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Original Research Paper

Pathology

Hematological Changes in Megaloblastic anemia: A study of 25 cases

Vijay Kumar Sinha	Assistant Professor, Department Of Pathology, Anugrah Narain Magadh Medical College, Gaya, Bihar.
Subhash Chandra Jha	Assistant Professor, Department Of Pathology, Government Medical College, Bettiah, West Champaran, Bihar
Parshuram Prasad*	Assistant Professor, Department Of Pathology, Anugrah Narain Magadh Medical College, Gaya, Bihar. *Corresponding Author
Dilip Kumar Pandey	Assistant Professor, Department Of Pathology, Anugrah Narain Magadh Medical College, Gaya, Bihar.

ABSTRACT

Materials and Methods: A teaching hospital-based retrospective study was done for a period of 1 year. 25 cases of megaloblastic anemia were analyzed and we correlated signs, symptoms, and hematological investigations.

Results: Totally, 25 patients with megaloblastic anemia in the above said period were studied. The patient's age group was ranged from 12 years to 87 years .Male female ratio was 4:1. Megaloblastic anemia was observed in all the cases. Pancytopenia was seen in all cases. 25 patients had isolated vitamin B12 deficiency. 3 patients had both Folic acid and vitamin B12 deficiency. One had only isolated Folic acid deficiency.

Conclusion: Megaloblastic anemia is one of the common causes of undiagnosed anemia, and the treatment is simple and easily affordable. If left untreated, it can lead to morbidity both because of anemia and attendant neurological involvement.

KEYWORDS : Megaloblastic anemia, Vitamin B12, Folic Acid, Pancytopenia

INTRODUCTION:

Megaloblastic anemia is a panmyelosis. The morphologic hallmark is nuclear-cytoplasmic dissociation, which is the best appreciated in precursor cells in the bone marrow aspirate. Megaloblastic nuclei are larger than normoblastic nuclei, and their chromatin appears abnormally dispersed due to its retarded condensation **(Figure 2]**). Giant band forms and metamyelocytes are usually seen.

There are various causes for the deficiency of both Vitamin B_{12} and folate that include parasitic infections like Diphyllo bothrium latum, alcoholism, vegetarianism, gastrectomy, pernicious anemia, drugs like oral contraceptives and anticonvulsants. With achlorhydria and loss of pepsin secretion, Vitamin B_{12} is not readily released from proteins in food. With gastrectomy and pernicious anemia, intrinsic factor is not available for transport to the ileum. The only dietary sources of vitamin B_{12} are foods of animal protein origin such as kidney, liver, heart, muscle meats, fish, eggs, cheese and milk. In contrast to folate, vegetables contain practically no vitamin B_{12}. Cooking has little effect on its activity. The humans are entirely dependent upon dietary sources. Vegetarians are more prone for megaloblastic anemia as compared to that of nonvegetarians.

The term macrocytosis refers to a blood condition in which red blood cells (RBC) are larger than normal. Macrocytosis is reported in terms of mean corpuscular volume (MCV). Normal MCV values range from 80 to 100 femtoliters (fl) and vary by age and reference laboratory (1).MCV is calculated according to the following formula:

$$MCV\ (fl)\ =\ [Hematocrit\ (percent)\ x\ 10]/[RBC\ count\ (10^6/\mu L)]$$

Macrocytosis can be identified by reviewing peripheral blood smears and/or by automated RBC indices. The peripheral blood smear is more sensitive than RBC indices for identifying early macrocytic changes because the MCV represents the mean of the distribution curve and is insensitive to the presence of small numbers of macrocytes(2).However, compared to the peripheral blood smear, MCV may underestimate macrocytosis in over 30% of cases (3)

Rarely hyperglycemia, marked leukocytosis and cold agglutinins may result in false elevations of the MCV (4, 6). Moreover, partial occlusion of the instrument aperture and/or leaving the blood sample at room temperature for several hours may also result in false elevations of the MCV value. Macrocytosis, defined as a mean corpuscular volume greater than 100 fL, occurs in approximately 3 percent of the general population (1). This article describes a strategy for the evaluation of patients with macrocytosis, as well as a brief discussion on treatment of vitamin B_{12} and folate deficiencies. The hypersegmented neutrophils and macroovalocytes are present in megaloblastic macrocytic anemia (**Figure 1**). Nonmegaloblastic anemia has round macrocytes or macroreticulocytes.When peripheral smear B_{12} level, and reticulocyte count have not lead to an obvious diagnosis, consider a comprehensive metabolic panel to look for liver and kidney disease, thyroid-stimulating hormone for thyroid disorders, and methylmalonic acid and homocysteine levels to assess for vitamin B_{12} deficiency, despite a normal vitamin B_{12} level. Bone marrow aspiration and biopsy are very helpful in confirming diagnosis of megaloblastic anemia in difficult cases.

35 percent of patients with alcoholism and macrocytic anemia are folate deficient, which can be caused by poor nutrition, malabsorption, hepatobiliary dysfunction, and possibly increased folate catabolism **(7, 8).**Some medications that are used to treat seizure disorders, cancer, and autoimmune diseases can lead to folate deficiency. For example, methotrexate directly inhibits dihydrofolate reductase, which leads to a functional folate deficiency. Serum folate levels are not much useful because they fluctuate rapidly with diet (**9**, 11). RBC folate levels more accurately correlate with folate stores and should be performed if folate deficiency is suspected. In differentiating the cause of megaloblastic anemia, a methylmalonic acid level that is within normal range also points toward a diagnosis of folate deficiency. Because of the limitations of measuring serum folate, RBC folate levels have been advocated as a more reliable source of measuring tissue stores of folate. RBC folate levels remain constant throughout the lifespan of the cell and are not

affected by short-term dietary changes that can alter serum levels. However, assays for measuring RBC folate levels have also been fraught with unreliability **(10, 11).** It should be noted that low RBC folate levels have been reported with alcohol use, pregnancy and anticonvulsant medications. Another important cause of low RBC folate levels is vitamin B12 deficiency(12,13).It is estimated that approximately 60% of patients with pernicious anemia have low RBC folate levels, presumably because vitamin B12 is necessary for normal transfer of methyltetrahydrofolate from plasma to RBC.

Vitamin B12 levels may be reported as normal or elevated in myeloproliferative disorders, liver disease, congenital transcobalamin II deficiency, intestinal bacterial overgrowth and antecedent administration of vitamin B12(14).Moreover, there are reports of falsely low vitamin B12 levels with folate deficiency, pregnancy, use of oral contraceptives, congenital deficiency of serum haptocorrins and multiple myeloma. The prevalence of vitamin B12 deficiency among the elderly ranges from 1.5% to 4.6%[25] and was reported to be as high as 15% in the population over the age of 60 years. The deficiency in many cases is associated with gastric achlorhydria, resulting in decreased synthesis and availability of intrinsic factor, a necessary binding protein that facilitates vitamin B12 absorption in the ileum.

Vitamin B_{12} is absorbed by the ileum when bound by intrinsic factor, which is produced by the parietal cells of the gastric mucosa. Nutritional deficiency is most common cause of Vitamin B12 in vegetarians and vegans in India , especially in Hindu community. In pernicious anemia, the loss of parietal cells leads to insufficient absorption of vitamin B_{12}, which then leads to vitamin B_{12} deficiency over time. It is common cause in western world.Pernicious anemia is most commonly caused by auto-immune atrophic gastritis, in which autoantibodies are directed against parietal cells and intrinsic factor. The diagnosis of pernicious anemia can be confirmed by identifying and measuring intrinsic antibody levels in the serum. Parietal cell antibodies, although not specific, are also commonly present Less commonly, pernicious anemia can be caused by nonautoimmune gastritis secondary to *H. pylori* infections and Zollinger-Ellison syndrome.

The spectrum of etiologies associated with macrocytic anemia includes nutritional deficiencies (e.g., vitamin B12 and folate), drugs , primary bone marrow disorders (e.g., myelodysplasia and leukemia) and other chronic illnesses. Macrocytosis due to vitamin B12 or folate deficiency is a direct result of ineffective or dysplastic erythropoiesis**.** These important vitamins and cofactors are required for normal maturation of all cells. When either of these two factors is deficient, RBC proliferation and maturation result in large erythroblasts with nuclear/cytoplasmic asynchrony. These abnormalities are caused by a defect in DNA synthesis that interferes with cellular proliferation and maturation. RNA synthesis and cytoplasmic components remain relatively unaffected. The marrow is hypercellular with all forms of the myeloid cell line being increased and erythroid elements being dominant on the marrow aspirate smear preparations. The erythroblasts become large, oval shaped and contain a characteristic immature, lacy nucleus. These bone marrow features are called "megaloblastic" and are highly suspicious of a vitamin B12 or folate deficiency. Megaloblastoid (megaloblastic-like) abnormalities of the marrow are frequently seen in other hematologic disorders not associated with vitamin B12 or folate deficiency, (e.g., myelodysplasia and leukemia) and a careful examination of the bone marrow is necessary to make this distinction.

Determining the underlying cause of the macrocytosis can be particularly challenging when thalassemia trait or iron deficiency or other nutritional deficiencies coexist with a vitamin B12 or folate deficiency. In these instances the peripheral blood smear may show a mixed population of microcytic and macrocytic RBCs with an elevated distribution width. In cases of macrocytosis related to alcoholism the elevated MCV may be due to the direct effect of the alcohol, liver disease and/or folate deficient.A review of the peripheral smear is imperative in determining the etiology of macrocytosis. The presence of macro-ovalocytes having an MCV >115 fl, anisocytosis, poikilocytosis and hypersegmented neutrophils suggests a megaloblastic disorder associated with a nutritional deficiency, i.e., vitamin B12 or folate deficiency. Round macrocytes are commonly seen in a variety of chronic illnesses, and round target-appearing macrocytes are characteristic of liver disease such as hepatitis, obstructive jaundice, and acute and chronic alcoholism with liver disease . For patients who present with disordered immaturity, hypogranulated or hyposegmented neutrophils, and cytopenias, a bone marrow examination is necessary to rule out or confirm a primary bone marrow disorder such as a myelodysplastic syndrome or leukemi A reticulocyte count should be obtained if there is evidence of hemolysis on the peripheral smear, i.e., increased polychromasia, nucleated RBCs, spherocytes or schistocytes.[18] The presence of increased polychromasia of the macrocytes on the peripheral smear and a reticulocyte count of >10% should raise suspicion of hemolysis or an acute bleed. These large polychromatophilic erythrocytes noted on the peripheral smear represent reticulocytes, immature RBCs that are larger than mature RBCs, and are indicative of increased erythropoiesis or RBC production and, if present in increased number, can raise the MCV. Additionally, the reticulocyte maturation parameters performed on the peripheral blood may also be helpful to differentiate megaloblastic from nonmegaloblastic causes of the macrocytosis.[19] An elevated reticulocyte maturation value is more suggestive of a megaloblastic rather than a non-megaloblastic anemia

Macrocytosis associated with a megaloblastic marrow is usually accompanied by anemia due to ineffective erythropoiesis. The bone marrow is hypercellular, showing evidence of abnormal proliferation and maturation of multiple myeloid cell lines. These abnormalities are most evident in the erythroid precursors with large megaloblastic erythroblasts present in increased numbers throughout the marrow. Similar morphologic abnormalities can be seen in the other myeloid elements, e.g., large or giant metamyelocytes and other granulocytic precursors. This ineffective erythropoiesis is accompanied by intramedullary hemolysis causing an elevated lactate dehydrogenase and indirect bilirubin in the serum(14,15).However, the reticulocyte count is low due to the abnormal maturation process. More severe degrees of abnormal proliferation and maturation are seen with myelodysplasia and myeloid leukemias. It is imperative that a hematologist or hematopathologist examine the marrow in order to appreciate these important, subtle, hematopoietic abnormalities. Patients with macrocytosis who are not anemic and have no other abnormalities noted on the peripheral blood smear do not usually need a bone marrow examination.Macrocytosis is the earliest abnormality seen in complete blood counts of patients with folate or vitamin B12 deficiency. In patients with elevated MCV values, laboratory tests for vitamin B12 and folate deficiencies are routinely ordered by physicians, although these tests are limited by their low sensitivity and specificit.Cobalamin and folate are cofactors in several important metabolic pathways in the cell. The hydroxylated form of cobalamin plays an important role in the metabolism of homocysteine and MMA. The conversion of homocysteine to methionine requires both vitamin B12 and folate as cofactors. However, the metabolism of L-methylmalonyl CoA to succinyl CoA, an enzymatic pathway involved in oxidative phosphorylation reactions within the cell, only requires vitamin B12. These metabolites provide

early information regarding the cellular state of vitamin B12 and folate and can be used to distinguish folate from vitamin B12 deficiency, since most patients with **folate deficiency have normal MMA** or mildly elevated levels. It should be kept in mind that nearly 50% of those with elevation of these metabolites will have normal serum vitamin B12 levels. HoloTC II is an emerging marker that may be useful in establishing a diagnosis of early vitamin B12 deficiency in cases where there is discordance between vitamin B12 levels and its metabolites, or in lieu of measuring vitamin B12, MMA and/or homocysteine concentrations.[41] HoloTC II can also be used in cases of renal failure or **myeloproliferative diseases in which vitamin B12 concentrations may be falsely elevated (30,31).**HoloTC II is a metabolically active protein that transports cobalamin to cell membrane receptors. Its serum concentration can be used to measure the amount of vitamin B12 attached to the binding protein transcobalamin II. Compared to measurements of serum vitamin B12, holoTC II seems to have greater sensitivity and specificity. However, routine ordering of this test as part of the work-up to establish the diagnosis of vitamin B12 deficiency is not currently recommended(16,17)

Patients with vitamin B12 deficiency from any cause have received cyanocobalamin intramuscularly/ intravenous 1000 to 1500 μg/ alternate day for one week, , twice weekly 2 weeks and weekly for 1 month and monthly, thereafter. This time-honored method remains an acceptable form of treatment for all causes of vitamin B12 deficiency, particularly when cognitive impairment or neurologic disease is present. Alternatively, hydroxocobalamin given in the same dose every 1–3 months intramuscularly is also effective therapy. This form of cobalamin remains in the tissues longer than the cyanocobalamin forms and can, therefore, be given less frequently.[46] In cases of deficiency due to inadequate intake, food-cobalamin malabsorption and pernicious anemia, oral cyanocobalamin administered at 1000–2000 μg/day for 1 month, followed by 125–500 μg/day is recommended and considered a safe and effective method of treatment.[47] Other oral administration regimens have demonstrated efficacy and have proven to be equally as effective as intramuscular administration.[48,49] Because of the inherent unreliability of measuring either serum or RBC folate, it is recommended that patients receiving treatment for vitamin B12 deficiency receive empiric folate supplementation of 400 g/day to 1 mg/day.[27,45,51] Maintenance therapy should continue until the underlying cause of the deficiency is corrected.In folate deficiency, responds well to short-term treatment with oral 5mg twice daily or intravenous 50 mg twice daily in acute and severe deficiency , especially in methotrexate toxicity. . Long-term treatment is not warranted except with chronic conditions such as malnutrition, exfoliative dermatitis or hemolysis .A complete blood cell count 10–14 days after starting the treatment for vitamin B12 or folate deficiency should reveal a rise in hemoglobin and a decrease in MCV. A full hematologic response should occur within 8 weeks. During treatment, further monitoring of the complete blood cell count or measuring vitamin B12 and folate levels or their metabolites is not necessary.[52] In patients taking long-term treatment for vitamin B12 deficiency, an annual complete blood cell count may be a reasonable consideration to monitor therapy.

MATERIALS AND METHODS:

Retrospective analysis of case records of all patients admitted and diagnosed as megaloblastic anemia was done. Those with a diagnosis of aplastic anemia and Myelodysplastic syndromes were excluded from the study. The data were collected, and multivariate analysis was done to determine the correlation between symptoms, signs, and hematological investigations.

Retrospective study of hematological, biochemical and bone marrow data of 25 patients of megaloblastic anemia were done in teaching medical colleges of Bihar. Salient clinical features were noted. Relevant photomicrographs were taken of PBS and BONE MARROW slides of classical cases of megaloblastic. Especial emphasis was given on CBC findings and relevant CBC parameters were notated and tabulated.

RESULT:

Total 25 megaloblastic anemia cases were included in this retrospective study. Male female ratio was 4:1 and age ranged from 12 years to 87 years . Pancytopenia was seen in all cases. 21 patients had isolated vitamin B12 deficiency. 3 patients had both Folic acid and vitamin B12 deficiency (Table 1) .One had only isolated Folic acid deficiency. All patients had MCV 100 or more than 100 fL (Table 1). Majority showed hypersegmented neutrophils on PBS (Figure 1). Classical sieve like nuclei and giant metamyelomyelocytes are found in most (Figure 2). Bone marrow revealed cellular bone marrow with megaloblastic change in erythroid, granulocytic and megakaryocytic series in majority. However few revealed depressed megakaryopoiesis. Patients who had severe anemia (hemoglobin <7 g %) was 22 (88%).All patients except one had moderate to severe thrombocytopenia (platelet count< 1, 00,000/μl.)

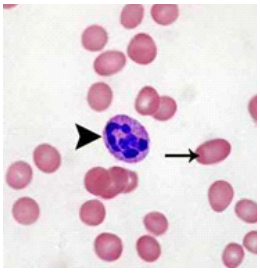

FIGURE: 1

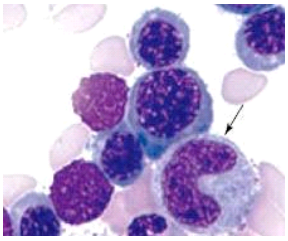

FIGURE: 2

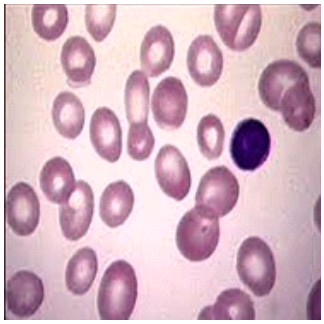

FIGURE: 3

Table 1.CBC parameters in megaloblastic anemia

CASE NO.	RBC	HB	MCV	MCH	WBC	PLT	B12	FOLIC ACID	AGE/GENDER
1	2.5	5.9	110	40.2	3.4	50	112		12Y/M
2	2.2	4.8	115	40.5	2.5	40	135		30Y/M
3	3.0	5.0	108	39.5	2.0	35	120		23Y/F

4	1.8	3.0	125	43.0	1.6	30	109		25Y/M
5	2.8	6.9	109	39.5	3.2	38	119		67Y/M
6	2.1	4.9	111	37.5	2.4	70	110		58Y/M
7	2.0	4.4	109	38.8	2.0	55	123		17Y/M
8	1.9	3.5	135	40.5	2.0	20	48	2.5	44Y/F
9	3.5	5.8	122	39.5	2.5	25	85	2.0	39Y/M
10	3.0	5.8	120	37.8	1.9	30	101		38Y/M
11	2.4	5.5	112	38.5	2.0	25	55		39Y/M
12	3.2	6.0	110	39.0	1.9	40	90		55Y/M
13	2.7	6.8	125	40.7	1.8	35	100		40Y/M
14	2.5	6.6	108	39.6	2.0	55	98		39Y/M
15	2.0	5.8	110	38	2.5	65	214	3.0	45Y/M
16	1.9	5.9	108	44.0	1.9	65	87		55Y/M
17	2.7	7.8	110	43.5	2.0	60	85		65Y/F
18	2.5	7.5	108	42.5	2.9	66	90		18Y/F
19	2.0	3.6	100	40.5	2.5	78	102		20Y/M
20	2.9	3.0	108	39.0	2.0	65	108		87Y/M
20	2.7	2.8	100	40.5	3.8	95	109		70Y/M
21	3.0	2.9	105	39.0	2.8	90	99		33Y/F
22	2.9	3.0	102	40.5	2.0	92	105	1.8	40Y/M
									38Y/M
23	3.0	2.8	100	39.0	1.8	99	108		30Y/M
24	3.8	7.8	108	40.5	2.0	100	110		59Y/M
25	2.5	6.4	110	43.5	2.7	75	85		60Y/M

Normal range:

B12: 211-911 pg/ml, FOLIC ACID :3.7-9.2mg/dl MCV: 83-100Fl,MCH: 27-33%, PLATELETS: 150-450X$10^3/\mu$l ,HEMOGLOBIN: (M) 12-15gm/dL, (F) 12-14gm/dL

DISCUSSION:

The adult age distribution and male predominance were expected given the demographics of vitamin B_{12} deficiency in Hindu population of India. Nutritional deficiency of vitamin B12 is more common .Drug induced folate deficiency is more common .Nutritional deficiency of Folic acid more commonly occur with vitamin B12 deficiency, especially in alcoholic (19). Low B_{12} levels are more common in vegans and alcoholic. Other causes of macrocytosis with or without anemia are hemolysis with reticulocytosis, drug-related macrocytosis, MDS, or other neoplasm. Myelodysplastic syndromes often present with pancytopenia, particularly 5q syndrome, present with a macrocytic anemia with normal platelet count or thrombocytosis. The dramatic treatment response of patients with full-blown vitamin B_{12} deficiency occurs in 3 to 5 days.The hypersegmented neutrophils were of no help in diagnosing vitamin B_{12} deficiency. Although most reports in the literature stating that the presence of hypersegmented neutrophils on the peripheral blood smear is neither sensitive nor specific for this purpose(20,21). Approximately 75% of serum B_{12} is bound to haptocorrin (transcobalamin I) whose function is unknown.[9] Therefore, total serum B_{12} levels largely reflect B_{12} that is not bio-available. Total transcobalamin, consisting of holoTC and apo-transcobalamin, is the major carrier protein in the plasma/serum that delivers B_{12} to the tissues.HoloTC should more accurately reflect intracellular B_{12} levels. If the vitamin B_{12} level s borderline low, methylmalonic acid and homocysteine levels should be ordered and, if elevated, may provide evidence of B_{12} deficiency.

Patients with vitamin B_{12} deficiency may describe paresthesias related to peripheral neuropathy. Neurologic signs such as ataxia, decreased proprioception, and vibratory sensation are more common. Patients may also have poor dentition or nonspecific oral stomatitis or glossitis.

Macrocytic anemia due to folic acid deficiency is more common in drug induced bone marrow depression and in alcoholic .Much less common is during pregnancy(23).Other uncommon causes include *Diphyllobothrium latum* (i.e., fish tapeworm) infection. Only 10 percent of persons with vitamin B_{12} deficiency are actually anemic (26.27).Oral therapy appears to be as effective as intramuscular therapy for the treatment of vitamin B_{12} deficiency, nutritional type. However intramuscular/intravenous B12 is more effective in pernicious anemia .Relapse of pernicious anemia occurs at a mean interval of 65 months after cessation of treatment. It is important for patients to adhere to long-term therapy because the deficiency will recur if treatment is stopped, unless a reversible cause is identified.

CONCLUSION:

Megaloblastic anemia is common cause of anemia amongst vegans and vegetarians .It is also common in alcoholic.Serum B12 testing without a clear clinical indication should be avoided; ie, macrocytosis (usually with anemia) or (less commonly) neurologic signs or symptoms potentially referable to vitamin B_{12} deficiency. We should obtain a vitamin B_{12} level for every patient with an elevated MCV.We should evaluate peripheral smear for megaloblastosis and perform a reticulocyte count in patients with suspected macrocytosis. Order methylmalonic acid and homocysteine levels if vitamin B_{12} level is borderline low (i.e., 100 to 400 pg/mL. Oral vitamin B_{12} may be as effective as intramuscular therapy for vitamin B_{12} deficiency. Obtain red blood cell folate level if other etiologies are not found, serum folate levels may be misleading.

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