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Clinicohaematological profile of Pancytopenia- A South Indiantertiary hospital experience

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ABSTRACT:

Introduction: *Pancytopenia refers to simultaneous presence of anaemia, leukopenia and thrombocytopenia. Multiple disorders either primarily or secondarily affecting bone marrow, manifest with various haematological derangements and is commonly presented as pancytopenia. Identification of the disease is of prime importance, since this is the key to appropriate management.*

Aim: *The main aim of this study is to diagnose and evaluate various causes of pancytopenia.*

Materials and methods: *Seventy five cases of pancytopenia were included in the study. Two millilitres of EDTA anticoagulated blood was collected and analyzed by automated haematology analyser ADVIA2120. Haematological parameters were recorded, peripheral smear and bone marrow smears studied.*

Results: *Bone marrow aspiration was helpful in identifying the cause of pancytopenia. There was female preponderance and the age ranged between 2yrs to 75 yrs. The most common cause of pancytopenia was megaloblastic anaemia (68%) followed by hypoplastic/ aplastic marrow (13.3%) and leukaemia/lymphoma (5.33%).*

Conclusion: *This study showed that the most common cause of pancytopenia was megaloblastic anemia and that is reversible by therapy. Thus in pancytopenia, thorough evaluation has to be done to identify the cause at the earliest, so the treatable causes are identified without delay and the patient is benefited.*

Keywords: *Anaemia, Pancytopenia, Leucopenia, Thrombocytopenia, Megaloblastic*

INTRODUCTION

Pancytopenia denotes simultaneous reduction in all three major formed elements of blood to levels below their normal limits leading to presence of anaemia, leucopenia and thrombocytopenia. It isnot a disease entity but a triad of findings that may arise from a number of disease processes.[1] Pancytopenia can be a striking feature of many serious and life threatening illnesses and may be caused by several disorders ranging from simple drug-induced bone marrow hypoplasia tomegaloblasticanaemia to fatal aplastic anaemia and leukaemia. The mechanism of development of pancytopenia varies from reduction in haematopoiesis as in aplastic anaemia, trapping of normal cells in hypertrophied and overactive reticuloendothelial system as in hypersplenism, ineffective haematopoiesis in megaloblastosisand replacement by abnormal or malignant tissue in themarrow.[1,2]

The severity of pancytopenia and the underlying pathology determine the management and prognosis of the patients,[3] thereby identifying the correct etiopathology, helps in planning the diagnostic and therapeutic approach in patients with pancytopenia. In India, the causes of pancytopenia are not well defined, so the present study has been undertaken to evaluate the various causes or disorders in pancytopenia and to correlate clinically.[3,4] Thereby, this data would help in planning the diagnostic and therapeutic approach in patients with pancytopenia.

MATERIALS AND METHODS

This is a prospective study conducted in the department of pathology in a tertiary hospital from January 2014 to May 2015. Seventy five cases which met the criteria of pancytopenia that is haemoglobin < 10 g/dL, total leukocyte count < 4×10^9 /L and platelet count < 100×10^9 /L, were selected for the study from all the cases referred to the clinical pathology. A systematic review of causes, clinical, haematological parameters and bone marrow findings were done. Detailed history, clinical examination and haematological parameters at presentation were recorded. Haematological profile included haemoglobin, red cell indices, total and differential leukocyte counts, platelet count, peripheral blood smear morphology and bone marrow aspiration/ biopsy.

Two millilitres of EDTA (ethylene diamine tetra-acetic acid) anticoagulated blood was collected and aspirated in an automated haematology analyzer ADVIA 2120; and haematological parameters were obtained. Peripheral smear was stained by Leishman stain for all the cases and examined in detail. Bone marrow aspiration was subsequently carried out under aseptic precautions after obtaining written consent from the patient or guardian. The slides were stained with Leishman stain and examined. Bone

marrow biopsy was done when necessary. The material was subjected to routine processing and Hematoxylin and Eosin stain done. Special stains like reticulin and Perls were done if required.

Inclusion criteria: Cases of all ages and both sexes which met the criteria of pancytopenia were included in the study.
Exclusion criteria: Cases that were on chemotherapy / radiotherapy were excluded from the study.

RESULTS

A total of 36,500 blood samples were received for complete blood counts during this period of study. Out of these, 75 consecutive samples which met the inclusion criteria were included. A definite female preponderance was seen overall and in all age groups with male to female ratio being 1:1.7. The minimum age was of 2yrs and maximum age 75yrs was seen. The maximum number of cases were found during the 6^{th} decade (15 cases 20%) followed by 5^{th} decade (14 cases 18.67%). The age sex distribution of our cases is depicted in Table 1. The most common presenting complaint/ physical finding in this study was splenomegaly (34.66%), followed by fever (30.66%). Table 2 shows the various presenting complaints and physical findings in our study. The predominant blood picture was dimorphic anaemia (38 cases 50.66%), followed by Normocytic normochromic anaemia (20 cases 26.66%). Normocytic hypochromic anaemia was seen in 13.34% (10 cases) and the least common was microcytic hypochromic anaemia in 9.34% (7 cases) of cases. Hyper segmented neutrophils were seen in 46.66% of cases. Leucopenia and thrombocytopenia were seen in all cases.

The most common cause of pancytopenia in our study was megaloblastic anaemia followed by hypoplastic / aplastic marrow. Megaloblastic anaemia was identified in 21 males and 30 females, their age ranging from 11 to 75 years, with a mean age of 38 years. Splenomegaly was observed in 23 cases of megaloblastic anaemia, 15 cases presented with fever and 13 cases with pallor. Plasmodium falciparum was positive in one case of megaloblastic anaemia. Bone marrow aspiration showed megaloblastic erythroid hyperplasia. Megaloblasts are of larger size, had the characteristic features of sieved nuclear chromatin, asynchronous nuclear maturation, bluish cytoplasm and with cytoplasmic blebs. Giant metamyelocytes and band forms were predominant in granulocyte series as shown in figure 1. As we could not estimate B_{12} and folate levels routinely, both folic acid and parenteral hydroxycobalamine therapies were administered to all, and they showed complete clinical and haematological remission. Hypoplastic / Aplastic anaemia was seen in 02 males and 08 females; their age ranged from 8 to 68 years, with a mean age of 30 years. In the present study, out of 10 cases of bone marrow hypoplasia, cause was not known in 09 cases and was grouped under idiopathic bone marrow hypoplasia. One case was secondary to infectious etiology. Bone marrow showed hypocellularity with suppression of erythropoiesis, myelopoiesis and megakaryopoiesis with relative lymphoplasmacytosis.

Three cases of leukaemia were seen along with a case of Non Hodgkin Lymphoma (NHL). One was a 55 yr old female patient who presented with generalised weakness, loss of appetite and weight loss. Peripheral smear revealed pancytopenia with relative lymphocytosis and increased tendency for rouleaux formation. Bone marrow aspiration revealed 35% of myeloblasts with 15% of immature plasma cells and was diagnosed as Acute Myeloid Leukaemia (AML) with reactive plasmacytosis-collision / composite hematolymphoid malignancy as shown in figure 2. The other case was of a 13 yr old female who presented with loss of appetite, fever, pallor, hepatosplenomegaly and lymphadenopathy. Peripheral blood picture was of pancytopenia and bone marrow aspiration showed features of Acute Lymphoblastic Leukemia-(ALL) L1 with 25% of lymphoblasts as shown in figure 3 & 4. The peripheral blood smear of a sixty year old female revealed pancytopenia with leucoerythroblastic picture with left shift and 597 NRBC/100 WBC. The bone marrow aspiration and biopsy showed features Chronic Myeloid Leukaemia (CML). We also encountered a 22 yr old female patient presenting with loss of appetite, weakness and generalised lymphadenopathy. Peripheral smear showed decrease in all the counts, bone marrow aspiration was a dry tap and bone marrow biopsy revealed features of NHL.

There were 4 cases of myelofibrosis in this study; 2 males and 2 females their age ranging from 35 yrs to 61 yrs with a median age of 52yrs. Bone marrow aspiration revealed suppression of all the three lineages with increase in marrow reticulin (Grade IV) as shown in figure 5.We encountered three cases of Immune Thrombocytopenic Purpura (ITP). All the three cases presented with bleeding manifestations. Bone marrow aspiration revealed increased megakaryocytes with hypolobated forms as shown in figure 6. There was a relative decrease in erythroid and myeloid series. Two cases of multiple myeloma were identified in this study with characteristic marrow findings as shown in figure 7. In one case of pancytopenia, the cause was not identified because of dry tap in bone marrow aspiration and inadequate yield in bone marrow biopsy.

Table 1: Age and Sex distribution

Age (yrs) / Sex	Male	Female	Total	Percentage (%)
0-10	2	2	4	5.33
11-20	2	10	12	16
21-30	4	8	12	16
31-40	5	7	12	16
41-50	6	5	11	14.67
51-60	4	14	18	24
61-70	4	1	5	6.67
71-80	0	1	1	1.33
Total	27 (36%)	48 (64%)	75	100

Table 2: Presenting complaints and physical findings in Pancytopenia

Symptoms	Number of cases	Percentage
Splenomegaly	28	37.33
Fever	23	30.66
Generalised weakness	21	28
Pallor	19	25.33
Hepatomegaly	10	13.33
Hepatosplenomegaly	07	9.33
Dyspnoea	07	9.33
Bleeding manifestations	06	8
Weight loss	05	6.66
Chills and rigor	02	2.66
Jaundice	02	2.66
Abdominal pain	02	2.66
Portal hypertension	02	2.66
Lymphadenopathy	01	1.33

Table 3: Distribution of various causes of Pancytopenia

Causes	Number of cases	Percentage
Megaloblasticanemia	51	68%
Hypoplastic /aplastic	10	13.3%
Leukemia / Lymphoma	04	5.34%
Myelofibrosis	04	5.34%
ITP	03	4%
Multiple myeloma	02	2.67%
Unknown	01	1.33%
Total	75	100%

Table 4: Age and sex etiology wise distribution

Age/ etiology	Sex	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	Total	Percentage
Megaloblastic anaemia	M	0	2	4	5	6	2	2	0	21	28%
	F	1	7	5	4	3	8	1	1	30	40%
Hypoplastic/ Aplastic	M	1	0	0	0	0	0	1	0	02	2.67%
	F	1	2	1	0	1	3	0	0	08	10.67%
Leukaemia /Lymphoma	F	0	1	1	0	0	2	0	0	04	5.34%
Myelofibrosis	M	0	0	0	0	0	1	1	0	02	2.67%
	F	0	0	0	1	0	1	0	0	02	2.67%
ITP	M	1	0	0	0	0	0	0	0	01	1.33%
	F	0	0	1	1	0	0	0	0	02	2.67%
Multiple myeloma	M	0	0	0	0	0	1	0	0	01	1.33%
	F	0	0	0	0	1	0	0	0	01	1.33%
Inadequate	F	0	0	0	1	0	0	0	0	01	1.33%
Total		04	12	12	12	11	18	05	01	75	100%

Table 5: Comparison of causes of Pancytopenia in various studies

Study	Country	Year	No of cases	Age	M:F	Most common cause	Second common cause
Tilak and Jain etal[3]	India	1999	77	5-70	1.1:1	MA (68%)	AA (7.7%)
Kumar etal[4]	India	2001	166	12-73	2.1:1	AA (29.5%)	MA (22.3%)
Khungeretal[5]	India	2002	200	2-70	1.2:1	MA (72%)	AA (14%)
Ashraf S et al[6]	Pakistan	2010	150	15-60	1.1:1	HS (68%)	MA (25.4%)
Gayathri BN etal[7]	India	2011	104	2-80	1.2:1	MA (74.04%)	AA (18.3%)
Vandana R etal[8]	India	2012	80	1-79	1:1.2	MA (41.2%)	DA (8.7%)
Swetaetal[9]	India	2014	100	5-80	1.56:1	MA (66%)	AA (18%)
Present study	India	2015	75	2-75	1:1.7	MA (56%)	AA (13.3%)

MA- Megaloblastic anaemia, AA-Hypoplastic/ Aplastic anaemia, HS-Hypersplenism,
DA- Dimorphic anaemia

DISCUSSION

In this study 75 cases of pancytopenia were encountered. Age and sex wise incidence, presenting complaints, physical signs, peripheral blood picture, bone marrow findings and various causes of pancytopenia were taken into consideration. These parameters were compared with other studies in literature. Pancytopenia is a feature of many transient illnesses or serious life threatening disorders.

The variation in the frequency of various diagnostic entities causing pancytopenia in different population groups has been attributed to differences in methodology and stringency of diagnostic criteria, period of observation, geographic area, age pattern, nutritional status, and prevalence of infective disorders, genetic differences, and varying exposure to myelotoxic agents amongst other factors.[4]In our study, the age of the patients ranged from 2yrs to 75yrs with maximum number of cases in 6th decade. There was female preponderance, while most of the studies showed male preponderance except for astudy done by Vandana R et al. which showed slight female preponderance.[5] The comparison of age sex and causes of pancytopenia with other studies are depicted in Table 5.

The most common clinical finding in our study was splenomegaly followed by fever and weakness. The presenting symptoms in other studies were attributed to anaemia or thrombocytopenia. Leucopenia was an uncommon cause of the initial presentation of the patient but can become the most serious threat to life during the course of the disorder.[6]

In this study hyper segmented neutrophils were noted in 46.6% of cases compared to 84.9% in a study by Tilak and Jain et al.[3] and Khunger JM et al. demonstrated no hyper segmented neutrophils in megaloblastic anaemia.[7]In 30% of hypoplastic / aplastic marrow, we noticed relative lymphocytosis compared to 50% in Tilak and Jain et al. study and 85.71% in Khunger JM et al. study.[3,7] The most common cause of pancytopenia in our study was megaloblastic anaemia, which is in contrast to various studies throughout the world where aplastic anaemia has been reported as the commonest cause. [3] Similar findings were observed in other studies conducted in India by Tilak and Jain etal, Vandana R etal, Gayathri BN etal, Khunger JM etal and Sweta etal[3,5,6,7,9] as depicted in Table 5. This seems to reflect the higher prevalence of nutritional anaemia in Indian subjects. Megaloblastic anaemia is a rapidly correctable disorder and should be promptly notified.[7] Bone marrow aspiration was indicated only when the diagnosis was not straight forward and haematological assays were not available.

The pathophysiology of aplastic/ hypoplasticanaemia is now believed to be due to triggering of aberrant immune response by viral infections, exposure to chemicals and drugs or endogenous antigens.[2] The mechanism is similar to tissue specific organ destruction mediated by lymphocytes seen in diabetes, multiple sclerosis, uveitis and colitis.[10,11] Incidence of aplastic/ hypoplastic anaemia varies from 10 to 52.7% among pancytopenic patients.[2] The incidence of hypoplastic anaemia in our study was 13.3% which correlated with the correspondingures in study done by Khunger JM et al., who observed an incidence of 14%.[7] A little higher incidence was reported by Gayathri BN et al (18.3%) and Sweta et al (18%).[6, 9]

We encountered 5.34% incidence of sub leukemicleukaemia and lymphoma, which is similar to incidence reported by Khunger JM et al (5%).[7] Whereas Kumar *R* et al. reported 12% incidence of aleukemic leukaemia. [4] The diagnosis of AML with relative plasmacytosis, CML and ALL was done on bone marrow aspiration. Also one case of NHL was reported by bone marrow biopsy. Myelofibrosis (5.2%), ITP (4%) and Multiple myeloma (2.67%) were other causes of pancytopenia in this study. Though these are rare causes of pancytopenia, they do occur as mentioned in various studies by Tilak and Jain et al, [3] and Kumar et al., [4]

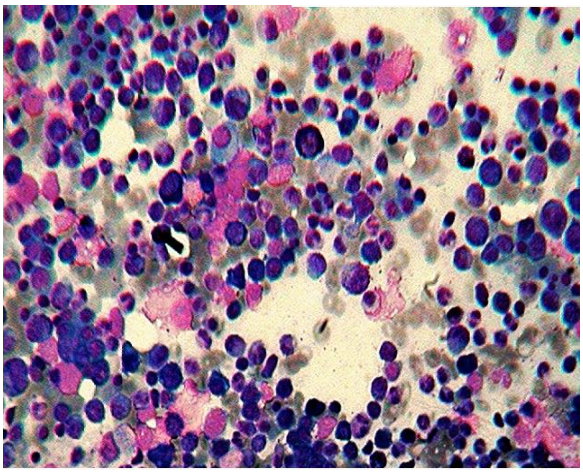

Figure 1: BMA Megaloblasts with sieve like chromatin and blue cytoplasm (10x40X- Leishman)

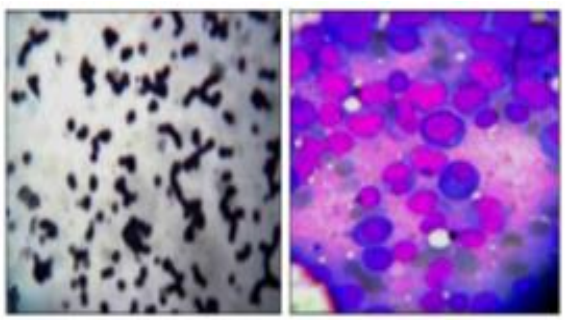

Figure 2: Peripheral smear with myeloblasts & BMA Myeloblasts with reactive plasma cells (1000X Leishman)

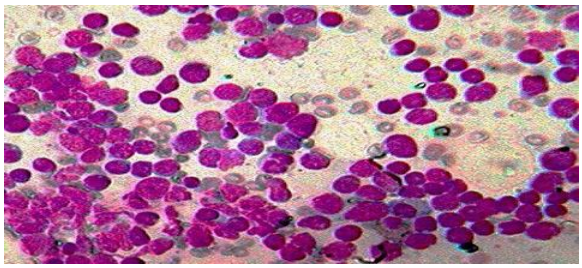

Figure 3: BMA Lymphoblasts in ALL 10x100X Leishman

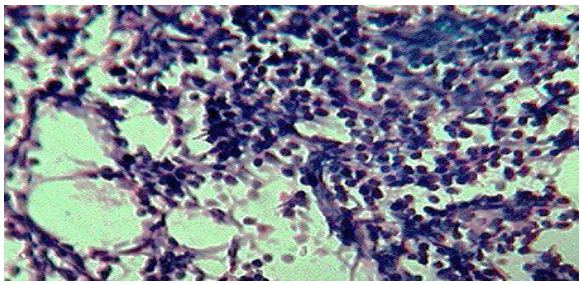

Figure 4: BMB in ALL 10x40X H&E

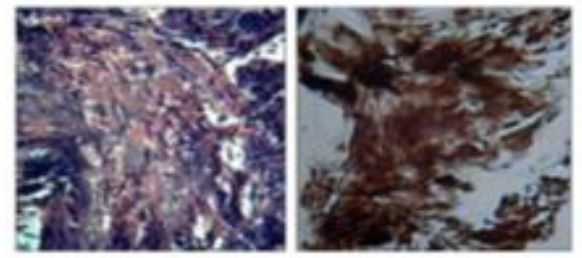

Figure 5: BMA showing increased fibrosis (10x40X- Leishman; Reticulin grade IV (10 x 40X)

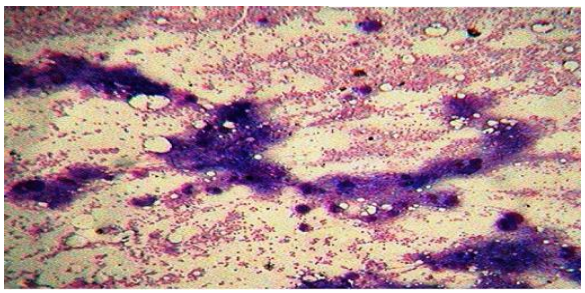

Figure 6: BMA showing increased megakaryocytes (10 x 40 Leishman)

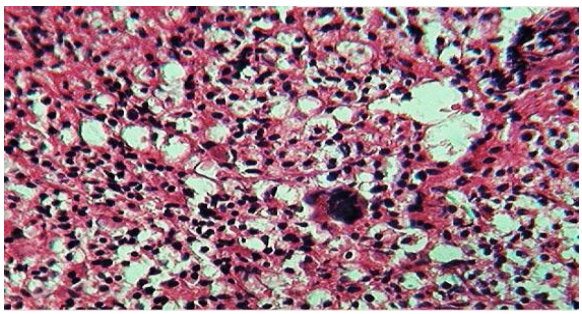

Figure 7: BMB Increase in plasma cells, some with binucleate forms 10x40X H & E

CONCLUSION

Pancytopenia is not a disease entity and should be suspected clinically when a patient presents with un-explained anaemia, prolonged fever and tendency to bleed. In this study, megaloblastic anaemia (56%) was the most common cause of pancytopenia, followed by hypoplastic/aplastic anaemia (13.3%) and leukaemia/ lymphoma (5.34%). Thus this study concludes that detailed primary haematological investigations along with bone marrow aspiration in cytopenic patients is helpful in understanding the disease process; to diagnose, or to rule out the less treatable causes of pancytopenia; and to plan for further investigations and management of cytopenic patients.

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