

## Haematological Profile of Macrocytic Anemia in Correlation with Serum Vitamin B 12 and Folate Levels

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### Original Research Article

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**Abstract:** Macrocytosis in adults is defined as a red blood cell (RBC) mean corpuscular volume (MCV) >100 FL. Macrocytic anemia is generally classified into megaloblastic and non-megaloblastic anemia. The objective of this study was to assess haematological findings in patients of macrocytic anemia based on complete blood count and peripheral blood smear and to classify them based on clinical, serum vitamin B 12 and folic acid levels. The study was carried out over a period of one year at a tertiary care teaching hospital. 100 patients were selected on simple random sampling with MCV >99 FL and peripheral blood smear showing features of macrocytosis. Out of the 100 patients studied of macrocytic anemia, 55 percent had megaloblastic anemia and remaining 45 percent had non-megaloblastic anemia due to various causes. In our study, megaloblastic anemia was the most common cause of macrocytic anemia and among non-megaloblastic causes liver disorders like hepatitis was the most important cause.

**Keywords:** Anemia, megaloblastic anemia, macrocytosis, nutritional anemia, non-megaloblastic anemia, vitamin B 12 and folic acid deficiency.

### INTRODUCTION

It is estimated in 2010 that the global prevalence of anemia is as high as 32.9 percent with more than 2.2 billion people affected by it [1].

The World Health Organization (WHO) defined anemia as hemoglobin count less than 13 g/L in men, less than 12 g/L in non-pregnant women, and less than 11 g/L in pregnant women and the elderly [1].

The cause of anemia varies widely and is affected by age, sex, race and geography. The most common anemia in developing countries is nutritional anemia while macrocytic anemia is one of the important cause of nutritional anemia causing significant morbidity [1].

Macrocytosis is found in 2.5-4% of adults who have a routine complete blood count and in up to 60% of cases, macrocytosis is not accompanied by anemia; however, isolated macrocytosis should always be investigated [2]

Macrocytic anemia is subdivided into megaloblastic and non-megaloblastic types. Megaloblastic anemia is caused by deficiency or impairment of utilization of vitamin B 12 or folate. Non megaloblastic anemia may be the result of liver

dysfunction, alcoholism, myelodysplastic syndrome or hypothyroidism [3].

The spectrum of disease varies from asymptomatic to life threatening pancytopenia or myelopathy [2]

The present study was done to study the haematological changes associated with macrocytic anemia and was correlated with serum vitamin B 12 and folic acid levels to know the etiology so that appropriately therapy can be initiated early.

### MATERIALS AND METHODS

The study was prospective observational cross-section study carried out over a period of 1 year from June 2016 to July 2017 at tertiary care hospital.

100 patients based on simple random sampling were selected with MCV>99 fl and peripheral blood smear showing features of macrocytosis like macro-ovalocytes, hyper segmented neutrophils or howell-jolly bodies.

Detailed clinical history including presenting complaints, dietary habits and alcohol with drug history were taken.

Serum vitamin B 12 and folic acid levels were done in all cases and other investigative findings were recorded.

Cases in which serum vitamin B 12 & folic acid levels were normal the investigation done were- serum thyroid levels(T3, T4 and TSH) , reticulocyte count, liver function test and bone marrow aspiration in few cases .

All the cases with MCV> 99 fl on complete blood count and showing macrocytosis on peripheral blood smear were included in the study. Vitamin B 12, folic acid levels were done in all the cases.

**Table-1: The causes of non-megaloblastic anemia were**

Serial No.	Cause	Percentage
1	Liver Disorders	30%
2	Thyroid disorder	7%
3	Bone marrow Disorders	5%
4	Haemolysis	5%
5	Miscellaneous (Drugs)	3%

**Table-2: Clinical features in megaloblastic anemia**

Clinical Features	Percentage
Pallor	60%
Fatigue/weight loss/wasting	45%
Jaundice/Hepatosplenomegaly	20%
Others (Bleeding manifestations, fever)	10%

**Table-3: Haematological Profile in Megaloblastic Anemia**

Sl No.	Findings	Percentage
1.	Leucopenia	25%
2.	Pancytopenia	50%
3.	Hypersegmented Neutrophils	15%
4.	Thrombocytopenia	10%

Out of 100 cases, 60 had decreased Vitamin B 12, 30 had combined Vitamin B 12 and folic acid

deficiency and only 10 had isolated folic acid deficiency.

**Table-4: Comparison of our study with various studies**

	Our Study	Kannan A <i>et al.</i> , Int J Res Med Sci. 2016 [3]	Srikanth <i>et al.</i> , 2016 [2]	Karlijn <i>et al.</i> , 2016 [4]	Yellinedi S <i>et al.</i> , Int J. Contemp Paediatr. 2016 [5]	Kaur. D <i>et al.</i> , 2015 [6]
Most common Cause	Megaloblastic Anemia	Non-Megaloblastic anemia	Megaloblastic anemia	Megaloblastic Anemia	Megaloblastic Anemia	Megaloblastic anemia
Most common non-Megaloblastic Cause	Liver disorder	Bone marrow disorders like acute leukemia and myelodysplastic syndrome	Bone marrow disorders	Liver disorders and alcohol	-----	Liver disorders
Least Important Cause of Non-megaloblastic Anemia	Drugs	Hypothyroidism	-----	Hemolysis	-----	Drugs

The cases where MCV<99 fl on complete blood count were excluded from the study and newborns with MCV> 99 fl were also excluded as it could be physiological. Cases where vitamin B 12 and folate levels were not done were excluded.

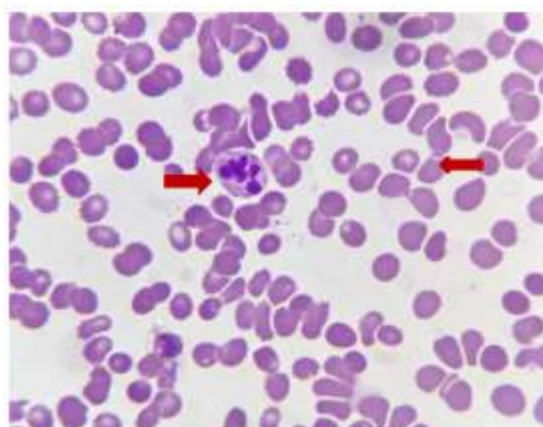
Bone marrow examination when done for diagnostic purpose was studied for megaloblasts and other abnormalities.

The findings were recorded on case proforma, master chart prepared, tabulated and statistically analysed.

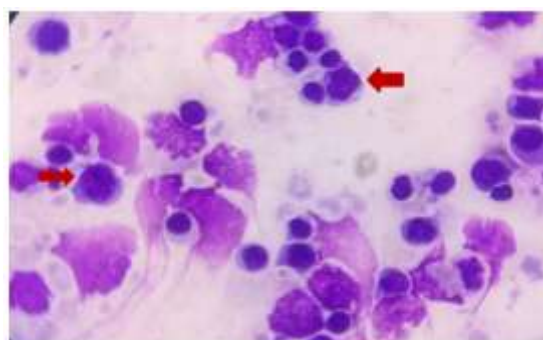
## RESULTS

Out of a total 100 patients studied the causes of macrocytic anemia were:

- Megaloblastic Anemia- 55% (Category I)
- Non-Megaloblastic Anemia- 45% (Category II)



**Fig-1: Peripheral blood smears showing macrocytosis and hypersegmented neutrophils (100X, oil Immersion)**



**Fig-2: Bone Marrow showing megaloblasts (100X, oil immersion)**

Bone marrow studied in cases of megaloblastic anemia done for confirmation of diagnosis (10 %) showed hyper cellularity with megaloblastic erythroid hyperplasia displaying sieve like chromatin, nucleocytoplasmic asynchrony and basophilic cytoplasm. Myeloid and megakaryocytes series were normal in all these cases. No hemoparasite noted.

## DISCUSSION

Macrocytic cells are red blood cells larger than the nucleus of a small lymphocyte. Although the presence of macro-ovalocytes, anisocytosis, and hyper segmented neutrophils suggest megaloblastic anemia associated with vitamin B 12 or folate deficiency, these morphological abnormalities may also be seen in myelodysplastic syndrome (MDS) or drug-induced disorders of DNA synthesis (Science Direct).

Megaloblastic anemia is found to be the most important cause of macrocytic anemia caused due to nutritional deficiency similar to our study. Normal level of vitamin B 12 is between 190 and 900 ng /ml [7]. Vitamin B 12 deficiency is the most common cause of megaloblastic anemia caused by insufficient intake especially in developing countries like India [2].

Megaloblastic anemia is a disorder in which erythroid hyperplasia is the prominent feature. The morphologic hallmark is nuclear-cytoplasmic asynchrony, which is seen in erythroid precursors in bone marrow aspirates [2].

The common symptoms reported in vitamin B 12 deficiency are fatigue, headache, palpitations and dyspnoea while neurological symptoms such as dysesthesia and hypoesthesia may also be present [Srikanth]. In our study, most patients presented with

pallor and palpitations while neurological symptoms were rare (Table-2).

The prominent peripheral smear findings typically seen in megaloblastic anemia are macro-ovalocytes and hypersegmented neutrophils similar to our study (Table-3).

Other haematological features include basophilic stippling, howell-jolly bodies, and cabot rings; however, these findings were not noted in our study (Science Direct).

It is said that bone marrow in megaloblastic anemia shows apart from changes in erythroblasts nuclear abnormalities of metamyelocytes and megakaryocytes resulting from impaired nuclear differentiation [3]. But in the present study, no significant changes were noted in myeloid and megakaryocytic series of cells.

Non megaloblastic anemia is caused by various diseases such as MDS, liver dysfunction, alcoholism, hypothyroidism, certain drugs, and by less commonly inherited disorders of DNA synthesis [1]. In our study, liver dysfunction and alcoholism was the most common cause followed by hypothyroidism. This is in concordance with other studies (Table-4).

Macrocytosis is said to be due to excess RBC membrane which occurs in patients with chronic liver disease when cholesterol esterification is defective. Macrocytosis with an MCV of 100 to 105 fl is said to occur in chronic alcohol use in the absence of folate deficiency [8].

Alcoholism is a well-known cause of macrocytic anemia and most important cause of non-megaloblastic anemia. It is said that chronic consumption of more than 80 grams of alcohol per day has adverse effects on the hematologic system (Makoto). Even in the present study, alcoholism was the most important cause of non-megaloblastic macrocytic anemia.

Further, mild macrocytosis is reported in aplastic anemia, especially in recovery phase. As RBC membrane moulding occurs in spleen after cell release from marrow, RBCs are macrocytic after splenectomy (Evan). In our study there were no cases of splenectomy or Aplastic Anemia (Table-1).

Many drugs reportedly cause megaloblastic anemia by impairing the cellular availability or the utilization of folic acid or vitamin B 12. Common drugs that cause macrocytosis are hydroxyurea, methotrexate, zidovudine, azathioprine, antiretroviral agents, valproic acid, and phenytoin. In our study, the drug related cases were few (5%) [1].

It is said that once macrocytosis has been identified, differential diagnosis should begin with determining the serum levels of vitamin B 12 and folate and examining the peripheral blood smear. It is said that, if the serum vitamin B 12 levels are <200 pg/mL, vitamin B 12 deficiency is very likely; however if levels are >300 pg/mL, vitamin B 12 deficiency is unlikely [1].

Reportedly, the absence of reticulocytosis suggests the possibility of alcoholism, liver dysfunction, hypothyroidism, or MDS. Macrocytic target cells are commonly seen on peripheral blood smears in liver disease, and hypolobulated or hypogranular neutrophils, large and/or abnormally granulated platelets, and monocytosis may be seen with MDS [1].

Of importance is the indication for bone marrow examinations. This is recommended for patients with abnormal cells in blood circulation or patients who do not respond to treatments, such as vitamin replacement (Srikanth). In the present study, bone marrow study was done only in cases of megaloblastic anemia done for confirmation and all of them showed typical features of megaloblastic erythroid hyperplasia in bone marrow morphology.

## CONCLUSION

In the present study, megaloblastic anemia is the most common cause of macrocytic anemia. Among non-megaloblastic causes, alcoholism is the most important cause and drugs are the least important. The difference noted with other studies may be due to dietary and regional variations.

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