

## Assessment of Vitamin B12 Deficiency in Patients Diagnosed with Pancytopenia in Bihar State

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Conflict of interest: Nil

### Abstract

**Aim:** The aim of the present study to assessment of vitamin B12 deficiency in patients diagnosed with Pancytopenia in Bihar state.

**Methods:** A descriptive observational study was conducted in the Department of General Medicine, Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar, India for 1 year. We included 100 patients of both sexes of age 14 years and above with pancytopenia. 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).

**Results:** Among 100 patients with pancytopenia, 54% of the patients were on vegetarian diet, 43% of the patients weresmokers 46% were alcoholics. All the patients in the study were noticed with history of fatigability. 32% of the patients had bleeding manifestations in addition to easy fatigability. Only 3% of the patients presented with neurological symptoms and signs like posterior column, pyramidal involvement and psychiatric manifestations. Only 7% of the patients gave positive history of intake of drugs like metformin, proton pump inhibitors, H<sub>2</sub> blockers etc. chronically. On hematological analysis of the patients presenting with pancytopenia, 67% of the patients were having MCV >100 fL and 33% of the patients are having MCV <100 fL. SGOT and SGPT was more than 40 in 75% and 66% of the patients respectively. On USG of the abdomen, 69% of the patients showed normal finding. Indications of hepatosplenomegaly were seen in 20% of the patients, splenomegaly in 8% of the patients. 3 patients found to have features suggestive of cirrhosis of liver with portal hypertension.

**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.

**Keywords:** pancytopenia, vitamin B12, Megaloblastic anemia

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## Introduction

Cytopenia is a reduction in any of the three types of peripheral blood cell. A reduction in all the three of cellular components is termed pancytopenia and this involves anemia, leukopenia, and thrombocytopenia. Initially mild impairment in marrow function may go undetected and pancytopenia may become apparent only during times of stress or increased demand (e.g. bleeding or infection). The presenting symptoms are usually attributable to anemia or thrombocytopenia.[1] Pancytopenia is a striking feature of many serious and life threatening illness ranging from simple drug induced bone marrow hypoplasia, megaloblastic marrow to fatal bone marrow aplasias and leukemias.[2] Etiological causes of pancytopenia often vary by geographical region, age, and gender. They include megaloblastic anemia, other nutritional anemia, aplastic anemia (AA), splenomegaly, sepsis, leukemia, lymphoma, multiple myeloma, myelodysplastic syndromes (MDSs), alcoholic diseases, HIV and hepatitis viruses, autoimmune diseases, endocrine diseases and bone marrow infiltrating diseases (such as Gaucher's disease).[3] Clinical features are those due to pancytopenia per se, and those due to underlying disorder with a different epidemiology, pathophysiology, clinical presentations, and clinical outcomes; identification of diseases is of primary importance, since this is the key to appropriate management.[4] Deficiency of vitamin B12 is a well-known cause of megaloblastic anemia. It is a reversible cause of bone marrow failure and demyelinating nervous system disorder, so early detection and prompt treatment of vitamin B12 deficiency is essential. As a large proportion of pancytopenia is of reversible aetiology (especially B12 deficiency), early & accurate diagnosis may be lifesaving. Whereas the severity of pancytopenia and the

underlying pathology determines the management and prognosis of the patients. So, present study to assessment of vitamin B12 deficiency in patients diagnosed with pancytopenia in bihar state.

## Materials and methods

A descriptive observational study was conducted in the Department of General Medicine, Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar, India. For 1 year after taking the approval of the protocol review committee and institutional ethics committee.

We included 100 patients of both sexes of age 14 years and above with pancytopenia. Patients who were not willing to participate in the study, patients on myelotoxic chemotherapy and radiotherapy and age below 14 years were excluded.

## Methodology

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. Chisquare 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 58 males and 42 females with a mean ratio of 1.38:1 and with a mean age of 33.06 years. Majority (50%) of the patients presenting with pancytopenia were laborer's.

Among 100 patients with pancytopenia, 54% of the patients were on vegetarian diet, 43% of the patients were smokers. 46% were alcoholics.

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. 32% of the patients had bleeding manifestations in addition to easy fatigability. Only 3% of the patients presented with neurological symptoms and signs like posterior column, pyramidal involvement and psychiatric manifestations. Only 7% of the patients gave positive history of intake of drugs like metformin, proton pump inhibitors, H<sub>2</sub> blockers etc. chronically. The most common clinical finding observed in all patients was pallor (100%). Only 10% of the patients had jaundice. Knuckle hyperpigmentation was seen in 45% of the patients, hepatomegaly in 22% of the patient's splenomegaly in 32% of the patients and lymphadenopathy in 5% of the

patients. Sternal tenderness was noticed in 5% patients.

On hematological analysis of the patients presenting with pancytopenia, 67% of the patients were having MCV >100 fL and 33% of the patients are having MCV <100 fL. SGOT and SGPT was more than 40 in 75% and 66% of the patients respectively.

Megaloblastic anemia was characterized by macrocytosis and hyper segmented neutrophils were seen in 60% of the cases. 15% of the patients were having peripheral smear finding suggestive of pancytopenia. 15% of the patients are having smear with dimorphic anemia with decrease in all cell lineages. 89% of the patients are having reticulocyte count less than 1% suggestive of hypocellular marrow. Rest 11% of the patients is having reticulocyte count less than 1%.

On USG of the abdomen, 69% of the patients showed normal finding. Indications of hepatosplenomegaly were seen in 20% of the patients, splenomegaly in 8% of the patients. 3 patient found to have features suggestive of cirrhosis of liver with portal hypertension.

About 59% 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 9 % of the patients presented with pancytopenia are found to be ICTC positive. Direct Coombs test was positive in 7% of the patients. 8% 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 ( $p=0.12$ ).

**Table 1: Demographic data of the patients (n=100).**

Variables	Number of patients	Percentage
Age (in years)		
Below 20	25	25
21 to 30	19	19
31 to 40	21	21
41 to 50	29	29
Above 51	6	6
Gender		
Male	58	58
Female	42	42
Occupation		
Nursing students	25	25
Labourer	50	50
Clerk	2	2
Housewife	20	20
Staff nurses	3	3
Diet history		
Non-vegetarian	46	46
Vegetarian	54	54
History of smoking		
No	57	57
Yes	43	43
History of alcoholism		
No	54	54
Yes	46	46

**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	68	68
Yes	32	32
Psychiatric manifestations		
No	95	95
Yes	5	5

Neurological manifestations		
No	97	97
Yes	3	3
History of drug intake		
No	93	93
Yes	7	7
Presence of pallor		
Yes	100	100
No	0	0
Presence of icterus		
No	90	90
Yes	10	10
Presence of Knuckle hyperpigmentation		
No	55	55
Yes	45	45
Presence of Hepatomegaly		
No	82	82
Yes	22	22
Presence of splenomegaly		
No	68	68
Yes	32	32
Lymphadenopathy		
No	95	95
Yes	5	5
Sternal tenderness		
No	95	95
Yes	5	5
MCV (fl/cell)		
Less than 100	33	33
More than 100	67	67
SGOT (U/l)		
Less than 40	25	25
More than 40	75	75
SGPT (U/l)		
Less than 40	34	34
More than 40	66	66
Peripheral smear		
Dimorphic anemia	15	15
Megaloblastic anemia	60	60
Pancytopenia	25	25
Reticulocyte count (%)		

Less than 1	89	89
More than 1	11	11
<b>Variables</b>	<b>Number of patients</b>	<b>Percentage</b>
Serum vitamin B12 (pg/ml)		
Less than 200	59	59
More than 200	41	41
USG of abdomen		
Normal	69	69
Hepatosplenomegaly	20	20
Cirrhosis with splenomegaly	3	3
Splenomegaly	8	8
Bone marrow aspiration		
Hypercellular marrow with no specific features	29	29
Hypercellular marrow with megaloblastic picture	57	57
AML	7	7
Hypocellular marrow with aplastic anemia features	7	7
ICTC		
Non- reactive	91	91
Reactive	9	9
Direct Coombs test		
Negative	93	93
Positive	7	7
INR		
Normal	92	92
Elevated	8	8

**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	64	98.46	27	77.14	91	91	X <sup>2</sup> =6.42 Df=1 P=0.012, Significant
More than 1	1	1.54	8	29.63	9	9	
Total	65	100	35	100	100	100	

**Discussion**

Pancytopenia is not a disease by itself but a constellation of hematological findings due to

anemia, neutropenia, and thrombocytopenia.[5] The severity and underlying pathology of the disease determine

the prognosis and management in these patients.[6] Timely diagnosis of etiology and intervention helps in reducing the morbidity and mortality rate in the patients with pancytopenia.

There were 58 males and 42 females with a mean ratio of 1.38:1 and with a mean age of 33.06 years which was similar to the observations of (34.9 years, 1.4:1), and (42 years, 1.2:1).[7,8]

The most common presenting features in patients presenting with pancytopenia was easy fatigability (100%) and 32% of the patients had bleeding manifestations, Only 3% of the patients presented with neurological symptoms and signs like posterior column, pyramidal involvement and psychiatric manifestations.[9]

In our study, The most common clinical finding observed in all patients was pallor (100%). Only 10% of the patients had jaundice. Knuckle hyperpigmentation was seen in 45% of the patients, hepatomegaly in 22% of the patient's splenomegaly in 32% of the patients and lymphadenopathy in 5% of the patients. Sternal tenderness was noticed in 5% patients. Similar observations were noted in the study.[10]

MCV values are >100 fl in 67% of study population. Increased MCV values are seen in all cases of megablasic anaemia and can be used as adjuncts in diagnosis of pancytopenia.[11] The findings of present study was consistent with the observations.[12]

Liver function test results are abnormal in pancytopenia. In our study, liver parameters were elevated in 66% of the patients. Elevation of these values is related to ineffective erythropoiesis and hemolysis.[13] Normal reticulocyte count ranges from 1-2%. It provides reliable measure of RBC production daily and helps in diagnosing the cause for pancytopenia.[14] In our study,

reticulocyte percentage analysis revealed 89% 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.[14] In our series, blood smear examination comprises megaloblastic anemia (60%), dimorphic anemia (15%), and pancytopenia (25%). This was comparable to findings.[15]

Vitamin B12 deficiency was considered as the frequent cause of pancytopenia.[16] In the present study, patients presenting with pancytopenia, found to have low serum vitamin B12 (91%) and found to have significant association with low reticulocyte percentage, ( $p=0.012$ ). 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%).[17] 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.[14] Other probable causes of pancytopenia related to infections are viral hepatitis, tuberculosis, dengue virus, Epstein-Barr virus, and cytomegalovirus.[14] In our study, among 100 cases of pancytopenia, 9 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.[18]

Bone marrow examination is always indicated in cases of pancytopenia to indicate increased cellular turnover.[19]

In our study, bone marrow aspiration findings revealed 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. Similar findings were noted in studies.[8,10,15,17] 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.

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