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Original Research Article

Clinical and haematological evaluation of Pancytopenia : A cross sectional study in a tertiary care centre of Western Odisha

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ABSTRACT

Background: Pancytopenia is the outcome of bone marrow abnormalities and progresses with thrombocytopenia, leukopenia, and anemia. The frequently reported clinical manifestations of pancytopenia include infections, hemorrhage, dyspnea, and pallor. This study aimed to improve the diagnostic assessment of pancytopenia by underlying its potential haematological and clinical parameters.
Materials and Methods: The enrolled patients underwent haematological and clinical assessments for pancytopenia at Veer Surendra Sai Institute of Medical Science and Research, Odisha, India. The pancytopenia assessment parameters included peripheral blood smear, reticulocyte count, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular volume, differential count, total platelet count, total leukocyte count, red blood cell count, and hemoglobin level. Patients also underwent bone marrow aspiration and biopsy for diagnostic investigation.
Results: The bone marrow study and peripheral smears were conducted on eighty-eight patients, and findings revealed the incidence of potential pancytopenia causes including, malaria (1%, n=1), multiple myeloma (1%, n=1), metastatic adenocarcinoma (1%, n=1), hemophagocytic lymphohistiocytosis (1%, n=1), tubercular granuloma (1%, n=1), sickle cell crisis (2%, n=2), hypersplenism (3%, n=3), myelodysplastic syndromes (3%, n=3), acute lymphoblastic leukemia (3%, n=3), acute myeloid leukemia (7%, n=6), aplastic anemia (32%, n=28) and megaloblastic anemia (43% n=38).
Conclusion: Pancytopenia is predominantly caused by megaloblastic anemia and aplastic anemia, respectively. The frequently reported clinical manifestations include bleeding, fever, and weakness. The diagnostic affirmations rely on bone marrow biopsy/aspiration.

1. Introduction

A marked reduction in all the cellular components of the blood below a normal reference range resulting in anemia, leucopenia and thrombocytopenia leads to the haematological condition, which is known as pancytopenia.[1] The diagnostic parameters of pancytopenia include a platelet count of less than 100,000/μl, total leukocyte count of less than 4,000/μl, and hemoglobin level of less than 10gm/dL. The most common clinical manifestations of pancytopenia include hemorrhage, dyspnea, and pallor.[2] The potential causes of pancytopenia include subleukemic leukemia, bone marrow aplasia, and drug-induced bone marrow changes. Pancytopenia may progress with hyperactivity of the reticuloendothelial system, blood cell peripheral sequestration, ineffective hematopoiesis, suppression of bone marrow due to antibody mediation, infiltration of malignant cells, and hematopoiesis failure. Pancytopenia is also a secondary manifestation of a range of potential conditions including

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systemic lupus erythematosus, infections, brucellosis, kala-azar, tuberculosis, folic acid/vitamin B12 deficiency, hypersplenism, bone marrow metastasis, paroxysmal nocturnal hemoglobinuria, myelodysplastic syndromes, hairy cell leukemia, nocturnal hemoglobinuria, and other systemic conditions. Blood smear in myelodysplastic syndrome shows pseudo-Pelger-Huet neutrophils, hypersegmentation of neutrophils and macro-ovalocytes are attributed to the development of megaloblastic anemia.[3] The diagnostic assessments for pancytopenia also rely on the trephine biopsy, bone marrow aspiration, and peripheral smear assessment.[4] Until 1919, pancytopenia had not acquired the status of a diagnosis and appeared to be the synonym for aplastic anemia. The hematological parameters for pancytopenia are not defined in India to date due to a high degree of geographical, environmental, and genetic variations across populations. Therefore, this study aimed to investigate the possible attributes that may standardize the diagnostic assessment of pancytopenia in the Indian subcontinent.

2. Materials and Methods

This cross-sectional prospective study was initiated in Veer Surendra Sai Institute of Medical Sciences and Research Burla, Odisha, India. The study continued for a duration of two consistent years (from 11-2018 to 11-2020). The duly written approval was obtained before study initiation by the Institutional Ethics Committee.

2.1. Inclusion parameters

This study included patients irrespective of their ages; the participants meeting the haematology criteria (platelet count<100000/μl, TLC <4000/μl, Hemoglobin<10gm/dL) were enrolled in the department of pathology.

2.2. Exclusion parameters

Patients with a present or past history of radiation therapy or chemotherapy were not included in this cross-sectional study. In addition, this study also excluded patients with potential comorbidities impacting the differential diagnosis for pancytopenia.

2.3. Diagnostic assessment

All patients were required to provide written informed consent for this study. After detailing all study procedures to the patients, written informed consent was taken from each of the participants. Subsequently, clinical assessments were performed that included both systemic and general body evaluations. The laboratory investigations included bone marrow aspiration, peripheral blood smear, reticulocyte count, and complete blood count. The complete blood counts were undertaken by the haematology analyzer SYSMEX XN-1000. Under aseptic conditions, after prepping and draping, the posterior superior iliac spine was approached for bone marrow aspiration. The clinical specimens were stained by Leishman's stain for the peripheral blood smears and bone marrow smear. Neutral-buffered formalin (10%) was used to preserve the biopsy specimens. The paraffin wax blocks were constructed for specimen analysis after processing. Thin sections were cut and stained with hematoxylin and eosin stain. Other special stains such as periodic acid schiff, myeloperoxidase, and Perl's stains were performed and the flowcytometry in acute leukemia was performed by immunophenotyping. SPSS-2016 (IBM) version was used for data analysis.

3. Results

The bone marrow biopsies and routine hematological assessments were undertaken for eighty-eight patients with pancytopenia. The study population was segregated into 55.68% (n=49/88) females and 44.31% (n=39/88) males. The female subjects superseded the male participants by a ratio of 1.3:1. The enrolled patients were largely from the group of 16-30 years. While the eldest patient was a female (age: 80 years), the youngest one was a male of age 3 years. Table 1 classifies the age and sex variations in the participants. Approximately 80% of the enrolled patients had a weakness as the most prevalent symptom; fever and breathlessness were the second (59%) and third (27%) most common symptoms in the participants. Abdominal distention and bleeding were reported in 5% and 20% of the subjects. Sternal tenderness was observed in 8%, lymphadenopathy in 10%, icterus in 14%, splenomegaly in 25%, hepatomegaly in 26%, and edema in 32% of patients. All patients reportedly had a pale appearance or pallor. Table 2 classifies the disease distribution in the enrolled patients based on aplasia, neoplasia, and miscellaneous causes.

The patients were further classified as mixed/plural dieters (54%) and pure vegetarians (46%). The hemoglobin count varied between 4.8 to 7.4 gm/dL in 43% of patients and within 2.5-9.8 gm/dL in overall patients; those with aplastic anemia had the lowest hemoglobin level. While the total leukocyte count varied within 1800-3000 cells/μL in 52.27% of patients, 600-3900 cells/μL was the variation for the total leukocytes count. Approximately 38% of patients had 12,000- 99,000 cells/μL reported on their platelet count reports; 0.2-4.2% depicted the reticulocyte range. The patients were further classified based on the bone marrow and peripheral smear outcomes. Patients with aplastic anemia were segregated into group 1 (32%, n=28) (Figure 1 a,b) and those with neoplastic conditions in group II (16%, n=14). In addition, patients were categorized based on metastatic adenocarcinoma (n=1), multiple myeloma (n=1), myelodysplastic syndromes (n=3), acute lymphoblastic leukemia (n=3), and acute myeloid

leukemia (n=6) (Figure 2 a,b,c,d). Patients with tubercular granuloma (1%, n=1), sickle cell crisis (2%, n=2), hemophagocytic lymphohistiocytosis (1%, n=1), malaria (1%, n=1), hypersplenism (3%, n=3), and megaloblastic anemia (43%, n=38) were categorized into group III (Figure 3a,b,c). Table 3 provides the clinical presentation of the study participants.

Table 4 categorizes patients based on their bone marrow/peripheral blood analysis. It depicts the incidence of sickled red blood cells (n=2), malaria parasites (n=1), immature white blood cells (n=9), hyper-segmented neutrophils (n=27), andanisopoikilocytosis/macrocytosis (40%). The bone marrow biopsy-based classification included patients with the neoplastic disorder (16%), aplastic anemia (32%), and megaloblastic anemia (43%). Importantly, patients with dry tap and marrow dilution also underwent bone marrow biopsy, leading to confirmation of tuberculosis (n=1) and myelodysplastic syndromes (n=1).

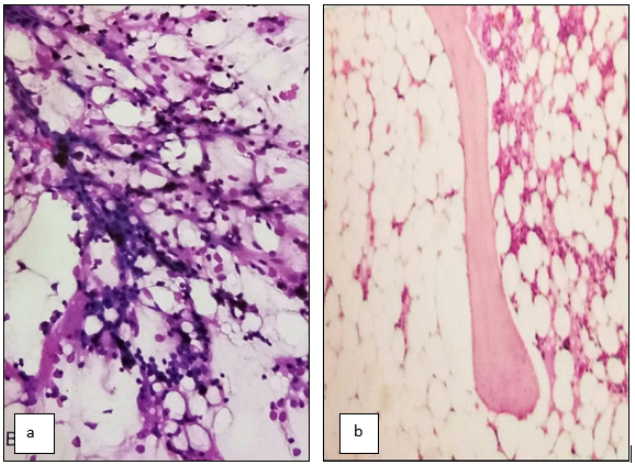

Figure 1: a: BMA showing marrow fragments with marked increase of fat (Leishman x1000); **b:** BMB showing Hypoplastic marrow with increase fat (H & E, X400)

4. Discussion

Of our studied patients (n=88), most belonged to the tribal/rural regions. The patient parameters, including bone marrow aspiration smears, peripheral blood picture, presenting indications, sex-based incidence, and age were studied and the respective findings were compared with the published results. Thirty-eight patients underwent bone marrow biopsy, while 87 agreed to bone marrow aspiration. One patient was excluded from the bone marrow aspiration due to a confirmed diagnosis of malaria based on a Plasmodium falciparum-positive peripheral smear result. Antimalarial treatment and follow-ups were performed for this patient until recovery. The mean age of the enrolled patients was 37 years (range: 3-80 years). The prevalence of the 31-45 years age group (25%) and 16-30 years age group

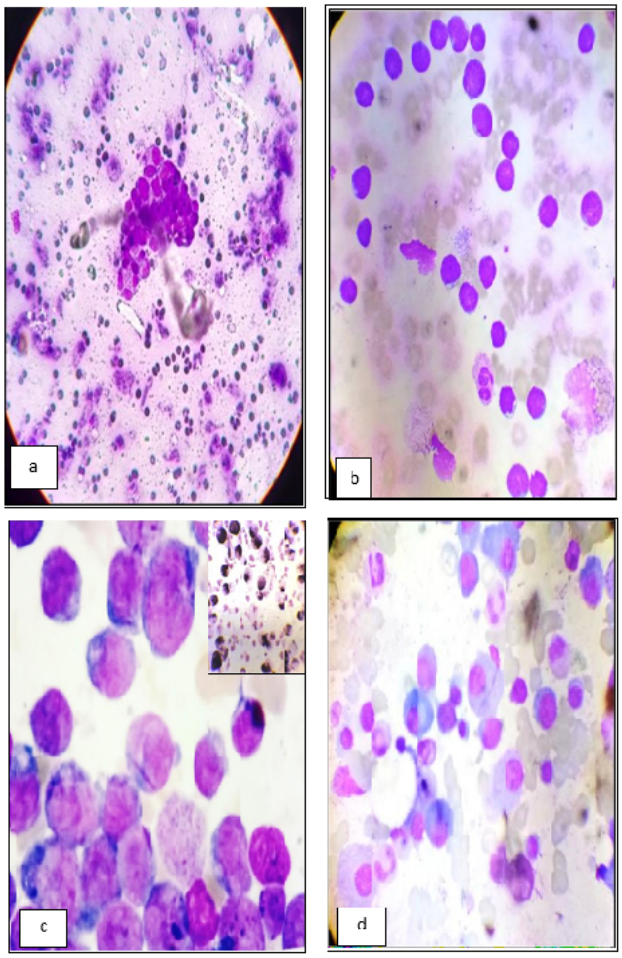

Figure 2: a: BMA showing maliganant epithelial cells in discohesive cluster (Leishman, X1000); **b:** BMA showing Lymphoblasts in ALL (Leishman, 1000); **c:** Leukemic promyelocytes with multiple auer rods (inset showing MPO Positive cells) (Leishman, X1000); **d:** BMA showing atypical plasma cells in multiple myeloma (Leishman, X1000)

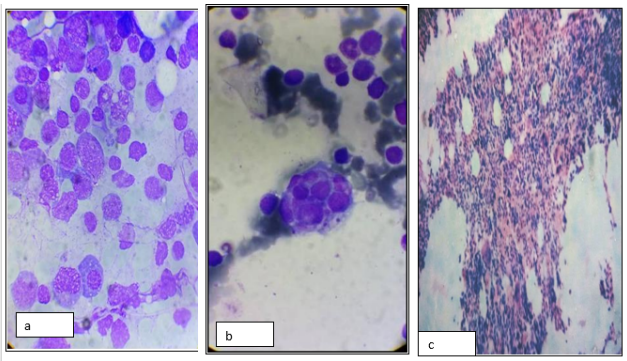

Figure 3: a: BMA aspiration showing Megaloblasts with sieve-like open chromatin (Leishman, X1000); **b:** BMA showing macrophages engulfing WBC, platelet and erythroid precursors (Leishman, X1000); **c:** BMB showing tubercular granuloma with giant cells (H & E, X400)

Table 1: Age and sex distribution of patients

Age (yrs)	Male	Female	Total
1-15	4(4.54%)	8(9.09%)	12(13.63%)
16-30	11(12.5%)	13(14.77%)	24(27.27%)
31-45	10(11.36%)	12(13.63%	22(25%)
46-60	7(7.95%)	9(10.22%)	16(18.18%)
>60	7(7.95%)	7(7.95%)	14((15.90%)
Total	39(44.31%)	49(55.68%)	88(100%)

Table 2: Distribution of disease in three groups (N=88)

Group	Disease	Number (%)
Aplastic	Aplastic anemia (primary)	28(31.81%)
	Total	28(31.88%)
Neoplastic	AML	6(6.81%)
	ALL	3(3.40%)
	Multiple myeloma	1(1.13%)
	MDS	3(3.40%)
	Metastatic adenocarcinoma	1(1.13%)
	Total	14(15.90%)
Miscellaneous causes	Megaloblastic anemia	38(43.18%)
	Hypersplenism	3(3.40%)
	Malaria	1(1.13%)
	HLH	1(1.13%)
	Sickle cell crisis	2(2.27%)
	Tubercular granuloma	1(1.13%)
	Total	46(52.27%)
Grand total		88(100%)

Table 3: Clinical presentation

Clinical manifestation	No(%)
Weakness	72(81.81%)
fever	52(59.09%)
breathlessness	24(27.27%)
Bleeding manifestation	18(20.45%)
Abdominal distension	4(4.54%)
palor	88(100%)
icterus	12(13.63%)
edema	28(31.81%)
Sternal tenderness	7(7.95%)
lymphadenopathy	9(10.22%)
hepatomegaly	23(26.13%)
splenomegaly	22(25%)

(27%) aligned with the outcomes of Graham et al.[5] and Azaad et al.[6] The pancytopenia prevalence in terms of male-female ratio (1:1.25), the incidence in males (44%), and incidence in females (56%) matched the findings of Pathak et al. (1:1.04)[7] and Aziz et al. (1:1.43);[8] however, these outcomes were contradicted by the results of Gayathri et al[9] indicating a high incidence of males. The incidences of symptoms revealed by this cross-sectional study [i.e., pallor (100%), breathlessness (27%), fever (59%), and generalized weakness (82%)] concorded with the outcomes of Raina et al.[10] and Gayathri et al.[9] Similarly the findings of Gayathri et al.[9] matched our results concerning variations in platelet count (12000-99000/μL), total leukocyte count (600-3800/μL), and hemoglobin levels (2.5-9.8gm%).

Thirty-eight patients underwent bone marrow biopsy and 87 were administered with bone marrow aspiration. The findings from various studies in the literature support the incidences of megaloblastic erythropoiesis and hypercellular marrow, reported by our study.[6,8–11] Our cross-sectional study revealed the incidence rates of various pancytopenia-related hematological conditions including malaria (1%), tubercular granuloma (1%), hemophagocytic lymphohistiocytosis (1%), metastatic adenocarcinoma (1%), multiple myeloma (1%), sickle cell crisis (2%),

Table 4: Peripheral blood and bone marrow picture

Disease	RBC	WBC	platelet	BM study
Aplastic anemia(28)	Anisopoikilocytosis4	Lymphocytosis28	Reduced	hypocellular
AML(6)	Anisopoikilocytosis4 Normoblast 1	Immature WBC 4	Reduced	Myeloblst& atypical promyelocytes>20%
ALL (3)	Anisopoikilocytosis2	Immature WBC 2	Reduced	Lymphoblast>20%
MDS (3)	Anisopoikilocytosis2 Normoblast 2	Immature WBC 3	Reduced	Ring sideroblast, Blast<20%
MM(1)	Anisopoikilocytosis1	Relative lymphocytosis	Reduced	Plasmacytosis
Metastatic (1)	Anisopoikilocytosis1	Depressed	Reduced	Malignant epithelial cell
Megaloblastic anaemia (38)	Anisopoikilocytosis with macro.-35, micro-macro-3	Hypersegmented neutrophil-27	reduced	Megaloblastoid change,hypercellular
Hyperspenism (3)	Anisopoikilocytosis3	Depressed	reduced	Normal
Malaria (1)	Anisopoikilocytosis1	Depressed	reduced	Not done
HLH (1)	Normocyti normo chromic1	Depressed	reduced	Hemophagocytic lymphohistiocytosis
Sickle cell Risis (2)	Anisopoikilocytosis2	Depressed	reduced	Hypocellular
TB (1)	Normocytic Normochromic-1	Depressed	reduced	Granuloma

hypersplenism (3%), myelodysplastic syndromes (3%), acute lymphoblastic leukemia (3%), and acute myeloid leukemia (7%). Our findings provided a clinical correlation between megaloblastic anemia and pancytopenia in 43% of patients. In addition, three patients were diagnosed with dimorphic anemia based on their iron deficiency. These outcomes concord with the results from Rathod et al.[11] and Sangwan et al.[12] indicating 27% and 72% incidences of pancytopenia, respectively. The results from our study and those listed in the literature signify the attribution of megaloblastic anemia to the pancytopenia events reported in India. Pancytopenia, however, can be managed and corrected with rapid diagnostic assessment and evidence-based treatments.

The results from this cross-sectional study differed from the findings of Barik et al.[13] indicating 74% of vegetarian patients compared with 53% with mixed diet and 47.3% of vegetarians with megaloblastic anemia. Our study reported 32 years (range: 3-76 years) as the mean age of patients with megaloblastic anemia. It further indicated <2% reticulocyte count (n=38/38) and mean corpuscular volume reduction (n=3/38) in megaloblastic anemia. While 11/38 patients had normal mean corpuscular volume, 24/38 had mean corpuscular volume elevations. In addition, hyper-segmented neutrophils were found in 27% of patients and macrocytic red blood cells with anisopoikilocytosis were observed in megaloblastic anemia (n=35/38). These observations correlate with the abnormal blood cell maturation mechanisms and are supported by Sharma et al.[14] Patients with megaloblastic anemia had expanded megaloblastic erythroblasts, erythroid precursors' abnormal maturation/proliferation, hyper-segmented neutrophils, giant meta-myelocytes, and erythroid hyperplasia.

The findings of Kumar et al.[15] based on a 30% incidence of aplastic anemia supported our results that revealed a 32% incidence in patients within the age range of 10-80 years. Contrarily, 14% aplastic anemia occurrence rates were recorded by Khunger et al.18 In addition, our findings indicating the occurrence of relative lymphocytosis in all patients matched the results of Khunger et al.[16] that revealed 86% incidence and the overall findings correlated with pancytopenia. Acute myeloid leukemia was reported as the third most common cause of pancytopenia by Graham et al.[5] This result supported our findings indicating a 7% (n=6) incidence of acute promyelocytic leukemia in patients with pancytopenia. The results of Vijay et al.[17] indicated acute lymphoblastic leukemia as the second most common cause of pancytopenia, and this finding aligned with our results indicating the occurrence of acute lymphoblastic leukemia in patients of age 57 years (n=1), 45 years (n=1), and 16 years (n=1) respectively. These patients also developed hepatomegaly (n=1), splenomegaly (n=2), and lymphadenopathy (n=2).

This cross-sectional study revealed a 3.4% incidence of myelodysplastic syndromes (n=3), including patients with myeloid maturation arrest, myelodysplasia, bone marrow blasts (14%), peripheral blood blasts (6%), and refractory anemia with excess blasts (n=2/3). In addition, refractory anemia with ring sideroblasts (>15%) was assessed in the Perls strain. These outcomes concord with the results of Raina et al.,[10] Devi et al.,[18] and Azaad et al.,[6] which indicate myelodysplasia as the third most recognizable pancytopenia attributor. Our study revealed an 1% incidence of pancytopenia in patients with multiple myeloma. Our

assessment of a 60 years old female with multiple myeloma indicated Bence-Jones protein in the urine, M-band in serum electrophoresis, and punched-out lesions in the x-ray of the skull. In addition, 49% of plasma cells were observed in the bone marrow aspirate. Similar findings regarding the occurrence of pancytopenia in multiple myeloma were revealed by Gayathri et al.,[9] Barik et al.,[13] and Graham et al.[5] The results from our study further indicated multiple myeloma manifestations, including joint pain, bony tenderness, pallor, and weakness. The bone marrow assessment in one patient revealed pleomorphic epithelial cell clusters without cohesion and acinar pattern with tumor giant cells. The iliac bone and sacrum of the patient reflected metastatic deposits. The findings of Sharma et al.[14] were similar to our case.

The outcomes from our study revealed hypersplenism in three patients with normal bone marrow (ages: 60, 24, and 22 years). The findings of Alamin et al.,[19] Manzoor et al.,[20] and Sangwan et al.[12] reconfirm hypersplenism as the frequently reported etiopathology in pancytopenia cases. Aplastic crises and sickle cell disease were reported in two patients (ages: 18 and 9 years) in this cross-sectional study. Hankins et al.[21] also reported similar outcomes and they probably correlate with geographical diversity. A patient developed miliary tuberculosis correlating with pancytopenia in our study. This result concorded with the outcomes of Khunger et al.,[16] Tilak et al.,[2] and Barik et al.[13] that revealed pancytopenia causation of miliary tuberculosis. Our study reported a case of hemophagocytic lymphohistiocytosis with infection in a male patient (age: 51 years). The symptoms included hypertriglyceridemia, splenomegaly, pallor, and fever. The bone marrow assessment of the patient revealed hemophagocytosis and megaloblastoid changes. Similar findings substantiating the pancytopenia attribution of hemophagocytic lymphohistiocytosis were indicated by Azaad et al.[6] The findings of our study further demonstrated pancytopenia in a patient with malaria and associated hypersplenism. These results were supported by the outcomes of Basavaiah et al.,[22] Javalgi et al.,[23] and Barik et al.,[13] which revealed malaria as a frequently reported condition in patients with pancytopenia.

5. Conclusion

The findings from this cross-sectional study indicated megaloblastic anemia as the most common cause of pancytopenia. This outcome warrants a comprehensive assessment of megaloblastic anemia cases to rule out the development of pancytopenia. The diagnostic workup can include a bone marrow analysis, a haematology assessment, and a complete clinical history. The other potential pancytopenia-causing factors include metastatic adenocarcinomas, multiple myeloma, acute lymphoblastic leukemia, acute promyelocytic leukemia, and other neoplastic conditions. However, the etiological factors of pancytopenia based on the Indian geography include sickle cell disease with aplastic crisis, malaria, and other infectious conditions.

6. Conflict of Interest

The authors declare that they have no conflict of interest.

7. Source of Funding

None

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