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Study of Clinical-Etiological Profile of Macrocytic Anemia and Correlation of Serum Vitamin B_{12} & folate level with Mean Corpuscular Volume (MCV)

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Abstract

Introduction: Macrocytic anemia is a group of diverse disorders and so it demands further clinical and laboratory assessment .This study was conducted to study the clinical and laboratory parameters of patients with macrocytic anemia and its etiology.

Materials and methods: This study was a cross-sectional retrospective study, done on 216 adult patients with macrocytic anemia. Macrocytic anemia was defined when peripheral blood examination showed anemia with a mean red blood corpuscular volume of >100 fl. After inclusion patients were subjected to various investigations to find out etiology of macrocytosis.

Result: The most common cause of macrocytic anemia was megaloblastic anemia (34.8%). The major causes of non-megaloblastic macrocytic anemia (65.2%) were alcoholism & alcoholic liver diseases (28.7%) primary bone marrow disorders (22.7%), and hemolytic anemia (5.6%). The megaloblastic anemia observed was due to vitamin B_{12} deficiency (54%), folic acid deficiency (25.6%) and combined B_{12} and folate deficiency (20.4%). We found no significant association of vitamin B12 level and folic acid with MCV value.

Conclusion: Systematic evaluation of macrocytosis is needed to distinguish megaloblastic and non – megaloblastic causes of macrocytosis. Megaloblastic

anemia due to Vitamin B 12 or folate deficiency remains the most important cause of macrocytic anemia.

Key words: Anemia, Macrocytosis, Macrocytic anemia, Megaloblastic anemia, MCV, Vitamin B_{12}

Introduction

Macrocytosis is commonly encountered during clinical setting. Macrocytosis is seen in 1.7–3.6% of patients seeking medical care. 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 95 femtoliters (fl) and vary by age and reference laboratory. [1]

Macrocytic anemia is not a specific disease but is an indicator of diverse underlying etiology and it requires further clinical and laboratory assessment. They may be classified as those resulting from disorders in DNA synthesis of erythrocyte precursors in the bone marrow (megaloblastic macrocytic anemia) or those caused by a variety of other mechanisms (non-megaloblastic macrocytic anemia). The causes of macrocytosis fall into two groups: (a) deficiency of vitamin B12 (cobalamin) or folate (or rarely abnormalities of their metabolism) in which the bone marrow is megaloblastic and (b) other causes, in which the bone marrow is usually normoblastic. [2]

Megaloblastic anemia (MA) due to Vitamin B12 or folate deficiency is the well-known cause of macrocytosis. Pernicious anemia is a major cause of megaloblastosis in Western world where their diet supplies the necessary nutrients. Nutritional megaloblastic anemia is very important in the Indian scenario. However non megaloblastic macrocytosis also forms an important group which includes alcoholic liver disease, hemolytic anemia, leukemia etc. [3]

The disorders that cause macrocytic anemia consist of heterogeneous groups that act through a variety of mechanisms. The purpose of this study was to evaluate clinical and laboratory parameters in patients presented with macrocytic anemia and its etiology.

Methods and Materials

This study was a cross-sectional descriptive study carried over a period of 32 months on 216 adult patients having macrocytic anemia presented to two large teaching hospitals of India, Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi and Indira Gandhi Institute of Medical Sciences, Patna.

Inclusion Criteria

1. Age ≥13vyears.
2. Anemia (hemoglobin of <13 g/dl in male & <12 g/dl in female) with mean corpuscular volume (MCV) ≥100fl.
3. Peripheral smear showing macrocytic picture.

Exclusion Criteria

1. Patient of macrocytic anemic on hematinic supplement.
2. Pregnant female

The detailed history was taken and a complete and thorough physical examination was carried out in all patients. Detailed information was sought on alcohol consumption, previous gastric surgery, malignant disease and drug therapy. All patients were investigated with a complete hemogram that included estimation of hemoglobin level, red cell indices, total leucocyte count, differential leucocyte count, platelet count and reticulocyte count, and peripheral smear examination and red cell distribution width estimation. A bone marrow aspiration was performed in all patients included. Serum bilirubin, total protein and albumin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamyl trans peptidase and LDH levels were obtained on a routine basis for all patients. Prothrombin time was estimated in patients with history of alcoholism or evidence of liver disease clinically. Thyroid

function tests were done in all the patients. The vitamin B12 and folic acid in the patients with megaloblastic anemia was done. An upper gastrointestinal endoscopy and gastric biopsy was performed in a few selected patients with megaloblastic anemia who gave consent for the same.

Observations and Results

In this study we found mean age of patients was 36.88 ± 8.67 years ranging from 16 years to 86 years. The study population (216) consists of 122 (56.5%) males and 94 (43.5%) females. Maximum patients were recorded in the range of 21-40 years (31.5%). (Figure-1)

The major symptoms at presentation were associated with anemia. Fatigability, generalized weakness, and dyspnea on exertion were most common complaints at presentation comprising 97% (210) of the group. Six patients presented without symptoms of anemia. They presented with symptoms like jaundice, abdominal pain, neurological symptoms and swelling of feet. Second most common symptom observed was fever (in 34.25% cases). Neurological complaints (burning sensation, disturbed gait, and weakness) were present in 15% (32) cases. Twelve patients came with bleeding manifestations as chief complaints. (TABLE-1)

On clinical examination, pallor was present in 91 % cases (196). Icterus was observed in 98 (45.5%) patients, 92(42.6%) patients had hepatomegaly, and 84(40%) had splenomegaly. Knuckle pigmentation, an important clinical sign of megaloblastic anemia, was present in 37.5% (81). Other signs included glossitis (26%), neurological signs (23%) and pedal edema (18%). (TABLE-1)

The hematological study showed mean hemoglobin of 6.88±3.21 g/dl. The mean MCV of these patients was 108.2± 8.86fl. And average MCH was 38.22± 6.28pg. (TABLE-2) These patients were subjected to a bone marrow examination and 78 (34.8%) of them were found to have megaloblastic erythropoiesis in the bone marrow. Peripheral blood smear showed hyper-segmented neutrophils, anisocytosis, macro-ovalocytosis, and nucleated red cells, were strongly associated with megaloblastic hematopoiesis. Sub-optimal level of vitamin B_{12} and folic acid level in this patient further suggest its megaloblastic etiology. (Figure-2)

Out of 78 megaloblastic cases, 54% (42) had vitamin B_{12} deficiency, 25.6% (20) had folic acid deficiency and 20.4% (16) had deficiency of both. We studied correlation between level of vitamin B_{12} & folic acid with MCV. And we found value of MCV is not significantly associated with blood level of these micronutrients (p value>0.26). It means elevated MCV doesn't confirm deficiency of this micronutrient or normal MCV value doesn't exclude megaloblastic anemia. (Figure-3, 4)

Among the non-megaloblastic anemia (65.2%) various diseases were found (figure-5). Alcoholism and alcohol related liver disease was leading cause followed by primary bone marrow disorders. Sixty two patients had either history of chronic alcohol abuse or laboratory and imaging evidence of liver disease. Alcoholic hepatitis and chronic liver disease were main etiology in the study group.

Among the patients with primary bone marrow disorders, twenty six had acute myeloid leukemia, fourteen patients had aplastic anemia of varying etiology; and nine patients had myelodysplastic syndrome (MDS). All were newly diagnosed case of primarily bone marrow disease.

Twelve cases had features of hemolytic anemia. Of these nine had autoimmune hemolytic anemia, two patients had thalassemia and one had paroxysmal nocturnal hemoglobinuria (PNH). Six patients were diagnosed with multiple myeloma on workup. These patients presented with symptoms of renal failure and anemia was not prominent features at presentation.

Five cases are categorized under drug induced macrocytosis as other parameters came out to be normal on investigations. Two female patients were on phenytoin therapy for last 8 years. Two patients were type 2 diabetes mellitus and on metformin for more than 15 years. One case was of rheumatoid arthritis and she was on methotrexate for more than two years. Four cases of hypothyroidism were documented as a cause of macrocytic anemia in our study.

Discussion

Macrocytosis, defined as a mean corpuscular volume greater than 100 fl, occurs in approximately 3 percent of the general population. Debate regarding the upper limit of normal values of mean corpuscular volume still persists. [4]

The causes of macrocytosis can be broadly classified as megaloblastic and non-megaloblastic. Megaloblastic processes are characterized on the peripheral smear by macro-ovalocytes and hypersegmented neutrophils, which are absent in non-megaloblastic macrocytic processes. Non-megaloblastic processes have round macrocytes or macroreticulocytes. [5]

In megaloblastic processes, erythrogenic precursors are larger than mature red blood cells (RBCs) because folate and vitamin B12 deficiencies result in defective RNA and DNA syntheses. Non-megaloblastic processes develop from multiple mechanisms and have not been fully outlined. Macrocytosis can occur when there is increased RBC production secondary to peripheral blood cell destruction (i.e., hemolysis) or loss (i.e., hemorrhage), leading to a reticulocytosis. In liver disease, the volume may increase due to excessive lipid deposition on red cell membrane and it is especially prominent in liver disease due to alcohol. [1, 6]

Many drugs can also leads to macrocytic anemia. Valproic acid which is an anticonvulsant inhibits mitochondrial folate metabolism. Hydantoin can lead to low folate levels. Antifolates such as methotrexate inhibit folate binding to dihydrofolate reductase and this limits the availability of tetrahydrofolate which is important in DNA metabolism. Sulfasalazine interferes with folate absorption. Chemotherapeutic agents interfering with nucleoprotein synthesis like hydroxyurea and 5 flourouracil cause macrocytic anemia. [7] In present study total 216 patients with macrocytic anemia were studied with male: female ratio of 1.29:1. Mean age of patients in this study is comparable to that in study of by Unnikrishnan et al. where mean age of the patients was 38.96±16.4 years.

Unnikrishnan et al. showed major symptoms at presentation were predominantly that due to anemia (91.7%), 8.3% of patients did not have the typical symptoms of anemia. This was similar to our study. But second most common symptom was bleeding manifestations (36%) in their study. In our study second most common symptoms was fever (34%) while only 5.6%% patients had presented with bleeding manifestations. This was due to less number of severe thrombocytopenia in our study.

Unnikrishnan et al. showed pallor was universally present in their study. Other signs included icterus, hepatomegaly, splenomegaly, neurological manifestations, knuckle hyperpigmentation and glossitis, pedal edema which was similar to our study. [3]

Symptoms associated with anemia, jaundice, neurological manifestations and skin pigmentation also found similar to finding of Kannan A et al. [8]

In a similar study by Niranjana S et al. majority of patients presented with generalized weakness and fatigue (100%) followed by dyspnea (83.8%) and palpitations (49.5%). Bleeding manifestations (14.1%) and diarrhea (1%) were other complaints. Pallor was present in all patients, Knuckle pigmentation (57.6%) and paraesthesias (36.4%) were prominent findings. [9]

Hematological profile of our patient was similar to that of Unnikrishnan et al. They reported hemoglobin (gm. %)-4.96±1.26, PCV (%)-14.65±3.83, MCV (fl)-111.18±9.56, MCH (pg.)-35.05±4.43, MCHC-34.2±2.35, Platelet count ($\times 10^3/mm^3$)-98.5±95.2, and Reticulocyte Count (%) 2.5±0.04.

Jain *et al* (2012) studied serum vitamin B12 and MCV in general population and concluded that every third person was vitamin B12 deficient in their region, but there was no correlation between vitamin B12 levels and MCV. Our study also didn't find any correlation between the two. This suggests that raised MCV should not be the only criterion to diagnose megaloblastic anemia. [10]

Kannan A et al. reported 38% had megaloblastic anemia and 62% had non-megaloblastic macrocytosis comparable to our study. [8]

Savithri MC et al. in their study showed alcoholic liver disease as the leading cause of macrocytosis, closely followed by primarily bone marrow disorders in non megaloblastic macrocytosis group. Hypothyroidism is reported in 3.33% of study group. This result is very similar to our study. [11] The etiological findings in our study parallel the results of the studies of Unnikrishnan et al. with megaloblastic anemia being the most common cause of macrocytic anemia, followed by primary bone marrow disorders, liver disease and hemolytic anemia. [3]

But primary bone marrow disorders were also came out to be the most common cause of macrocytosis in non megaloblastic group in different study. Kannan A reported primary bone marrow disorders in 46% as most common cause followed by megaloblastic anemia(38%), hemolytic anemia (6%), drug induced (5%), alcoholism and liver disease (4%) and idiopathic thrombocytopenic purpura (1%). Drug induced macrocytosis were seen associated with, hydroxyurea, phenytoin and methotrexate like in our study. [8]

Figure-1 Composite Age - Sex Distribution Of The Patients

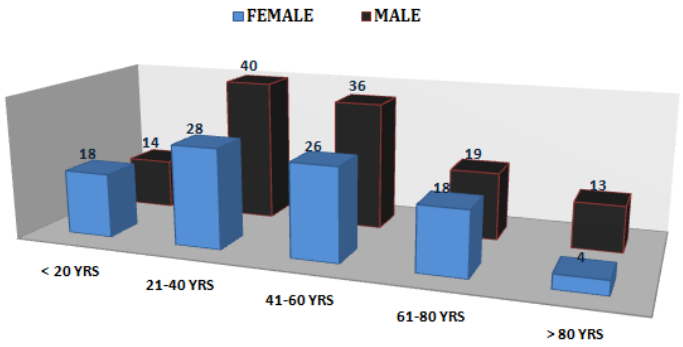

Figure-2 Etiology Of Macrocytic Anemia

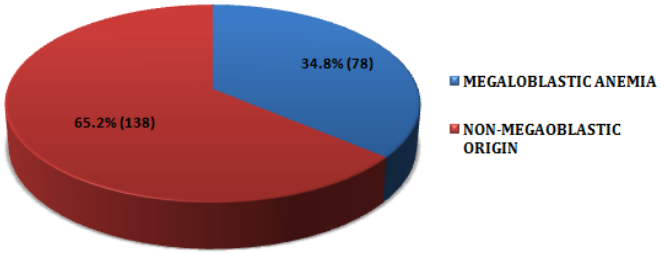

FIGURE-3 Variation Of MCV With Serum Vitamin B_{12} Level

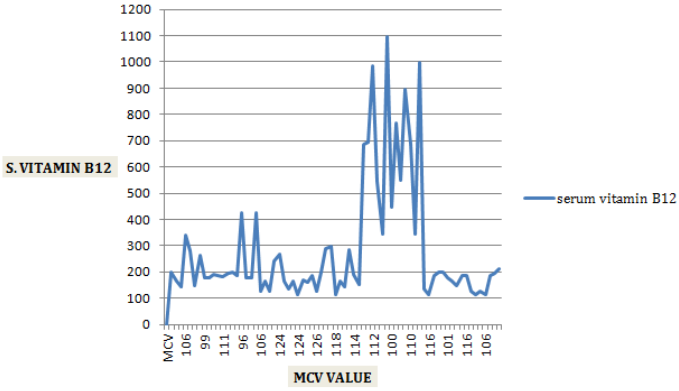

FIGURE- 4 Variation of MCV value with S. folic acid level

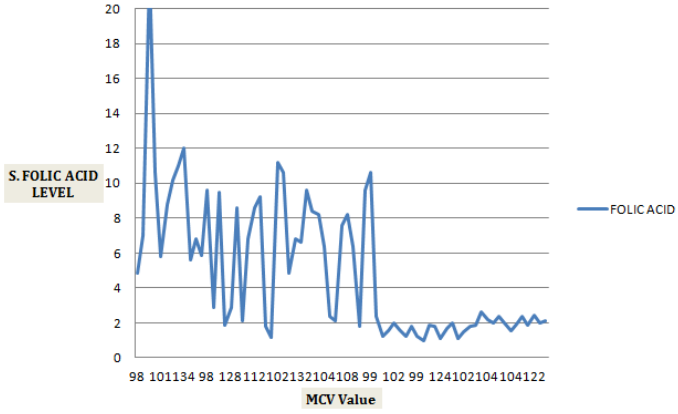

FIGURE-5 DISTRIBUTION OF PATIENTS AMONG NON MEGALOBLASTIC(TOTAL-138) ETIOLOGY

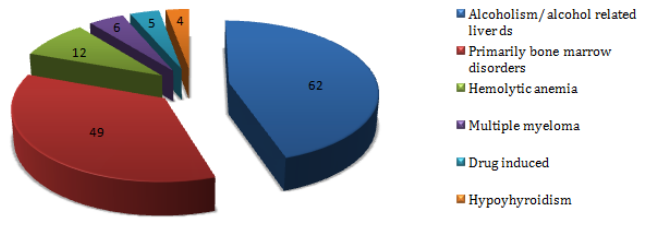

TABLE-1 COMMON CLINICAL FEATURES OF THE STUDY GROUP

SYMPTOMS	PERCENTAGE	SIGNS	PERCENTAGE
FATIGABILITY	97	PALLOR	91
GENERALIZED WEAKNESS		ICTEUS	45.5
DYSPNEA ON EXERTION		HEPATOMEGALY	42.6
FEVER	34.25	SPLENOMEGALY	39
NEUROLOGICAL SYMPTOMS	15	KNUCLE PIGMENTATION	37.5
BLEEDING MANIFESTATION	5.6	GLOSSITIS	26
		PEDAL EDEMA	18

TABLE-2 HEMATOLOGICAL CHARACTERICS OF THE PATIENT

PARAMETERS	VALUE±SD
Hemoglobin	6.88±3.21
Total leukocyte count	9.86±6.18
Platelet count	1.32±2.38
MCV	108±8.86
MCH	38.22±6.28
Reticulocyte count	2.38±0.08
LDH	1684±684

Conclusion

The pathological conditions associated with macrocytic anemia are much more diverse than is often appreciated and macrocytosis should not always equate with megaloblastosis, since there is various non-megaloblastic cause of macrocytosis. Megaloblastic anemia however still remains the most important cause of macrocytic anemia. With one of the highest per capita consumption of alcohol in India, alcohol related problems are on the rise. In our study after megaloblastic anemia, alcoholism and alcohol related liver disease was common disorder associated with macrocytic anemia.

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