International Journal of Research in Medical Sciences
*Kannan A et al. Int J Res Med Sci. 2016 Jul;4(7):2670-2678*
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20161930

**Research Article**

# Evaluation of clinical, biochemical and hematological parameters in macrocytic anemia

**Aarthi Kannan[1,2]*, Vijai Tilak[2], Madhukar Rai[3], Vineetha Gupta[4]**

[1]Department of Pathology, Chettinad medical college and research institute, Kelambakkam, Tamilnadu, India
[2]Department of Pathology, Institute of medical sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India
[3]Department of General Medicine, Institute of medical sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India
[4]Department of Pediatrics, Institute of medical sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

**Received:** 30 May 2016
**Accepted:** 02 June 2016

***Correspondence:**
Dr. Aarthi Kannan,
E-mail: aarthi.kanna@gmail.com



## ABSTRACT

**Background:** Macrocytosis is a common finding encountered in automated coulters and evaluation of clinical, biochemical and haematological parameters in macrocytic anemias will provide a clue to diagnosis. This study was done to evaluate the clinical and laboratory parameters in macrocytic anemias and their utility in differentiating megaloblastic and non-megaloblastic anemia.
**Methods:** 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 and reticulocyte count was done in all cases and Vitamin $B_{12}$ and folate levels, bone marrow aspiration and bone marrow biopsy in some cases. After evaluation, a provisional diagnosis was made and patients with megaloblastic anaemia was given vitamin $B_{12}$ and folic acid treatment and followed up after 2 weeks and 1 month.
**Results:** Primary bone marrow disorders were the most common cause of macrocytosis (46%). The other causes in decreasing order of frequency were megaloblastic anaemia (38%), hemolytic anemia (6%), drug induced (5%), alcoholism and liver disease (4%) 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 >1345.2 IU/L or MCV>121fl (criterion values of ROC curve) with reticulocyte count <2% was taken as criteria, the sensitivity was 92.1% and specificity was 93.5% 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:** Megaloblastic anemia, Macrocytosis, Non-megaloblastic macrocytosis, Serum LDH, Mean corpuscular volume, Anemia

## INTRODUCTION

After the introduction of the automated cell coulters, the incidence of detecting macrocytosis have increased and has varied from 1.7 to 3.6% in several reported series.[1-4] Detectable macrocytosis may not be present in peripheral smear in about 33% of cases with MCV >100fl.[1] Mild increase in MCV (MCV of 100 – 110fl) is particularly common and most often remains unexplained though it cannot be ignored. So, the evaluation of macrocytosis

needs a systemic approach.[5] Macrocytosis can be seen in many hematological and non-hematological disorders and more than one cause may co-exist in an individual. Serum vitamin $B_{12}$ and folic acid tests are routinely ordered but they are limited by their low sensitivity and specificity.[5-8] Methyl malonic acid and homocysteine levels, though sensitive is costly and not available in all places. 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 parameters.

## METHODS

A prospective cross-sectional descriptive study was conducted for a period of 18 months on adult and pediatric cases presenting with macrocytosis to the department of pediatrics and General medicine of Sir Sunderlal Hospital, Banaras Hindu University, Uttar Pradesh, India.

Cases with age >12 years having MCV≥100fl at presentation and cases with age ≤12 years 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 Mindray BC 3000 and 5000 automated hematology analyzers (manufactured in China). The analyzer has been standardized with internal and external quality control.

External quality control was done regularly using samples from BIO-RAD Liquicheck - hematology 16 controls manufactured by BIO – RAD, Irvine, CA, USA. Peripheral blood smear and reticulocyte count was done in all cases. Bone marrow aspiration and biopsy were done in necessary cases. Biochemical parameters including bilirubin levels, serum iron, TIBC and serum LDH were done in all cases using flexor open analyzer (manufactured in USA) in which internal control was done using manufacturer control and external control was done from Christian Medical College, Vellore samples. Thyroid function tests, vitamin $B_{12}$ 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 $B_{12}$ and folate levels, megaloblastic erythropoiesis, dysplasia and blastoid cells were initially placed in the megaloblastic group. A trial of hematinic therapy with vitamin $B_{12}$ and folic acid was given and response was seen after 1 week, 2 week and after 1 month. Response was seen with reticulocyte count, CBC reports and serum LDH values. Those who did not respond to the treatment were further evaluated and diagnosis was reclassified. Observations were recorded and analysis was done using SPSS V.19 software and medcalc software. p values were calculated by Fisher's exact test or Mann Whitney U test wherever necessary.

### *Ethical clearance*

This study has been approved by the Institute Ethical Committee Board (Institute of Medical Sciences, BHU) and therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All studies followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008(5). Informed consent have been taken from all patients involved in the study.

## RESULTS

100 cases of macrocytosis were included in the study. Out of 100 cases, 38% had megaloblastic anemia and 62% had non-megaloblastic macrocytosis. Primary bone marrow disorders (46%) were the most common cause of macrocytosis in our study. This category included acute and chronic leukemia's, aplastic anemia, multiple myeloma, myelofibrosis 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 | 38 | 38% |
| Acute leukemia | 18 | 18% |
| Myelodysplastic syndrome | 12 | 12% |
| Aplastic anemia | 10 | 10% |
| Hemolytic anemia | 6 | 6% |
| Drug therapy induced | 5 | 5% |
| Alcoholism and liver disease | 4 | 4% |
| Multiple myeloma | 2 | 2% |
| Myelofibrosis | 2 | 2% |
| CML in chronic phase | 1 | 1% |
| Hairy cell leukemia | 1 | 1% |
| Idiopathic thrombocytopenic purpura | 1 | 1% |
| **Total** | **100** | **100%** |

One patient of aplastic anemia had associated Paroxysmal nocturnal hemoglobinuria. Hypothyroidism was seen in one case of megaloblastic anemia and one case of myelodysplastic syndrome. Drug induced macrocytosis were seen associated with Zidovudine, hydroxyurea, phenytoin and methotrexate.

***Clinical parameters***

The minimum age at presentation was 4 years and maximum age was 75 years with a mean of 29.69±18.15years. The mean age at presentation in non – megaloblastic group was 32.69±20.54years which is approximately 8 years later than the megaloblastic group (24.79±1.21years). Around half of the cases (55%) of megaloblastic anemia were between 11-20 years of age. Out of 100 cases studied 48 cases were males and 52 were females with a slight female preponderance (1:1.08).

Symptoms of anemia were present in 87% of the cases. Symptoms of anemia included breathlessness, easy fatigability, and generalized weakness. Bleeding episodes were present in 18 cases; out of which 14 cases belonged to the non-megaloblastic group. Only 4 cases of megaloblastic group presented with bleeding episodes. Neurological manifestations like ataxia, parasthesia were significantly associated with megaloblastic anemia. (p= 0.00). History of jaundice was present in 26 cases; out of it 18 cases belonged to the megaloblastic group. Visual disturbances like blurring of vision and photophobia was present in 4 cases out of which 1 case belonged to megaloblastic group. Other symptoms seen infrequently in the non-megaloblastic group were abdominal fullness, blackish discoloration of skin, bone pains, hepatic failure, headache, dragging sensation in left abdomen and loss of consciousness. Past history of surgery for gastric perforation and ileal resection for tuberculosis was present in the megaloblastic group. History of alcohol intake was present in 5(8.1%) cases of non – megaloblastic macrocytosis. Most of the cases in the megaloblastic group (30/38) were vegetarians and there was a significant difference (p=0.0017) in diet between the two groups with 78.9% cases of vegetarians in megaloblastic group.

***Haematological and biochemical parameters***

Though most of the cases (98%) presented with anemia, 2 cases did not have anemia. One case had alcohol induced macrocytosis and the other case was drug induced (hydroxyurea). Skin pigmentation in the knuckles and fingers was seen in 8 cases (21.1%) of megaloblastic anemia and was significantly associated with this group (p = 0.0011). Icterus was present in 19 cases (50%) of megaloblastic anemia and was significantly associated with it and glossitis was seen exclusively in this group. In megaloblastic anemia 5 cases (13.0%) had fundal abnormalities in the form of bilateral fundal bleeds (5.2%), Roth spots (5.2%), and splinter hemorrhages (2.6%).

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

| Parameter | Megaloblastic (n = 38) | | | | Non – megaloblastic (n = 62) | | | | p value |
|---|---|---|---|---|---|---|---|---|---|
| | Min | Max | Mean | STD | Min | Max | Mean | STD | |
| TLC (cellsX10^3/µL) | 1400 | 15000 | 5264.47 | 2713.39 | 600 | 270000 | 19727.10 | 46929.66 | 0.47 |
| Hb (g/dl) | 1.6 | 8.9 | 4.72 | 1.79 | 1.3 | 16.2 | 5.26 | 2.82 | 0.58 |
| PLT (cellsX10^3/µL) | 9000 | 287000 | 108605.3 | 69023.51 | 6000 | 632000 | 109951.61 | 148718.28 | *0.00* |
| HCT (%) | 5.2 | 25.7 | 14.23 | 5.57 | 4.0 | 46.8 | 16.02 | 8.85 | 0.51 |
| RBC (cellsX10^6/µL) | 0.42 | 2.24 | 1.24 | 0.52 | 0.35 | 4.49 | 1.47 | 0.84 | 0.24 |
| MCV (fl) | 101.8 | 141.4 | 118.59 | 10.35 | 100.3 | 126.8 | 110.08 | 7.01 | *0.00* |
| MCH (pg) | 30.0 | 54.0 | 39.23 | 5.74 | 29.5 | 54.9 | 37.43 | 4.87 | 0.12 |
| RDW (%) | 13.9 | 34.9 | 19.17 | 5.02 | 13.0 | 33.9 | 18.59 | 3.98 | 0.94 |
| CRC (%) | 0.02 | 2.59 | 0.71 | 0.54 | 0.02 | 14.0 | 1.42 | 2.76 | 0.50 |
| LDH (IU/L) | 753.9 | 19740.0 | 4660.26 | 4490.09 | 58.0 | 4470.0 | 682.72 | 615.68 | *0.00* |

*p value is significant

The mean Hb value was 5.06 and a standard deviation of ±2.48g/dl. The values in MCV ranged from 100.3 to 141.4 fl with a mean of 113.31fl and standard deviation of ±9.36fl. The serum LDH values ranged from 58.0 U/L to 19740.0 U/L with a mean value of 2194.19U/L and standard deviation of ±3396.10U/L. The hematological values are listed in Table 2. A significant difference was seen in the values of serum LDH (p=0.00) and MCV (p = 0.00) values between the two groups. Box plot graph was drawn with the MCV values in megaloblastic and non – megaloblastic anemia. The central line of the box represents the median values and the box represents the cases between the 25th and 75th percentile (interquartile range). The median value in megaloblastic group (MGB) was 118.59fl with the inter quartile range of 110.7 to 126fl. The median value in the non – megaloblastic group (non- MGB) was 108.4fl with the interquartile range of 104.3–116.4 fl. There was significant difference in the

means of MCV between the two groups. (p value=0.00) (Figure 1). MCV values were in the range of 100.1-110 in 45 cases, out of which 36 cases had non-megaloblastic macrocytosis. No cases of non-megaloblastic macrocytosis had MCV >130fl. Maximum MCV value was seen in megaloblastic anemia (141.1fl) (Table 3).

**Table 3: Percentage of cases in megaloblastic and non-megaloblastic group under different MCV values.**

| MCV Range In fl | All cases (n = 100) | | Megaloblastic ( n = 38) | | Non – megaloblastic (n = 62) | |
|---|---|---|---|---|---|---|
| | No. of. Cases | Percentage | No. of Cases | Percentage | No. of Cases | Percentage |
| 100.1–110 | 45 | 45% | 9 | 23.7% | 36 | 58.1% |
| 110.1–120 | 31 | 31% | 12 | 31.6% | 19 | 30.6% |
| 120.1–130 | 17 | 17% | 10 | 26.3% | 7 | 11.3% |
| 130.1–140 | 6 | 6.0% | 6 | 15.8% | - | - |
| 140.1–150 | 1 | 1% | 1 | 2.6% | - | - |

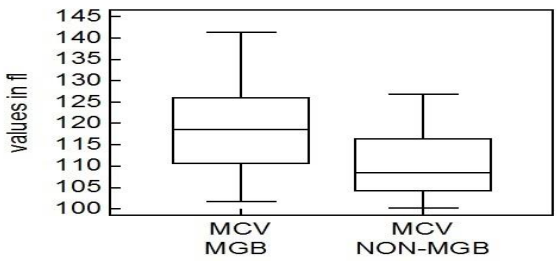


**Figure 1: Box plot graph demonstrating the median and the interquartile range for the MCV values in megaloblastic and the non-megaloblastic group.**

Another Box–plot graph was drawn with serum LDH values in megaloblastic and the non – megaloblastic group. The median value of serum LDH in megaloblastic anemia was 2990.5 IU/L and the interquartile range was 1925 – 5240 IU/L. 4 outliners were present in megaloblastic anemia that had much higher values than the rest of the group. Their values were 12168IU/L, 15102IU/L, 16920IU/L and 19740IU/L. This show that patient with megaloblastic anemia can have very extreme values, as high as 63 times the normal values.

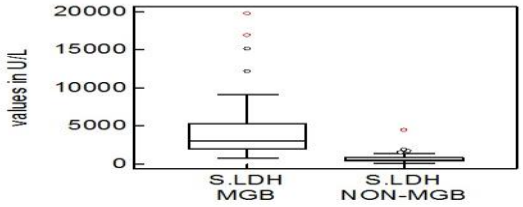


**Figure 2: Box plot graph demonstrating the median and the interquartile range for the serum LDH values in megaloblastic and the non-megaloblastic group.**

**Table 4: Percentage of cases in megaloblastic and non – megaloblastic group under different serum LDH values.**

| LDH range in IU/L | Increased by (times) | Total (n = 100) | | Megaloblastic (n = 38) | | Non – megaloblastic (n =62) | |
|---|---|---|---|---|---|---|---|
| | | No. of Cases | Percentage | No. of Cases | Percentage | No. of Cases | Percentage |
| <300 | Normal | 12 | 12% | - | - | 12 | 17.4% |
| 301-600 | 1 | 27 | 27% | - | - | 27 | 43.5% |
| 601-900 | 2 | 15 | 15% | 2 | 5.3% | 13 | 21.0% |
| 901-1200 | 3 | 2 | 2% | 1 | 2.6% | 1 | 1.6% |
| 1201-1500 | 4 | 6 | 6% | 1 | 2.6% | 5 | 8.1% |
| 1501-1800 | 5 | 7 | 7% | 5 | 13.2% | 2 | 3.2% |
| 1801-2100 | 6 | 3 | 3% | 2 | 5.3% | 1 | 1.6% |
| 2101-2400 | 7 | 3 | 3% | 3 | 7.9% | - | - |
| 2401-2700 | 8 | 4 | 4% | 4 | 10.5% | - | - |
| 2701-3000 | 9 | 1 | 1% | 1 | 2.6% | - | - |
| >3001 | 10 | 20 | 20% | 19 | 50% | 1 | 1.6% |

The median value in the non-megaloblastic group was 497.50IU/L with the interquartile range of 381-805IU/L. 4 outliners were present in which one case had very high value of 4470IU/L, this patient had hemolytic anemia. The other cases were aplastic anemia with PNH (1902.5IU/L), CML in chronic phase (1725IU/L) and autoimmune hemolytic anemia (1508IU/L) (Figure 2). Serum LDH values were normal in 12 cases of non-

megaloblastic anemia (17.4%). No cases of megaloblastic anemia had serum LDH <600 IU/L, but 27 cases of the non – megaloblastic group (43.5%) had serum LDH between 301-600 IU/L.

20% cases in total had serum LDH >3001U/L (10 times higher than normal) out of which 19 cases belonged to megaloblastic group and 1 case of hemolytic anemia in non - megaloblastic group had LDH of 4470 U/L (Table 4).

Serum bilirubin was raised in 27 cases of megaloblastic anemia (71.1%) with a mean value of 2.83mg/dl and a standard deviation of 1.34mg/dl.

**Table 5: Sensitivity and specificity for megaloblastic anemia at different levels of serum LDH values.**

| Serum LDH Values (IU/L) | Sensitivity | Specificity |
|---|---|---|
| >682 | 100.00 | 70.97 |
| >803 | 94,74 | 74.19 |
| >1020 | 92.11 | 85.48 |
| >1217 | 92.11 | 87.10 |
| >1345.2* | 92.11 | 93.55 |
| >1473 | 83.33 | 91.30 |
| >1508 | 86.84 | 95.16 |
| >1925 | 73.68 | 98.39 |
| >4470 | 31.58 | 100.00 |

*criterion value of ROC

Pancytopenia was present in 36 cases (36%), out of which 12 cases (31.6%) belonged to megaloblastic group and 24 cases (38.7%) 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.

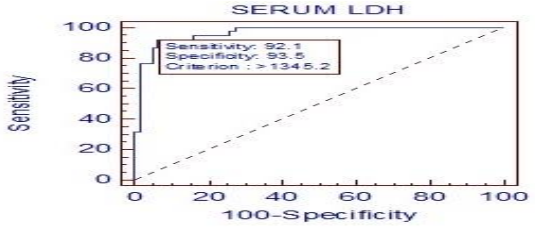


**Figure 3: ROC curve for serum LDH values.**

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 >1345.2 U/L for megaloblastic anemia (Table 5), at which the sensitivity was 92.11% specificity was 93.55 % with a positive predictive values of 87.5% and an accuracy of 92%.

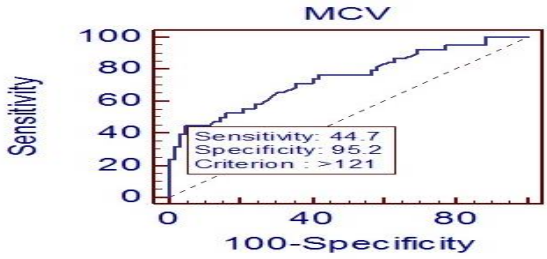


**Figure 4: ROC curve for MCV values.**

The area under the curve was 0.966 and the p value was <0.0001 (Figure 3). From the chart, it is inferred that as patient's LDH value rises above 1345.2U/L, there is more probability of having megaloblastic anemia than non-megaloblastic anemia and cases having serum LDH >4470U/L will most probably not have non-megaloblastic macrocytosis. ROC curve analysis for MCV values gave a criterion of >121fl for megaloblastic anemia (Table 6), at which the sensitivity was only 44.7% but specificity was 95.2% with a positive predictive value of 80.95% and an accuracy of 75%. The area under the curve was 0.743 and the p value was <0.0001 (Figure 4). From this curve, it is inferred that cases having MCV>126.8fl will probably not have non-megaloblastic macrocytosis.

**Table 6: Sensitivity and specificity for megaloblastic anemia at different values of MCV.**

| MCV values (fl) | Sensitivity | Specificity |
|---|---|---|
| >101.5 | 100 | 11.29 |
| >110 | 76.32 | 58.06 |
| >115.3 | 60.53 | 72.58 |
| >121* | 44.7 | 95.2 |
| >126.8 | 23.68 | 100 |
| >131 | 15.79 | 100 |

*criterion value of ROC

When LDH>1345.2U/L or MCV >121fl was taken as a criteria along with Reticulocyte count <2%, the sensitivity was 92.1%, specificity was 93.5%, positive predictive value was 89.7%, negative predictive value was 95%, and accuracy was 93%. Since the sensitivity is 92.1% and specificity is 93.5%, 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 (Table 7).

**Table 7: Sensitivity, specificity, positive predictive value, negative predictive value and accuracy for diagnosing megaloblastic anemia at ROC criterion values.**

| Condition | Sensitivity | Specificity | Positive predictive value | Negative predictive value | Accuracy |
|---|---|---|---|---|---|
| Serum LDH>1345.2IU/L (criterion Value) | 92.1% | 93.5% | 87.5% | 95% | 92% |
| When MCV>121fl (criterion Value) | 44.74% | 95.2% | 80.6% | 73.4% | 75% |
| When S.LDH>1345.2IU/L Or MCV>121fl | 94.7% | 87% | 81.8% | 96.4% | 90% |
| When S.LDH>1345.2IU/L Or MCV>121fl And CRC<2% | 92.1% | 93.5% | 89.7% | 95% | 93% |

## DISCUSSION

The incidence of macrocytosis has varied from 1.7 to 3.6% in several reports.[1-4] Though 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 were 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 [9] used MCV>115fl, Unnikrishnan et al used MCV>95fl) used along with varied environmental and demographic factors.[10]

The various studies on macrocytosis are listed in the Table 8. 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.[5]

The lowered mean age at presentation in megaloblastic anemia may be due to increased demand during growth spurt and puberty. There was female preponderance in megaloblastic anemia in our study which was similar to the study conducted by Khanduri U and Sharma A.[14]

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 Unnikrishnan et al.[10]

The presence of fundal abnormalities as seen in our case has been reported in anemia by few authors.[15-17] The mean haemoglobin in present study was 5.06g/dl with standard deviation of 2.48g/dl which was similar to Unnikrishnan V et al where the mean Hb was 5.6g/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.[1,10]

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 113.31±9.36fl which is much higher than that of Davidson et al and Unnikrishnan V et al.[1,10]

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 patient in other disorders.[5,9]

But if MCV exceeds 120fl, megaloblastic erythropoiesis was most likely. In our case too, we found that when MCV was between 110-120fl, almost equal number of cases were present in both the 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 98.6%.

In present study, it was found that the specificity for megaloblastic anaemia at MCV>121fl was 95.16% and this was comparable with the above mentioned study. So, if the MCV>121fl, there is 95% chance that the disease is not a non-megaloblastic. Study found a weak positive correlation (r = 0.324) between MCV and serum LDH, which was significant at 0.01 level. There was a significant difference between means of serum LDH between megaloblastic and non – megaloblastic group.

The maximum value of serum LDH was 19740 IU/L which was 54 times the normal value. Values as high as 28,125 units/L have been reported and the serum LDH values reduced after treatment.[18] A correlation between reticulocytosis and decreasing LDH activity, followed later by rise in haemoglobin has been found in few studies.[19,20]

**Table 8: 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[9] | Connecticut | 1973 | 100 | Adult patients | >115fl | Vitamin B12 and Folic Acid deficiency 50%) | Liver disease (15%) |
| Wymer et al[4] | 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[5] | Colorado | 2000 | 300 | Adult cases | ≥100fl | Drug induced (37%) | Alcoholic liver disease & alcohol abuse (each 13.33%) |
| Unnikrishnan V et al[10] | Puducherry, India | 2008 | 60 | Adult cases (age ≥ 13 years) | >95fl, and patients with anaemia | Megaloblastic anaemia (38.4%) | Primary bone marrow disorders (35%) |
| Breeveld et al[2] | The Netherlands | 1981 | 70 | All cases | ≥105fl | Vitamin B12 and Folic acid deficiency (38.57%) | Alcohol Abuse (27.14%) |
| Present study | Varanasi, India | | 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 anaemia (38%) |

In present study too, there is a significant difference between LDH values before and after treatment. 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 451-3000 IU/L can be seen in megaloblastic anaemia with early megaloblastic change, dimorphic anaemia and hemolytic anemia.[21] Emerson et al found that the relationship between Hb and serum LDH is not linear.[22] 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.

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%.[5] In present study, we found that when serum LDH was taken as >1345.2 IU/L (criterion value of ROC curve), the sensitivity was 92.1%, specificity was 93.5% with positive predictive value of 87.5% and a negative predictive value of 95% and a total accuracy of 92%.

When LDH value >1345.2 IU/L or MCV >121fl were taken along with corrected reticulocyte count <2%, the sensitivity and specificity became >90%. 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.

Two cases of megaloblastic anaemia had increased blastoid cells in the marrow but those patients responded to treatment. Cases of megaloblastic anaemia having blastoid changes have been reported. Aitelli et al had stated that Vitamin B12 deficiency can cause profound alterations in the bone marrow and may mimic acute leukaemia or myelodysplasia.[23] However, after further studies, they were both found to have vitamin $B_{12}$ deficiency, and parenteral vitamin $B_{12}$ administration resulted in normalization of the bone marrow. So, a trial of Vitamin $B_{12}$ and folic acid therapy should be given before labelling a case as myelodysplasia.

12 cases in present study, which initially showed megaloblastic erythropoiesis in bone marrow were given treatment with $B_{12}$ and folic acid and was followed up. They didn't respond to treatment even after 1 month of therapy and repeat marrow aspirations were done in these patients. All of them showed megaloblastic erythropoiesis with features of dysplasia in one or more lines with increased number of blasts. When evaluated retrospectively these patients had only mild increase in serum LDH levels when compared to their megaloblastic anaemia counterpart.

With the advent of automated cell coulters, the red cell indices form an intergral 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.

## ACKNOWLEDGEMENTS

Authors would like to acknowledge the help given by the medicine residents and the technical staff of hematology department, IMS, BHU.

*Funding: No funding sources*
*Conflict of interest: None declared*
*Ethical approval: This study has been approved by the Institute Ethical Committee Board (Institute of Medical Sciences, BHU)*

## REFERENCES

1. Davidson RJL, Hamilton PJ. High mean red cell volume: its incidence and significance in routine haematology. J Clin Pathol. 1978;31(5):493-8.
2. Breedveld FC, Bieger R, van Wermeskerken RK. The clinical significance of macrocytosis. Acta Med Scand. 1981;209(4): 319-22.
3. Colon-Otero G, Menke D, Hook CC. A practical approach to the differential diagnosis and evaluation of the adult patient with macrocytic anemia. Med Clin North Am. 1992;76(3):581-97
4. Wymer A, Becker DM: Recognition and evaluation of red cell macrocytosis in the primary care setting. J Gen Intern Med. 1990;5(3):192-7.
5. Savage DG, Ogundipe A, Allen RH, Stabler SP, Lindenbaum J. Etiology and diagnostic evaluation of macrocytosis. Am J Med Sci. 2000;319(6):343-52.
6. Savage DG, Lindenbaum J, Stabler SP, Allen RH. Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosing cobalamin and folate deficiencies. Am J Med. 1994;96(3):239-46.
7. Savage DG, Allen RH, Gangaidzo IT, Levy LM, Gwanzura C, Moyo A, et al. Pancytopenia in Zimbabwe. Am J Med Sci. 1999;317(1):22-32.
8. Savage DG, Gangaidzo IT, Lindenbaum J, Kiire C, Mukiibi JM, Moyo A, et al. Vitamin B12 deficiency is the primary cause of megaloblastic anemia in Zimbabwe. Br J Haematol. 1994;86(4):844-50.
9. McPhedran, P, Barnes MG, Weinstein JS, Robertson JS. Interpretation of electronically determined macrocytosis. Annals of Internal Medicine. 1973;78(5):677-83.
10. Unnikrishnan V, Dutta TK, Badhe BA, Bobby Z, Panigrahi AK. Clinico – aetiologic profile of macrocytic anemias with special reference to megaloblastic anemia. Indian J. Hematol. Blood Transfus. 2008;24(4):155-65.
11. Bridgen ML. A systemic approach to macrocytosis. sorting out the cases. Postgrad Med. 1995;97(5):171-2, 175-7, 181-4 passim.
12. Lee GR. Anemia: a diagnostic strategy. In: Lee GR, Foerster J, Lukens J, Paraskevas F, Greer JP, Rodgers GM, Wintrobe MM editors. Wintrobe's clinical hematology, 10th ed. Baltimore: Williams & Wilkins. 1998;908-40.
13. Mahmoud MY, Lugon M, Anderson CC. Unexplained Macrocytosis in Elderly Patients. Age and ageing. 1996;25(4):310-2.
14. Khanduri U and Sharma A. Megaloblastic anaemia: prevalence and causative factors. The national medical journal of india. 2007;20(4):172-5.
15. Marshall RA. A review of lesions in the optic fundus in various diseases of the blood. Blood. 1959;14(7):882-91.
16. Aisen ML, Bacon BR, Goodman AM, et al. Retinal abnormalities associated with anemia. Arch Ophthalmol. 1983;101(7): 1049-52.

17. Merin S, Freund M. Retinopathy in severe anemia. Am J Ophthalmol. 1968;66(6):1102-6.
18. Goldfarb TG, Papp BJ. Excessively high levels of lactic acid dehydrogenase activity in pernicious anemia. Am J Med. 1963;34:578-622.
19. Gordin R, Eanri TM. Lactic dehydrogenase in vitamin B12 deficiency. Acta Hematol. 1959;21(1):16-22.
20. Emerson PM, Wilkinson JH. Lactate dehydrogenase in the diagnosis and assessment of response to treatment of megaloblastic anemia. Brit J Hematol. 1966;12(6):678-87.
21. Jaswal TS, Mehta HC, Gupta V, Singh M, Singh S. Serum Lactate Dehydrogenase in diagnosis of megaloblastic anemia. Indian J. Pathol. Microbiol. 2000;43(3): 325-9.
22. Emerson PM, Withycome WA, Wilkinson JH. The origin of the elevated serum lactate dehydrogenase in megaloblastic anemia. Brit. J. Hematol. 1967;13(5):656-64.
23. Aitelli, Cristi BS, Wasson, Lori DO, Ray Page, DO. Pernicious Anemia: Presentations Mimicking Acute Leukemia. Southern Medical Journal. 2004;97(3):295-7.

**Cite this article as:** Kannan A, Tilak V, Rai M, Gupta V. Evaluation of clinical, biochemical and hematological parameters in macrocytic anemia. Int J Res Med Sci 2016;4:2670-8.