

Original research article

Descriptive observational study to determine the incidence and demographic profile of vitamin B12 deficiency in patients with pancytopenia.

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Abstract

Aim: To assess the incidence of vitamin B12 deficiency in patients with pancytopenia.

Material and methods: This was a descriptive observational study carried out at Department of General Medicine, Jannayak Karpuri Thakur Medical College and Hospital, Madhepura, Bihar, India for 1 year. We included 100 patients of both sexes of age 12 years and above with pancytopenia. 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. 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.

Results: A total of 100 patients with pancytopenia were included in the study. There were 62 males and 38 females with a mean ratio of 1.63:1 and with a mean age of 33.12 years. Majority (50%) of the patients presenting with pancytopenia were laborer's. All the patients in the study were noticed with history of fatigability. 28% of the patients had bleeding manifestations in addition to easy fatigability. Only 5% of the patients presented with neurological symptoms and signs like posterior column, pyramidal involvement and psychiatric manifestations. Only 9% 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%).

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 diagnosed when there is a reduction in all three hematopoietic cell lines. This is seen as reduction in the white cell count, hemoglobin, and platelet count which is most often the result of bone marrow infiltration or failure, anticancer chemotherapy, hypersplenism, systemic diseases, and infections like HIV, tuberculosis, leishmaniasis, and so forth.^{1,2} While there are several published studies on the hematological diagnosis of pancytopenia on basis of bone marrow morphology, few have attempted to explore the underlying etiology and clinical course of the disorders leading to this condition. Vitamin B12 is an essential micronutrient for DNA synthesis and proliferation of haematopoietic cells of bone marrow, gastrointestinal cells, epithelial cells, cervico-vaginal cells and testicular germ cells. The daily requirement of vitamin B12 is 5–30 µg and daily absorption is 1- 5 µg.³ Liver stores 2000 - 5000µg of B12 which lasts for 3-5 years. Nutritional cobalamin deficiency is common in India.⁴ This may be attributed either due to lack of proper diet or

malabsorptive states. The other causes of vitamin B12 deficiency being intrinsic factor deficiency, chronic gastritis, H. Pylori infection, blind loop syndrome, transcobalamin II deficiency, and fish tape worm infestation. Vitamin B12 deficiency may present in multiple ways from a haematological manifestation to a neurological disorder. Manifestations involving cardiac, cutaneous and skeletal systems are also noted.⁵ The most common hematologic hallmark of B12 deficiency is megaloblastic anaemia.⁶ Megaloblastic anaemia of B 12 deficiency is frequently observed in clinical practice but remains underestimated.⁷ 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.

Material and methods

This was a descriptive observational study carried out at Department of General Medicine, Jannayak Karpuri Thakur Medical College and Hospital, Madhepura, Bihar, India for 1 year. We included 100 patients of both sexes of age 12 years and above with pancytopenia.

Methodology

Patients who were not willing to participate in the study, patients on myelotoxic chemotherapy and radiotherapy and age below 12 years were excluded. 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.

Statistical Analysis

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 100 patients with pancytopenia were included in the study. There were 62 males and 38 females with a mean ratio of 1.63:1 and with a mean age of 33.12 years. Majority (50%) of the patients presenting with pancytopenia were laborer's.

Table 1: Demographic profile of the patients

Variables	Number of patients	Percentage
Age (in years)		
Below 20	23	23
20 to 30	19	19
30 to 40	23	23

40 to 50	27	27
Above 50	8	8
Gender		
Male	62	62
Female	38	38
Occupation		
Students	22	22
Labourer	50	50
Clerk	5	5
Housewife	19	19
Staff	4	4
Diet history		
Non- vegetarian	46	46
Vegetarian	54	54
History of smoking		
No	55	55
Yes	45	45
History of alcoholism		
No	53	53
Yes	47	47

Among 100 patients with pancytopenia, 54% of the patients were on vegetarian diet, 45% of the patients weresmokers and 47% were alcoholics.

Table 2: Clinicopathological findings in study participants

Variables	Number of patients	Percentage
History of fatigability		
Yes	100	100
No	0	0
History of bleeding		
No	72	72
Yes	28	28
Psychiatric manifestations		
No	95	95
Yes	5	5
Neurological manifestations		
No	95	95
Yes	5	5
History of drug intake		
No	91	91
Yes	9	9
Presence of pallor		
Yes	100	100
No	0	0
Presence of icterus		
No	89	89
Yes	11	11
Presence of Knuckle hyperpigmentation		

No	57	57
Yes	43	43
Presence of hepatomegaly		
No	79	79
Yes	21	21
Presence of splenomegaly		
No	68	68
Yes	28	28
Lymphadenopathy		
No	93	93
Yes	7	7
Sternal tenderness		
No	97	97
Yes	3	3
MCV (fl/cell)		
Less than 100	35	35
More than 100	65	65
SGOT (U/l)		
Less than 40	27	27
More than 40	73	73
SGPT (U/l)		
Less than 40	35	35
More than 40	65	65
Peripheral smear		
Dimorphic anemia	14	14
Megaloblastic anemia	59	59
Pancytopenia	27	27
Reticulocyte count (%)		
Less than 1	86	86
More than 1	14	14
Variables	Number of patients	Percentage
Serum vitamin B12 (pg/ml)		
Less than 200	60	60
More than 200	40	40
USG of abdomen		
Normal	72	72
Hepatosplenomegaly	17	17
Cirrhosis with splenomegaly	3	3
Splenomegaly	8	8
Bone marrow aspiration		
Hyper cellular marrow with no specific features	29	29
Hyper cellular marrow with megaloblastic picture	57	57
AML	7	7
Hypocellular marrow with aplastic anemia features	7	7
ICTC		
Non- reactive	89	89

Reactive	11	11
Direct Coombs test		
Negative	93	93
Positive	7	7
INR		
Normal	91	91
Elevated	9	9

Table 3: Comparison of pre-treatment reticulocyte percentage with of post treatment reticulocytepercentage distribution

Reticulocyte percentage	Post treatment reticulocyte percentage						Statistical inference
	Less than 1		More than 1		Total		
	N	%	N	%	n	%	
Less than 1	68	97.14	18	60	86	86	X ² =6.550 Df=1 P=0.011, Significant
More than 1	2	2.86	12	40	14	14	
Total	70	100.0	30	100	100	100	

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. 28% of the patients had bleeding manifestations in addition to easy fatigability. Only 5% of the patients presented with neurological symptoms and signs like posterior column, pyramidal involvement and psychiatric manifestations. Only 9% 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 11% of the patients had jaundice. Knuckle hyperpigmentation was seen in 43% of the patients, hepatomegaly in 21% of the patient's splenomegaly in 28% of the patients and lymphadenopathy in 7% of the patients. Sternal tenderness was noticed in 3% patients.

On hematological analysis of the patients presenting with pancytopenia, 65% of the patients were having MCV>100 fL and 35% of the patients are having MCV <100 fL. SGOT and SGPT was more than 40 in 73% and 65% of the patients respectively. Megaloblastic anemia was characterized by macrocytosis and hyper segmented neutrophils were seen in 59% of the cases. 27% of the patients were having peripheral smear finding suggestive of pancytopenia. 14% of the patients are having smear with dimorphic anemia with decrease in all cell lineages. 86% of the patients are having reticulocyte count less than 1% suggestive of hypocellular marrow. Rest 14% of the patients is having reticulocyte count less than 1%.

On USG of the abdomen, 72% of the patients showed normal finding. Indications of hepatosplenomegaly were seen in 17% of the patients, splenomegaly in 8% of the patients. 3 patient found to have features suggestive of cirrhosis of liver with portal hypertension. About 60 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 57 patients, hypocellular marrow with aplastic anemia features in 7 patients, hypercellular marrow alone was noticed in 29 patients and features of acute myeloid leukemia in 7 patients. About 11% of the patients presented with pancytopenia are found to be ICTC positive. Direct Coombs test was positive in 7% of the patients. 9% 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 ($p=0.11$)

Discussion

Pancytopenia is not a disease by itself but a constellation of hematological findings due to anemia, neutropenia, and thrombocytopenia.⁸ The severity and underlying pathology of the disease determine the prognosis and management in these patients.⁹ 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 33.12 years with a definite male dominance in the study (M:F-1.63:1) which was similar to the observations of (34.9 years, 1.4:1), and (42 years, 1.2:1).^{10,11}

The most common presenting features in patients presenting with pancytopenia was easy fatigability (100%) and bleeding (28%). Neurological manifestations like paraparesis observed in 5 cases. Psychiatric manifestations were observed in 5 cases which were comparable to the presenting feature in studies.^{12,13}

In our study, The most common clinical finding observed in all patients was pallor (100%). Only 11% of the patients had jaundice. Knuckle hyperpigmentation was seen in 43% of the patients, hepatomegaly in 21% of the patient's splenomegaly in 28% of the patients and lymphadenopathy in 7% of the patients. Sternal tenderness was noticed in 3% patients. Similar observations were noted in the study.¹³

65% of the patients were having $MCV > 100$ fL and 35% of the patients are having $MCV < 100$ fL. Increased MCV values are seen in all cases of megaloblastic anaemia and can be used as adjuncts in diagnosis of pancytopenia.¹⁴ The findings of present study was consistent with the observations.¹⁵ Liver function test results are abnormal in pancytopenia. In our study, liver parameters were elevated in 72% of the patients. Elevation of these values is related to ineffective erythropoiesis and hemolysis.¹⁶ Normal reticulocyte count ranges from 1-2%. It provides reliable measure of RBC production daily and helps in diagnosing the cause for pancytopenia.¹⁷ In our study, reticulocyte percentage analysis revealed 86% 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 (59%), dimorphic anemia (14%), and pancytopenia (27%). This was comparable to findings.¹⁸ 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.¹⁹ In the present study, patients presenting with pancytopenia, found to have low serum vitamin B12 (86%) and found to have significant association with low reticulocyte percentage, ($p=0.011$). 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%).²⁰ But in our study we could not found 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.¹⁷ Other probable causes of pancytopenia related to infections are viral hepatitis, tuberculosis, dengue virus, Epstein-Barr virus, and cytomegalovirus.¹⁷

On analyzing relationship between chronic drug exposure to drugs like metformin, proton pump inhibitors revealed only 9% 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.²¹ Bone marrow examination is always indicated in cases of pancytopenia to indicate increased cellular turnover.²²

In our study, bone marrow aspiration findings revealed hypercellular marrow with megaloblastic features in 57 cases, hypercellular marrow with no specific features in 29 cases, hypocellular marrow suggestive of aplastic anemia in 7 cases. 7 cases were found to have acute myeloid leukemia. Similar findings were noted in studies.^{11,13,18,20}

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

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.

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