Original Research Paper

Volume-9 | Issue-3 | March-2019 | PRINT ISSN - 2249-555X

Human Genetics

ETIOLOGICAL PROFILE OF PANCYTOPENIA IN SOUTHERN URBAN REFERRAL CENTRE.

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ABSTRACT Pancytopenia is defined as reduction of all the three formed elements of blood below the normal reference range. Various studies are available in literature, reported aplastic anemia as the commonest cause of pancytopenia. Studies done in developing nations like India had revealed megaloblastic anemia as the commonest cause of pancytopenia.

Aim and Objectives : The aim of our study is to find out the incidence, various causes of pancytopenia and to do clinicopathological correlations.

Materials and Methods : Patient selection was based on clinical features and supported by laboratory evidence. The relevant clinico-hematological parameters, physical examination, Blood film, bone marrow and trephine biopsy were examined by a panel of pathologists and recorded.

Results : The mean age of incidence was 39.5 years, comparatively high in women. Megaloblastic anemia was the most common cause of pancytopenia in our study group.

Conclusion : Detailed primary haematological investigations along with bone marrow examination in cytopenic patients is helpful, for better understanding of the disease process and very much useful in planning further investigations and management of cytopenic patients.

KEYWORDS : Pancytopenia, Reticulin Megaloblastic Anaemia

INTRODUCTION

Cytopenia is a disorder with reduced production of one or more blood cell types. Pancytopenia is a disorder, in which all three major formed elements of blood (red blood cells, white blood cells and platelets) are decreased than normal[1]. The presenting symptoms are usually attributable to anemia, thrombocytopenia and rarely leucopenia with striking feature of many saerious and life threatening illnesses[2].

Manifestations of peripheral pancytopenia are due to a wide variety of disorders which primarily or secondarily affects the bone marrow, which ranges from simple drug induced bone marrow hypoplasia to fatal bone marrow aplasia and leukemias[3, 4, 5]. Varying factors like, geographic distribution, genetic factors, nutritional status and the prevalence of infective disorder may cause variation in the incidence of pancytopenia [6]. The severity of pancytopenia and the underlying pathology determine the management and prognosis of these patients[4].

The complete hematological work up including a good peripheral blood smear examination, bone marrow aspiration and trephine biopsy with clinical correlation is of utmost importance to evaluate the cause of pancytopenia and to plan further investigations and treatment[1]. We present our experience with 50 cases of pancytopenia, over a period of one year.

Further, this study was carried out with an aim to obtain further information to evaluate the various causes of pancytopenia and to correlate the peripheral blood findings with bone marrow aspirate and trephine biopsy along with special stain for elastic fibers.

METERIALS AND METHODS

The present study was carried in the Heamatology Unit, Department of Pathology, at a tertiary care center, urban south India over a period of one year. Patient selection was based on clinical features and supported by laboratory evidence. The relevant clinico-hematological parameters were recorded. Details of physical examination were obtained from medical records of patient. The study was conducted in a routine hematology laboratory at the same hospital. Three ml of blood sample was collected aseptically from each subject into tri-potassium ethylenediamine tetra-acetic acid (K3EDTA) anticoagulant bottle. This was thoroughly mixed for complete blood count (CBC) analysis. Blood sample was divided into 2 parts as follows: 2ml for manual method and 1 ml for automated method using hematology auto analyzer Sysmex KX-21. The laboratory tests performed were:

1. CBC using Sysmex KX-21 automated analyser and the analysis was done following the manufacturer's operational guidelines.
2. In cases of very low counts and abnormal cells, a manual review of the results was performed using the improved Neubauer counting chamber with appropriate diluting fluids.
3. A blood film was stained by the Leishman stain and evaluated for red cell morphology, platelet count and white cell morphology.
4. Reticulocyte count using 1% Brilliant Cresyl Blue for supravital staining.
5. Bone marrow aspiration smear was stained by Leishman stain for all the cases and examined in detail.
6. Bone marrow aspiration and trephine biopsy was subsequently carried out after obtaining written consent from the patient or the guardian. Bone marrow aspiration was performed by using Salah needle either from posterior iliac crest or stenum and biopsy with Jamshidi needle from posterior iliac crest, under aseptic precaution. The bone marrow aspiration smears were stained with Leishman's stain and the trephine biopsy core was decalcified, routinely processed, embedded in paraffin and sections stained with Hematoxylin and Eosin.
7. Added to it reticulin stain was done and the extent of fibrosis was graded.

The causes of pancytopenia are analyzed based on clinico-hematological parameters, including peripheral blood film, bone marrow aspiration, bone marrow biopsy (in cases of dry tap), clinical features, age, gender and compared with the various studies published in literature.

Inclusion criteria

Presence of three of the following

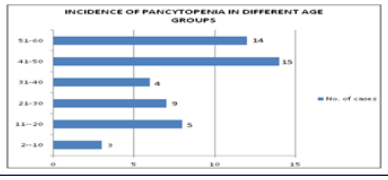
- Haemoglobin < 9 g/dl
- TLC <4000/cumm and
- Platelet count <1,00,000 / cumm
- Patients whose bone marrow had diagnostic aspirate

Exclusion criteria:

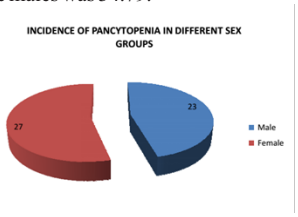
- Patients on myelotoxic chemotherapy.
- Age <2years and >60 years.
- Refractory to treatments

RESULTS

The study group includes age group of 2-60 years with a mean age of 39.5 years. It is observed that the incidence of pancytopenia was high (30%) in the age group of 41-50, and lowest in the age group 2-10 (6%) (Fig1).



Moreover that the incidence of pancytopenia was comparatively high in women54% than men 46% (Fig 2). The average age of the women is 35 .5 years, and the men was 39yrs with the standard deviation17.44 and 13.56 respectively. The coefficient of variation for the females was 49.11 while for the males was 34.79.



For this study of pancytopenia, various physical complaints and findings were taken into account. All the patients were affected by the generalized weakness. Nearly 46% of them had dyspnoea and Fever, nearly 86% of the cases had Pallor; 40% cases had hepatomegaly, 36% had splenomegaly, 10% of the cases had weight loss, 8% had jaundice and 2% had bony tenderness, lymphadenopathy and bleeding manifestations.

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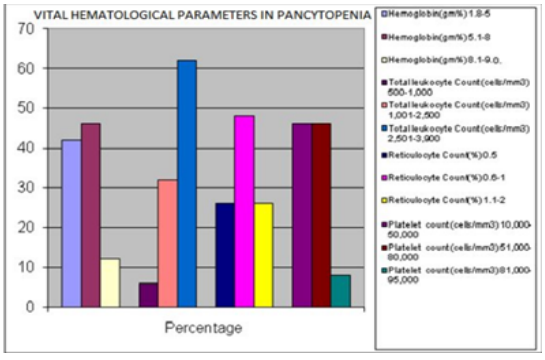
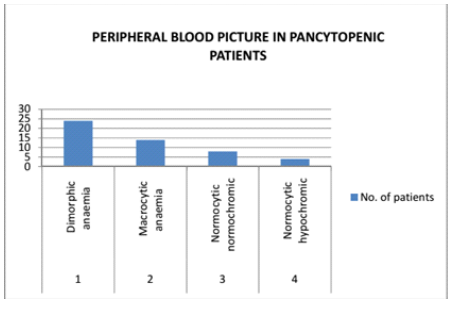
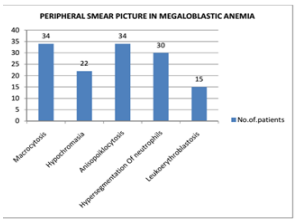
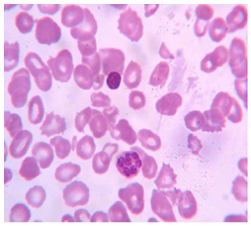


In peripheral blood picture, the incidence of Dimorphic anaemia was higher (46%) than Macrocytic anaemia (24%), Normocytic normochromic anemia (20%) and normocytic hypochromic anemia (8%) (Fig 4). While all the 34 patients had macrocytosis and anisopoiklocytosis, whereas 88% had hypersegmentation of neutrophils. Two-third of the patients had hypochromasia (Fig 5, 6)

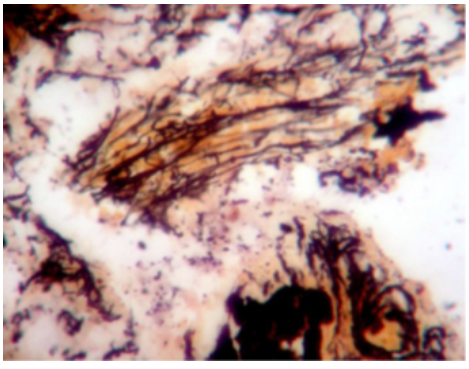






While assessing the bone marrow, hypercellularity is the commonest finding, accounting for about 78%, followed by hypocellularity(20%) and few with normocellularity (2%) . Megaloblastic anemia shows the highest incidence of 34% in the age group of 41-50years, equal incidence 23.5% in age groups of 21-30years and 51-60years followed low incidence in other age group patients. When considering the sex predilection, it is slightly more in men in a ratio of 1:1.1.While all the 34 patients had macrocytosis and anisopoiklocytosis, 30%shows hypersegmentation of neutrophils. Two-third of the patients had hypochromasia .Assessing vital parameters in megaloblastic anemia shows, Hemoglobin percentage varied from 1.8 % - 9.0g %. Half of the patients had Hemoglobin percentage between 5.1 – 8g %. While 12 patients had Hemoglobin percentage≤ 5g%.Total leukocyte count ranges from 500 – 3,900 cells/mm^3, 24 patients had white cell count in the range of 2,501 – 3,900 cells/mm^3. While seven patients had leukocyte count ≤ 2,500 cells/mm^3. Only 3 patients had white cell count in the range of ≤1,000. In Reticulocyte count 65% of the patient shows the count between 0.6 – 1 %. Among the remaining 12 patients, 6 of them had the Reticulocyte count< 0.5. Platelet count varied from 10,000 – 95,000 cells/mm^3. Half of the patients show their Platelet count ≤50,000 cells/mm^3, an equal number of the patients show the count≥50,000 cells/mm^3.

Bone marrow hypoplasia showed its highest incidence (58%) in the age group 41-60 followed by the age groups 21-40 (28%), and 2-20 (14 %). The incidence of Bone marrow hypoplasia is comparatively higher in women than men, approximate male to female ratio being 2:3. Among 7 cases 44% of the patient had dimorphic anemia, normocytic hypochromic anemia in another 44% of the patients and macrocytic anemia in 12% of cases. Grade of myelofibrosis by reticulin stain reveals, 44 % of the patients had Grade 2 (Fig.7), 28 % had Grade 1, 20 % had Grade 0, 8 % had Grade 3. None of the patients had Grade 4 myelofibrosis.



Statistics

On the basis of the statistical significance values provided by the Chi-Square Tables, we arrive at the following conclusions: 1. The count of MA, AA, MF, ALL, AML, MDS in the final diagnosis are statistically related to the count of them in the Peripheral Smear.2. The count of MA, AA, MF, ALL, AML, MDS in the final diagnosis are statistically related to the count of them in the Bone Marrow Aspiration (BMA).3. The count of MA, AA, MF, ALL, AML, MDS in the final diagnosis are statistically related to the count of them in the Bone Marrow Trephine (BMT). The above statistical findings establish the strong correlation between the peripheral blood findings with bone marrow aspirate.

DISCUSSION

Pancytopenia is the simultaneous presence of anaemia, leucopenia and thrombocytopenia and therefore it exists when there is a pathology which affects hematopoietic stem cells before they get differentiated [7]. Pancytopenia can be due to decrease in hemtaopoietic cell production in the bone marrow e.g. By infections, toxins, malignant cell infiltration or suppression or can have normocellular marrow or even hypercellular marrow, without any abnormal cells, e.g. Ineffective hematopoiesis and dysplasia, maturation arrest of all cell lines and peripheral sequestration of blood cells[8]. In other situations, however, the marrow may be normally cellular or even hypercellular and no abnormal cells may be present. The mechanisms leading to pancytopenia in these conditions may be due to ineffective haemopoiesis with cell death in the marrow, formation of defective cells that are rapidly removed from the circulation, sequestration or destruction of cells by the action of antibodies, and trapping of normal cells in a hypertrophied and overactive reticuloendothelial system[9].

Age range was similar in almost all the studies and female predominance was noted in our study like those of others, except in the

study by Kumar R et al, where male predominance was noted[3].

Sl.No.	Authors	No.of.cases	Age range	M : F
1	Khunger JM et al5	200	2-70	1.2:1
2	Kumar R et al3	166	12-73	2.1:1
3	Khodke K et al6	50	3-69	1.3:1
4	Tilak V et al4	77	5-70	1.14:1
5	Gayathri et al	104	2-80	1.2:1
6	Present study	50	2-60	1:1.2

The onset of this disease is insidious, manifestations depending on the severity of anaemia, leucopenia, and thrombocytopenia[10]. Initial presenting symptoms include mild progressive weakness and fatigue attributable to anaemia. Also patients are predisposed to various infections because of neutropenia. Haemorrhage from skin, nose, and gums is due to thrombocytopenia. Physical examination reveals fever, pallor, petechiae and ecchymotic patches over the skin, mucous membranes and conjunctiva[10]. Presence of splenomegaly and lymphadenopathy calls for attention to the possibility of leukemia, lymphoma, myelofibrosis and storage diseases. On the other hand, lack of these signs as well as lack of evidence of vitamin B12 or folate deficiency should suggest multiple myeloma or aplastic anaemia. Finally, rare presentations include diarrhea, jaundice and weight loss[1]. When the physical findings noted in our case series was compared to other studies and tabulated.

Diseases	Physical Findings								
	Spleenomegaly			Hepatomegaly			Lymphadenopathy		
	A	B	C	A	B	C	A	B	C
Megaloblastic Anemia	40	22	11	42	23	13	1	3	-
Aplastic anemia	-	4	-	1	3	-	-	1	-
Myelofibrosis	-	2	5	-	1	5	-	-	-
Leukemia	8	1	1	10	-	1	6	-	1

A – Khunger JM et al's study [5] B – Tilak V et al's study [4], C – Present Study(n=50)

The commonest cause of pancytopenia, reported from various studies throughout the world has been aplastic anaemia[4]. Idiopathic aplastic anaemia accounts for more than 70% cases of pediatric anaemia and it is imperative to search for an etiology in all cases of aplastic anaemia before labellind it as idiopathic[11].A child with hereditary spherocytosis who acquired human parvovirus B19 infection developed transient pancytopenia[12]. Seronegative hepatitis precedes the diagnosis of aplastic anemia in 3 to 5% of cases and is recognized as hepatitis associated aplastic anemia[13].

This is in sharp contrast with the results of various Indian studies including our study where the commonest cause of pancytopenia is megaloblastic anaemia[1,3,5,6] This seems to reflect the higher prevalence of nutritional anemias indeveloping countries like India. Though bone marrow aspiration study is uncommon in a suspected megaloblastic anaemia, if the diagnosis does not appears straight forward or if the patient requires urgent treatment and the haematological assays are not available, bone marrow aspiration is indicated (fig 3). 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[4].

The total white cell count in acute leukemia ranges between subnormal to markedly elevated values. In about 25% of patients the total white cell count at the onset is reduced ranging between 1-4 x 109/L[14]. Blast cells may be present in very small numbers in peripheral blood. Buffy coat smear will help in detecting blasts under these circumstances[14]. Bone marrow examination provides the diagnosis[14].

The myelodysplastic syndromes are a heterogeneous group of clonal stem cell disorders characterized by cytopenias due to impaired blood cell production, a hypercellular and dysplastic bone marrow, and an increased risk of leukemic transformation[15]. A Leukemia Research Fund (LRF) -UK based study puts the annual incidence of MDS as 3.6 per 100000[16]. One group has suggested a prevalence of 1 in 500 in those who presented with pancytopenia[17]. In a clinical study of primary myelodysplastic syndrome (MDS) in 33 children, it was noted that pancytopenia was the predominant presenting feature[18]. In a study of the haematological spectrum of myelodysplastic syndrome in 31 cases, pancytopenia constituted 16.1%[19].

Primary myelofibrosis is a clonal myeloproliferative neoplasm of the pluripotent haematopoietic stem cell in which the proliferation of multiple cell lineages is accompanied by progressive bone marrow fibrosis characterized by splenomegaly, leucoerythroblastic picture, bone marrow fibrosis and extramedullary haematopoiesis[20]. Diagnostic criteria of myelofibrosis depends on the following factors ; Reticulin grade ≥3(on a 0-4scale), presence or absence of mutation in JAK2,palpable spleen, unexplained anemia,tear drop cells and or leukoerythroblastic blood film,histological evidence of extramedullary hematopoeisis[20]

Marrow destruction by tumor plasma cells in multiple myeloma results in anaemia, leucopenia and Thrombocytopenia[21]. Metastatic carcinoma related pancytopenia can be a direct result of tumor invasion of the bone marrow, or indirect result of tumor therapy or systemic symptomatology, or an incidental finding resulting from other pathology in the patient[22]. Pancytopenia is a rare haematological finding in disseminated tuberculosis and its degree is influenced more by the duration of infection than its severity[23]. Secondary hypersplenism due to haematological malignancies, storage disease, infections like malaria, typhoid, brucellosis, leishmaniasis, collagen vascular diseases, congestive splenomegaly and splenic tumors is also an important etiology for pancytopenia[24]. When the causes of pancytopenia was compared with those seen in the literature the following was observed

Study group	Country	Commonest cause
IAASG	Israel & Europe	Hypoplastic anemia
Keisu&Ost	Israel	Post radiation
Hossain et al	Bangladesh	Hypoplastic anemia
Verma &Dash	India	Hypoplastic anemia
Tilak & Jain 4	India	Megaloblastic anemia
Kumar et al3	India	Hypoplastic anemia
Khodke et al	India	Megaloblastic anemia
Bajracharya et al	Nepal	Hypoplastic anemia
Present study	India	Megaloblastic anemia

CONCLUSION

When the causes of Pancytopenia was evaluated, high prevalence of Megaloblastic anaemia was noted in our study which indicates that the incidence of nutritional anaemia is high in our region. The other common causes were hypoplastic/aplastic marrow. However, uncommon and rare causes such as multiple myeloma, storage disease should be kept in mind while planning investigation for complete work up of cytopenic patients. Tuberculosis being highly prevalent and endemic in India, it is essential to be aware of its manifestation as pancytopenia. Present study concludes that detailed haematological investigations along with bone marrow examination in cytopenic patients are helpful.

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