,11,12].

The variations in the frequency of various diagnostic entities causing pancytopenia has been attributed to difference in methodology and stringency of diagnostic criteria, geographic area, period of observation, genetic differences and varying exposure to myelotoxic agents, etc[6].

In the present study megaloblastic anaemia constituted the most frequent underlying etiology in 75(50%) patients of pancytopenia followed by hypersplenism diagnosed in 27(18%) cases. Aplastic anaemia was reported in 17(115), Acute leukemia in 13 (9%), MDS in 15 (10%) and nutritional anaemia is 3(2%). The commonest cause of pancytopenia, reported from various studies throughout the world has been aplastic anaemia. On the contrary most studies conducted in the Indian subcontinent observed megaloblastic anaemia to be the major cause of pancytopenia.However, occasional studies reported from India observed aplastic anaemia as the most common underlying etiology of pancytopenia with megaloblastic anaemia [6,9,11,13,14,15].

The incidence of megaloblastic anaemia noted in the present study was 50%. This correlated with the high incidence ranging from 44% to 74.04% reported by various Indian studies. The increased incidence of megaloblastic anaemia reflects the high prevalence of nutritional deficiency in Indian subjects. As facilities for estimating folic acid and vitamin B12 levels are not routinely available in most centers in India, the exact deficiency is usually not identified[3].

Table-7: Comparison of the findings of this study with other studies in India.

S. No	Study	Country	Year	No. of cases	Commonest cause	Second most Common cause
1	TilakV, Jain R [6]	India	1998	77	Megaloblastic anaemia (68%)	Aplastic anaemia (7.7%)
2	Khodke et al[12]	India	2000	50	Hypoplastic anaemia(29.51%)	Megaloblastic anaemia (22.3%)
3	Kumar R etal [5]	India	2001	166	Aplastic anaemia (29.5%)	Megaloblastic anaemia (22.3%)
4	Khunger et al[3]	India	2001	200	Megaloblastic anaemia (72%)	Aplastic anaemia (14%)
5	Gupta et al [17]	India	2008	105	Aplastic anaemia (43%)	Acute leukemia (25%)
6	Santra and Das [16]	India	2010	111	Aplastic anaemia (22.72%)	Hypersplenism (11.7%)
7	Gayathri and Rao [13]	India	2011	104	Megaloblastic anaemia (74%)	Aplastic anaemia (18%)
8	Naseem et al [18]	India	2011	571	Aplastic anaemia (43%)	Megaloblastic anaemia (13.7%)
9	Present study	India	2018	150	Megaloblastic anaemia (50%)	Hypersplenism (18%)

In present sudy mean peripheral blood findings of the study population included haemoglobin level (6.25 g/dL), total leukocyte count (2,786/uL), and platelet count (42,032/uL). Whereas, mean values for red cells indices included red blood cells count (2.4 million/µL), mean corpuscular volume (122 fL), mean corpuscular haemoglobin (26.9 pg) and mean corpuscular haemoglobin concentration (31.1g/dL). Such results are in agreement with otherpublished reports. 3,16,19–21. In the present study, the bone marrow was hypercellular with reduction of fat cells in most of the patients of pancytopenia associated megaloblastic anemia cases (82%). Bone marrow was normocellular in (15%) and hypocellular in (3%) cases. Erythroid hyperplasia with megaloblastic maturation was seen in all the patients. In aplastic anaemia cellularity of bone marrow is very much reduced. It may be hypocellular or acellular. Lymphocytes and plasma cells are prominent.

Bone marrow examination is an important diagnostic tool in hematology it is also helpful in differential diagnosis of other diseases and excluding a primary marrow involvement and suggesting alternative investigations for diseases which is instrumental in confirming the underlying diagnosis.

Conclusion

Pancytopenia is not an uncommon haematological problem encountered in clinical practice and should be suspected on clinical grounds when a patient presents with unexplained anaemia, prolonged fever and tendency to bleed. Most studies in the literature report megaloblastic anaemia, hypersplenism and aplastic anaemia as the most frequently diagnosed underlying cause. Majority of the studies reported from Indian subcontinent report megaloblastic anaemia as a predominant cause of pancytopenia in contrast to western studies where aplastic anaemia and malignancies are common. The physical findings and peripheral blood picture play an important role in planning the investigations in pancytopenic patients. Clinical and haematological parameters in the major causes of pancytopenia show considerable overlap, making specific tests mandatory to arrive at a diagnosis.

The severity of pancytopenia and the underlying pathology determine the management and prognosis of the patients. A large proportion of pancytopenia is attributable to megaloblastic anaemia which is amenable to treatment. Hence, early and accurate diagnosis is life-saving.

Findings: Nil; **Conflict of Interest**: None initiated
Permission from IRB: Yes

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