

Original Research Article

A study on incidence of vitamin B12 deficiency in patients with pancytopenia

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ABSTRACT

Background: Pancytopenia is a serious and life threatening illness presented with multiple etiologies. The current study was done with the objective to evaluate the clinic-pathological factors responsible for incidence of pancytopenia in patients with vitamin B12 deficiency and their response to the therapy with vitamin B12.

Methods: It was an observational study carried out at Department of Medicine, Mahatma Gandhi Memorial Government Hospital, Trichy, Tamilnadu during the period between December 2016 to December 2017. A total 50 patients with pancytopenia were clinically evaluated along with hematological parameters and bone marrow aspiration.

Results: A total of 50 patients were included in the study. The mean age of the patients was 32.84 years with a male to female ratio of 1.5:1. All patients had history of fatigability. Commonest physical presentation was pallor (100%). Megaloblastic anaemia was the predominant blood picture in 58% patients. The common bone marrow finding was hyper cellular marrow with megaloblastic picture. Management with cyanocobalamin preparations and folate supplementations, significantly improved the reticulocyte count percentage in patients with pancytopenia ($p=0.01$)

Conclusion: The study concluded that the most common cause of pancytopenia was megaloblastic anaemia. Detailed haematological investigations along with bone marrow aspiration in patients with cytopenia provided a clear understanding of disease process to identify the etiologies of pancytopenia.

Keywords: Pancytopenia, Reticulocyte percentage, Vitamin B12 deficiency

INTRODUCTION

Pancytopenia is one of the most commonly encountered hematological entities in the clinical practice. It is characterized by reduction in the number of all blood cell lines namely red blood cells, white blood cells, platelets.¹ For all the practical purposes, it should have hemoglobin <10 g%, absolute neutrophil count <1500 mm³, platelets <100,000/mm³. It is labeled as severe pancytopenia when hemoglobin <7 g%, absolute neutrophil count <500 mm³, platelet <20,000 cumm.²

The incidence of pancytopenia may be due to several congenital and acquired causes. It may be due to either

decreased bone marrow production or increased peripheral destruction/sequestration. The most common causes of acquired decreased bone marrow production are megaloblastic anemia, cytotoxic chemotherapy, radiotherapy, bone marrow infiltration, myelofibrosis, myelodysplasia, idiopathic aplastic anemia. Other common acquired causes are systemic lupus erythematosus, HIV, mycobacterial infection, acute viral infections like Epstein Barr virus etc.³

Uncommon causes of decreased bone marrow production are paroxysmal nocturnal hemoglobinuria, anorexia nervosa, transfusion associated graft versus host disease, heavy metal poisoning, infections like *Parvovirus B19*,

Human herpes virus6, CMV in transplant recipients and Legionnaires' disease.^{4,5}

Common causes of pancytopenia due to increased destruction are due to liver disease, portal hypertension. Other causes are drug induced pancytopenia, hypersplenism due to myeloid, lymphoproliferative disease, Evans syndrome, infections like brucellosis, visceral leishmaniasis and hemophagocytic syndrome.¹

Congenital causes of pancytopenia associated with complex multisystem disorders such as Fanconi anemia, dyskeratosis congenita, congenital amegakaryocytic thrombocytopenia, Shwachman syndrome and Gauchers disease. The incidence and severity of the disease condition depends on underlying gene mutation and environmental influences such as lifestyle, exposure to toxins and infections.⁶

Only few studies have been done in India regarding incidence and causes of pancytopenia. Among several causes of pancytopenia, megaloblastic anemia is one of the most commonly cited causes for pancytopenia.^{7,8} Megaloblastic anemia is one of the easily treatable causes for pancytopenia. Its early recognition and treatment helps in reducing morbidity and mortality.

The study was undertaken with the aim to assess the clinic-pathological factors responsible for incidence of pancytopenia in patients with vitamin B12 deficiency and their response to the therapy with vitamin B12.

METHODS

This was a descriptive observational study carried out at Department of Medicine, Mahatma Gandhi Memorial Government Hospital, Trichy, Tamil Nadu during the period between December 2016 to December 2017. We included 50 patients of both sexes of age 13 years and above with pancytopenia. Patients who were not willing to participate in the study, patients on myelotoxic chemotherapy and radiotherapy and age below 13 years were excluded.

After getting approval from institutional ethics committee, a written informed consent was obtained from all the patients after having fully explained the purpose and protocols of the study. Detailed history was obtained from all the participants using predesigned proforma. Complete physical and detailed clinical examination to detect pallor, jaundice, lymphadenopathy, sternal tenderness, hepatosplenomegaly was done for all the patients. Detailed neuropsychiatric evaluation was done. The blood samples were collected from all the patients and sent for basic routine tests like complete blood count including red cell indices like mean corpuscular volume (MCV). Peripheral smear study was done along with reticulocyte count. Serum samples were sent for vitamin B12 estimation and for biochemical investigations like liver function test, and coagulation profile testing by

PT/INR, activated partial thromboplastin time. All patients' blood samples were subjected to direct Coombs test and HIV testing. Ultrasonography (USG) of the abdomen was done to all the patients. Bone marrow aspiration was done in all the patients using Salah's bone marrow aspiration needle from posterior iliac crest under strict aseptic precautions with local infiltration with xylocaine. From the aspirate, approximately eight to ten smears were made and sent to the pathological study.

Data was entered and analysed with help of statistical software tool SPSS Chicago. Data was presented in number and percentages for categorical variables. Chi square test was used to test the significance. P value less than or equal to 0.05 was considered to be statistically significant.

RESULTS

A total of 50 patients with pancytopenia were included in the study. There were 30 males and 20 females with a mean ratio of 1.5:1 and with a mean age of 32.84 years. Majority (48%) of the patients presenting with pancytopenia were laborer's.

Table 1: Demographic data of the patients (n=50).

Variables	Number of patients	Percentage
Age (in years)		
Below 20	12	24
21 to 30	9	18
31 to 40	12	24
41 to 50	13	26
Above 51	4	8
Gender		
Male	30	60
Female	20	40
Occupation		
Nursing students	13	26
Labourer	24	48
Clerk	2	4
Housewife	9	18
Staff nurses	2	4
Diet history		
Non-vegetarian	24	48
Vegetarian	26	52
History of smoking		
No	27	54
Yes	23	46
History of alcoholism		
No	26	52
Yes	24	48

Among 50 patients with pancytopenia, 52% of the patients were on vegetarian diet, 46% of the patients were smokers.48% were alcoholics.

Table 2: Clinicopathological findings in study participants.

Variables	Number of patients	Percentage
History of fatigability		
Yes	50	100.0
No	0	0
History of bleeding		
No	35	70.0
Yes	15	30.0
Psychiatric manifestations		
No	48	96.0
Yes	2	4.0
Neurological manifestations		
No	48	96.0
Yes	2	4.0
History of drug intake		
No	46	92.0
Yes	4	8.0
Presence of pallor		
Yes	50	100.0
No	0	0
Presence of icterus		
No	44	88.0
Yes	6	12.0
Presence of Knuckle hyperpigmentation		
No	28	56.0
Yes	22	44.0
Presence of hepatomegaly		
No	40	80.0
Yes	10	20.0
Presence of splenomegaly		
No	35	70.0
Yes	15	30.0
Lymphadenopathy		
No	47	94.0
Yes	3	6.0
Sternal tenderness		
No	48	96.0
Yes	2	4.0
MCV (fl/cell)		
Less than 100	17	34.0
More than 100	33	66.0
SGOT (U/l)		
Less than 40	13	26.0
More than 40	37	74.0
SGPT (U/l)		
Less than 40	18	36.0
More than 40	32	64.0
Peripheral smear		
Dimorphic anemia	8	16.0
Megaloblastic anemia	29	58.0
Pancytopenia	13	26.0
Reticulocyte count (%)		
Less than 1	44	88.0
More than 1	6	12.0

Variables	Number of patients	Percentage
Serum vitamin B12 (pg/ml)		
Less than 200	31	62.0
More than 200	19	38.0
USG of abdomen		
Normal	35	70.0
Hepatosplenomegaly	10	20.0
Cirrhosis with splenomegaly	1	2.0
Splenomegaly	4	8.0
Bone marrow aspiration		
Hypercellular marrow with no specific features	15	30.0
Hypercellular marrow with megaloblastic picture	29	58.0
AML	3	6.0
Hypocellular marrow with aplastic anemia features	3	6.0
ICTC		
Non- reactive	45	90.0
Reactive	5	10.0
Direct Coombs test		
Negative	46	92.0
Positive	4	8.0
INR		
Normal	45	90.0
Elevated	5	10.0

Table 3: Comparison of pretreatment reticulocyte percentage with of post treatment reticulocyte percentage distribution.

Reticulocyte percentage	Post treatment reticulocyte percentage						Statistical inference
	Less than 1		More than 1		Total		
	N	%	N	%	n	%	
Less than 1	31	96.9	13	72.2	44	88.0	X ² =6.630 Df=1 P=0.010, Significant
More than 1	1	3.1	5	27.8	6	12.0	
Total	32	100.0	18	100.0	50	100.0	

Clinical symptoms and pathological findings in the patients were given in Table 2. All the patients in the study were noticed with history of fatigability. 35% of the patients had bleeding manifestations in addition to easy fatigability. Only 4% of the patients presented with neurological symptoms and signs like posterior column, pyramidal involvement and psychiatric manifestations. Only 4% of the patients gave positive history of intake of drugs like metformin, proton pump inhibitors, H₂ blockers etc. chronically. The most common clinical finding observed in all patients was pallor (100%). Only 12% of the patients had jaundice. Knuckle hyperpigmentation was seen in 44% of the patients, hepatomegaly in 10% of the patient's splenomegaly in 30% of the patients and lymphadenopathy in 6% of the patients. Sternal tenderness was noticed in 4% patients.

On hematological analysis of the patients presenting with pancytopenia, 66% of the patients were having MCV >100 fL and 34% of the patients are having MCV <100

fL. SGOT and SGPT was more than 40 in 74% and 64% of the patients respectively.

Megaloblastic anemia was characterized by macrocytosis and hyper segmented neutrophils were seen in 58% of the cases. 13% of the patients were having peripheral smear finding suggestive of pancytopenia. 8% of the patients are having smear with dimorphic anemia with decrease in all cell lineages. 88% of the patients are having reticulocyte count less than 1% suggestive of hypocellular marrow. Rest 12% of the patients is having reticulocyte count less than 1%.

On USG of the abdomen, 70% of the patients showed normal finding. Indications of hepatosplenomegaly were seen in 20% of the patients, splenomegaly in 8% of the patients. One patient found to have features suggestive of cirrhosis of liver with portal hypertension.

About 62% of the patients were identified with deficiency of vitamin B12 (<200 pg/ml). Bone marrow aspiration was done in all the cases. Hypercellular marrow with

megaloblastic picture was seen in 29 patients, hypocellular marrow with aplastic anemia features in 3 patients, hypercellular marrow alone was noticed in 15 patients and features of acute myeloid leukemia in 3 patients.

About 10% of the patients presented with pancytopenia are found to be ICTC positive. Direct Coombs test was positive in 8% of the patients. 5% of the patients had elevated INR.

In our study, treatment was given to the patients presenting with pancytopenia with low reticulocyte count, low serum vitamin B12, with appropriate doses of parenteral cyanocobalamin preparations along with supplements such as folate, ferrous sulphate tablets to meet proliferating marrow demand. Post treatment reticulocyte count taken after one week of treatment before discharge. Significant improvement in hematological parameters was in patients after parenteral cobalamin administration ($p=0.10$).

DISCUSSION

Pancytopenia is not a disease by itself but a constellation of hematological findings due to anemia, neutropenia, and thrombocytopenia.⁹ The severity and underlying pathology of the disease determine the prognosis and management in these patients.¹⁰ Timely diagnosis of etiology and intervention helps in reducing the morbidity and mortality rate in the patients with pancytopenia.

The mean age of the patients in our study was 32.4 years with a definite male dominance in the study (M:F-1.5:1) which was similar to the observations of (34.9 years, 1.4:1), and (42 years, 1.2:1).^{11,12}

The most common presenting features in patients presenting with pancytopenia was easy fatigability (100%) and bleeding (30%). Neurological manifestations like paraparesis observed in 2 cases. Psychiatric manifestations were observed in 2 cases which were comparable to the presenting feature in studies.^{13,14}

In our study, the most common clinical finding observed in all patients was pallor (100%), followed by knuckle hyperpigmentation (44%), splenomegaly (30%), hepatomegaly (20%), icterus (20%), generalised lymphadenopathy (6%), and sternal tenderness (4%). Similar observations were noted in the study.¹⁴

MCV values are >100 fl in 66% of study population. Increased MCV values are seen in all cases of megaloblastic anaemia and can be used as adjuncts in diagnosis of pancytopenia.¹⁵ The findings of present study was consistent with the observations.¹⁶

Liver function test results are abnormal in pancytopenia. In our study, liver parameters were elevated in 64% of the patients. Elevation of these values is related to

ineffective erythropoiesis and hemolysis.¹⁷ Normal reticulocyte count ranges from 1-2%. It provides reliable measure of RBC production daily and helps in diagnosing the cause for pancytopenia.¹⁸ In our study, reticulocyte percentage analysis revealed 88% of the patients have values less than 1%.

Peripheral blood smear findings give important information about premature release of reticulocytes and their evaluation should be done before blood transfusion.¹⁸ In our series, blood smear examination comprises megaloblastic anemia (58%), dimorphic anemia (16%), and pancytopenia (26%). This was comparable to findings.¹⁹ In his study, anisocytosis was seen in most of the cases (58%) followed by megaloblastosis (25%), and normocytic normochromic anemia (34%).

Vitamin B12 deficiency was considered as the frequent cause of pancytopenia.²⁰ In the present study, patients presenting with pancytopenia, found to have low serum vitamin B12 (88%) and found to have significant association with low reticulocyte percentage, ($p=0.015$). These findings concluded that reticulocyte percentage can be taken as surrogate marker for patients presenting with pancytopenia due to vitamin B12 deficiency. In a study vitamin B12 deficiency was considered as frequent cause of pancytopenia in younger adults (22%).²¹ But in our study we could not find a significant relationship between age and serum vitamin B12 levels.

Pancytopenia is very common in advanced stages of HIV and the etiology was found to be multifactorial which included high viral load, use of antiretroviral drugs, and use of acute or chronic opportunistic infections.¹⁸ Other probable causes of pancytopenia related to infections are viral hepatitis, tuberculosis, dengue virus, Epstein-Barr virus, and cytomegalovirus.¹⁸ In our study, among 50 cases of pancytopenia, 5 patients were found to be HIV reactive with incidence of pancytopenia.

On analyzing relationship between chronic drug exposure to drugs like metformin, proton pump inhibitors revealed only 8% of these patients had pancytopenia. 13.5% cases of pancytopenia secondary to chronic use of drugs including chemotherapy in their study of 111 patients, which is comparable to our study.²²

Bone marrow examination is always indicated in cases of pancytopenia to indicate increased cellular turnover.²³

In our study, bone marrow aspiration findings revealed hypercellular marrow with megaloblastic features in 29 cases, hypercellular marrow with no specific features in 15 cases, hypocellular marrow suggestive of aplastic anemia in 3 cases. 3 cases were found to have acute myeloid leukemia. Similar findings were noted in studies.^{12,14,19,21}

In our study, the patients with pancytopenia of having low reticulocyte count, low serum vitamin B12 were treated with parenteral cyanocobalamin and folate supplementations. All the patients were recovered with the treatment and a significant improvement in the reticulocyte count ($p=0.01$) was observed in the study.

CONCLUSION

Pancytopenia is a hematological feature of varying etiologies with male preponderance. Megaloblastic anemia due to vitamin B12 deficiency is the most common cause of pancytopenia and can be prevented by improving the nutritional status of the population. Bone marrow aspiration is to be considered as an important investigative tool to investigate underlying cause and prognosis in patients with pancytopenia. Early identification and management of pancytopenia due to megaloblastic anemia can be reversed and treated successfully with parenteral cyanocobalamin therapy.

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