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Original Research Article

Evaluation of clinical, biochemical and hematological parameters in macrocytic anemia

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ABSTRACT

Background: Macrocytosis can be seen in many hematological and non-hematological disorders and more than one cause may co-exist in an individual. Serum vitamin B12 and folic acid tests are routinely ordered but they are limited by their low sensitivity and specificity. This study is done to analyze the clinical, hematological and biochemical parameters in macrocytic anemia and to study the difference between megaloblastic and non-megaloblastic anemia in these parameters.
Methods: There were 100 patients presenting with macrocytosis were taken in to study. A detailed clinical history and physical examination was done in all cases. CBC, biochemical investigations, peripheral blood examination, Vitamin B12, folate levels, bone marrow aspiration and reticulocyte count was done in all cases.
Results: Primary bone marrow disorders were the most common cause of macrocytosis (45%). The other causes in decreasing order of frequency were megaloblastic anemia (36%), alcoholism and liver disease (15%), drug induced (2%) and idiopathic thrombocytopenic purpura (1%). There was a significant difference in the mean values of MCV and serum LDH between megaloblastic and non-megaloblastic macrocytosis. When serum LDH >1124.5IU/L or MCV>120.5fl (criterion values of ROC curve) with reticulocyte count <2% was taken as criteria, the sensitivity was 94.4% and specificity was 93% for diagnosing megaloblastic anemia.
Conclusions: Systematic evaluation of macrocytosis will help us to distinguish megaloblastic and non-megaloblastic macrocytosis. The blood and biochemical parameters especially CBC, RC, and serum LDH along with supporting clinical features help us in diagnosing megaloblastic anemia in a setup where vitamin and metabolite levels are difficult to obtain.

Keywords: Anemia, Macrocytosis, Mean corpuscular volume, Megaloblastic anemia, Non-megaloblastic macrocytosis, Serum LDH

INTRODUCTION

The term Macrocytosis refer to a blood condition in which Red Blood Cells (RBC) are larger than normal. Macrocytosis is defined in terms of Mean Corpuscular Volume (MCV). Normal MCV values range from 80 to 100 fl. MCV is calculated according to following formula

MCV (fl) = Hemototrit (%) * 100 / RBC Count ($10^{6/ml}$)

Macrocytosis can be identified by peripheral blood smear or by automated RBC indices. The incidence of detecting macrocytosis have increased and has varied from 1.7% to 3.6% in several reported series.[1]

Compare to peripheral blood smear MCV may underestimate macrocytosis in over 30% of cases.[2,3] No complication arise from macrocytosis as isolated finding however if identification can provide important

information regarding presence of underlying disease state.[4-6] Serum vitamin B12 and folic acid tests are routinely ordered but they are limited by their low sensitivity and specificity.[5-8]

Large circulating RBC's are not always associated with pathological condition. In fact, RBC's of new born and infant tend to be larger than adults (mean MCV 108fl).[9-11] Mild increase in MCV (100-110fl) is particularly common and most often reminds unexplained. So, the evaluation of macrocytosis needs a systemic approach.[5]

Macrocytic anemia can usually be divided into two categories, megalobalstic and non-megaloblastic based on examination of bone marrow. This categorization is important and frequently aids in determining the etiology of anemia.[9]

This study was done to analyse the clinical, haematological and biochemical parameters in macrocytic anemia and to study the difference between megalobalstic and non-megaloblastic anemia in these patients.

METHODS

A cross-sectional study was conducted for a period of 6 months from 1st June 2018 to 1st December 2018 on adults presenting with macrocytosis to the department of General medicine of Basaveshwara Medical College and Hospital, Chitradurga, Karnataka, India.

Cases with age >12 years having MCV≥100fl at presentation or having MCV more than two standard deviations for that particular age group were taken into study. Those cases who were recently transfused and those already on hematinic therapy were excluded from the study. A detailed clinical history and thorough physical examination was done in all cases. Complete hemogram for all patients were taken from sysmex sp 100 automated hematology analyzers.

Peripheral blood smear, reticulocyte count, and bone marrow aspiration were done on all cases. Biochemical parameters including bilirubin levels, serum iron, and serum LDH were done in all cases. Thyroid function tests, vitamin B12 and folate assays, Ultrasound examination and coombs test were done wherever necessary.

A provisional diagnosis was made, and patients were classified into megaloblastic and non-megaloblastic group. Patients with low vitamin B12 and folate levels, megaloblastic erythropoiesis, dysplasia and blastoid cells were initially placed in the megaloblastic group.

This study has been approved by the Institute Ethical Committee Board (BMCH) and therefore been performed in accordance with the ethical standards. Informed consent has been taken from all patients involved in the study.

RESULTS

There were 100 cases of macrocytosis were included in the study. Out of 100 cases, 36% had megaloblastic anemia and 64% had non-megaloblastic macrocytosis. Primary bone marrow disorders were the most common cause of macrocytosis in our study. This category included aplastic anemia, myelofibrosis, Chronic leukemia and myelodysplastic syndrome. The various causes of macrocytosis in our study are detailed in Table 1.

Table 1: Etiology of macrocytosis and percentage of cases in each diagnostic group.

Diagnosis	Number of patients	Percentage
Megaloblastic anemia	36	36%
Acute leukemia	0	0%
Myelodysplastic syndrome	4	4%
Aplastic anemia	14	14%
Hemolytic anemia	2	2%
Drug therapy induced	2	2%
Alcoholism and liver disease	15	15%
Multiple myeloma	0	0%
Myelofibrosis	2	2%
CML in chronic phase	1	1%
Hairy cell leukemia	0	0%
Idiopathic thrombocytopenic purpura	1	1%
Others	23	23%
Total	**100**	**100%**

Hypothyroidism was seen in six cases of megaloblastic anemia. Drug induced macrocytosis were seen associated with anti tubercular drug, phenytoin and metformin.

Clinical parameters

The minimum age at presentation was 16 years and maximum age was 84 years with a mean of 39.76years. The mean age at presentation in non-megaloblastic group was 45.86years which is approximately 7 years later than the megaloblastic group (39.97years). Out of 100 cases studied 52 cases were males and 48 were females.

Symptoms of anemia were present in 85% of the cases. Symptoms of anemia included breathlessness, easy fatigability, and generalized weakness. Neurological manifestations like ataxia, parasthesia were significantly associated with megaloblastic anemia. History of jaundice was present in 43 cases; out of it 28 cases belonged to the megaloblastic group. History of alcohol intake was present in 15 cases of non-megaloblastic macrocytosis.

Hematological and biochemical parameters

There were 15 cases had alcohol induced macrocytosis and the other 4 cases were drug induced. Skin pigmentation in the knuckles and fingers was seen in 8 cases of megaloblastic anemia and was significantly associated with this group. Icterus was present in 28 cases of megaloblastic anemia and was significantly associated with it and glossitis was seen exclusively in this group.

The mean Hb value was 5.156 and a standard deviation of ±1.69g/dl. The values in MCV ranged from 105.4 to 143.2 fl with a mean of 126.41fl and standard deviation of ±9.62fl. The serum LDH values ranged from 630.0 U/L to 9700.0U/L with a mean value of 2616.3U/L and standard deviation of ±2001.08U/L. The hematological values are listed in Table 2. To compare mean between 2 groups we use unpaired T-test for Hb, PC, MCV and MCH. Mann whitney U-test for TLC and LDH.

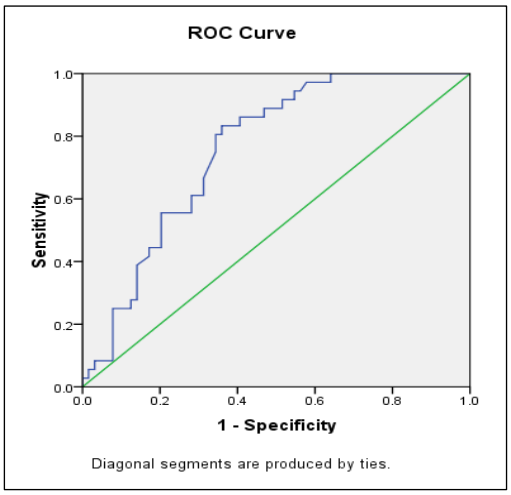
Table 2: Comparisons of various complete blood count parameters between megaloblastic and non-megaloblastic group.

	Megaloblastic (n=36)				Non megaloblastic (n=64)				
Parameter	**Min**	**Max**	**Mean**	**STD**	**Min**	**Max**	**Mean**	**STD**	**p-value**
Hb	2.1	8.4	5.156	1.6922	2.1	9.4	6.134	1.8688	0.011*
TLC	1100	10600	4070.00	2126.102	600	34200	7575.47	5568.177	0.0001*
PC	0.22	2.40	1.2392	0.53057	0.40	5.00	2.0981	1.30068	0.0001*
MCV	105.4	143.2	126.461	9.6221	100.8	141.0	112.253	8.5354	0.0001*
MCH	22.6	40.8	35.353	3.6138	18.3	42.8	34.552	4.7015	0.34
LDH	630	9700	2616.33	2001.816	140	7810	1411.59	1630.770	0.001*

*p value is significant at 5% level of significance

Pancytopenia was present in 28 cases, out of which 22 cases belonged to megaloblastic group and 6 cases had non-megaloblastic macrocytosis. Macrocytes and macroovalocytes were seen in all the cases of megaloblastic anemia and hypersegmented neutrophils in 60% of the cases. Nucleated RBCs were present in 11 cases of megaloblastic anemia (28.9%).

The other findings seen less frequently were tear drop cells, target cells and schistiocytes but they were more commonly associated with megaloblastic group.



Area under the curve =0.76 and p=0.0001 (sig), Inference: Diagnostic accuracy is good

Figure 1: ROC curve for serum LDH values.

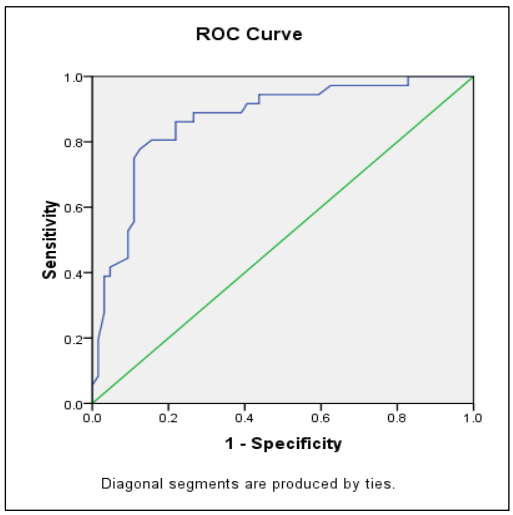
ROC curve analysis was done for MCV values and LDH values to get a cut-off value which can be used as screening tests in macrocytic anemia to differentiate between megaloblastic and non-megaloblastic macrocytic anemia. ROC Curve analysis for serum LDH values gave a criterion value of >1124.5U/L for megaloblastic anemia, at which the sensitivity was 83.3% specificity was 64.10% with a positive predictive value of 56.6% and an accuracy of 71%.

When LDH>1124.5U/L or MCV >20.51fl was taken as a criterion along with reticulocyte count <2%, the sensitivity was 94.4%, specificity was 93.1%, positive predictive value was 88.5%, negative predictive value was 94.6%, and accuracy was 95%. Since the sensitivity is 94.4% and specificity is 93.1%, all three parameters combined together can be used as screening test to distinguish between the 2 groups of macrocytic anemia (megaloblastic and non-megaloblastic) and further evaluation can be done depending upon the categorization.

ROC curve analysis for MCV values >121fl for megaloblastic anemia at which sensitivity was only 44.7% but specificity was 95.2%. With the positive predictive value of 74.4% and an accuracy of 83%. The area under the curve was 0.87 with the p value 0.000 (sig).

When LDH>1124.5U/L or MCV >120.5fl was taken as a criteria along with reticulocyte count <2%, the sensitivity was 100%, specificity was 48.4%, positive predictive

value was 50%, negative predictive value was 100%, and accuracy was 64%.



Area under the curve =0.76 and p=0.0001 (sig), Inference: Diagnostic accuracy is good

Figure 2: ROC curve for MCV values.

When LDH >1124.5U/L or MCV >120.5fl were taken together sensitivity was 94.4% and specificity was 54.7%, with accuracy of 69%.

Since sensitivity is 100% with all 3 parameters combined together it can be used as screening test to distinguish between the 2 groups of macrocytic anemia (megaloblastic and non-megaloblastic) and further evaluation can be done depending upon the categorization (Table 3).

Table 3: Sensitivity and specificity for diagnosing megaloblastic anemia at ROC criterion values.

Condition	Sensitivity	Specificity
Serum LDH>1124.5 (criterion value)	83.3%	64.1%
When MCV>120.5 (criterion value)	80.6%	84.4%
When S LDH and gt; 1124.5IU/L, MCV and gt; 120.5	94.4%	54.7%
When S LDH>1124.5IU/L, MCV>120.5 and reticulocyte count <2%	100%	48.4%

DISCUSSION

Macrocytosis is a common finding seen with the use of automated counters and presents as a common clinical problem, proper evaluation protocols have not been established. The etiology and demographic profile varies among various western and Indian studies. Drug therapy, vitamin B12 and folate deficiency, alcohol abuse was described as common causes of macrocytosis.[4,5,9-12]

But, in present study primary bone marrow disorders was the most common cause. This variation might be due to the difference in the selective criteria (Mcphedran et al) used MCV>115fl, Unnikrishnan et al used MCV>95fl) used along with varied environmental and demographic factors.[5] Symptoms associated with anemia, history of jaundice, neurological manifestations and bowel disturbances were similar to other studies.[10-14] Icterus and skin pigmentation were significantly associated with megaloblastic anemia which was similar to the finding of Savage DG et al.[15]

The various studies on macrocytosis are listed in the Table 4. Savage et al, stated that most patients with megaloblastic erythropoiesis and primary bone marrow disorders were 70 years of age or older, but in discordance to this study, mean age at presentation in our study was between 20-40 years of age.[16-18]

The lowered mean age at presentation in megaloblastic anemia may be due to increased demand during growth spurt and puberty. The mean hemoglobin in present study was 5.16g/dl with standard deviation of 1.69g/dl which was similar to Unnikrishnan V et al where the mean Hb was 5.16g/dl with standard deviation of 2.12g/dl. 85% of the patients had severe anaemia with the haemoglobin of ≤7g/dl. In a study by Davidson RJL et al in 1978 with the criteria of MCV>100fl, no cases had haemoglobin <7g/dl.[6,19,20]

They also found that the severity of macrocytosis increased in proportion to the degree of anaemia. But in our study as well as Unnikrishnan V et al study, no such significant increase could be found (p value = 0.234). The mean MCV in the whole series was 112.25±9.36fl which is much higher than that of Davidson et al and Unnikrishnan V et al.[6,20] The difference between means of MCV between megaloblastic and non-megaloblastic group was significant with those of megaloblastic group having higher MCV than the non-megaloblastic group. Majority of the cases in our study had an MCV between 100-110fl.

So, if we would have taken the cut-off as 115fl as Mcphedran et al, lot of cases would have been missed. Savage et al stated that though MCV values >110fl were seen commonly in patients with megaloblastic anaemia, this degree of MCV elevation was also noted in about 1 in every 3 patients in other disorders.[5,16]

If MCV exceeds 120fl, megaloblastic erythropoiesis was most likely. In our case too, we found that when MCV was between 110-120fl, majority of cases were present in non-megaloblastic anemia groups, the probability of a case being megaloblastic increased when the MCV was >120fl. Also, they found that when MCV was >120fl, the specificity for diagnosing the case as megaloblastic was 82.4%.

In present study, it was found that the specificity for megaloblastic anemia at MCV>121fl was 84.4% and this was comparable with the above-mentioned study. So, if the MCV>121fl, there is 84% chance that the disease is not a non-megaloblastic.[7]

Table 4: Common causes of macrocytosis in various national and international studies.

Name of the study	Place	Year	No. of cases	Age criteria used	MCV criteria used	Most common cause	2nd most common cause
McPhedran P et al[5]	Connecticut	1973	100	Adult patients	>115fl	Vitamin B12 and folic acid deficiency 50%)	Liver disease (15%)
Breeveld et al[7]	The Netherlands	1981	70	All cases	≥105fl	Vitamin B12 and folic acid deficiency (38.57%)	Alcohol Abuse (27.14%)
Wymer et al[8]	Virginia	1990	72	Adult patients (age ≥17 years)	>98.5fl	Alcohol abuse (65.2%)	Hemolysis (12.5%)
Mahmoud MY et al[13]	London	1996	124	>75 years	>95fl	Megaloblastic anaemia (26.6%)	Alcohol abuse (13.7%)
Savage DG et al[15]	Colorado	2000	300	Adult cases	≥100fl	Drug induced (37%)	Alcoholic liver disease and alcohol abuse (each 13.33%)
Unnikrishnan V et al[20]	Puducherry, India	2008	60	Adult cases (age ≥ 13 years)	>95fl, and patients with anaemia	Megaloblastic anaemia (38.4%)	Primary bone marrow disorders (35%)
Kannan A study et al[21]	Varanasi, India	2016	100	Adult (>18yrs) and pediatric cases (age≤18 years)	≥100fl and in <12years according to age, including patients without anaemia	Primary bone marrow disorders (46%)	Megaloblastic anemia (38%)
Present study	Chitradurga Inida	2018	100	Adult (>12Yrs)	≥100fl	Primary bone marrow disorders (45%)	Megaloblastic anemia (36%)

Emerson et al found that the relationship between Hb and serum LDH is not linear.[3] The activity of this enzyme increased disproportionately when anaemia was very severe. They also found that the serum LDH decreases after treatment and suggested that post treatment serum LDH measurement is a good and accurate method for assessing early response to treatment. The elevated serum LDH was due to ineffective hematopoiesis in bone marrow, and not due to the peripheral hemolysis.[15-17]

Jaswal et al stated that raised serum LDH levels were seen in all types of macrocytic anaemia, but serum total LDH values >3000IU/L are diagnostic of megaloblastic anaemia and values between 451-3000IU/L can be seen in megaloblastic anaemia with early megaloblastic change, dimorphic anaemia and hemolytic anemia.[17]

Savage et al found that LDH elevations >220IU/L were not specific or sensitive for megaloblastic anaemia. But when LDH >1000U/L were taken, they got sensitivity of 22.2%, specificity of 97.5%, positive predictive value of 36.4% and negative predictive value of 95.1%.[16]

In present study, we found that when serum LDH was taken as >1124.5IU/L (criterion value of ROC curve), the sensitivity was 83.3%, specificity was 64.1% with positive predictive value of 56.6% and a negative predictive value of 87.2% and a total accuracy of 71%.

When LDH value >1124.5 IU/L or MCV >121fl were taken along with corrected reticulocyte count <2%, the sensitivity was 100% and specificity was 43.8%. So, this criterion can be used as a screening test to differentiate the two groups and further investigation can be done according to the categorization.

Aitelli et al, had stated that vitamin B12 deficiency can cause profound alterations in the bone marrow and may

mimic acute leukaemia or myelodysplasia.[18] However, after further studies, they were both found to have vitamin B12 deficiency, and parenteral vitamin B12 administration resulted in normalization of the bone marrow. So, a trial of vitamin B12 and folic acid therapy should be given before labelling a case as myelodysplasia.[21]

With the advent of automated cell coulters, the red cell indices form an integral part of diagnosis. Abnormally high values of MCV are common, but their precise clinical significance may be difficult to establish. With a step-wise diagnostic approach, a definitive diagnosis can be reached in most cases. In a resource limited set up, where facilities to measure vitamin B12 and folic acid are not available, a trial of hematinic therapy can be given to those presenting with typical clinical features along with raised MCV and moderate to markedly elevated serum LDH and peripheral blood findings (anisopoikilocytosis, macrocytes, macroovalocytes, hypersegmented neutrophils) and reduced to normal reticulocyte count. Response can be assessed using serum LDH, CBC and reticulocyte count. Those not responding to therapy can be further evaluated.

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