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EVALUATION OF BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS IN MACROCYTIC ANEMIA

Hematology

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ABSTRACT

Background: In the age of calibrated automatic analysers, biochemical and hematological parameter evaluation form the yardstick to define macrocytic anemias. The size of red cells in relation to mean corpuscular volume (MCV) has been used to categorize anemia. Aim of current study was to evaluate the role of routine blood parameters and biochemical parameters in differentiating megaloblastic anemia and non megaloblastic anemia. **Methods:** A retrospective study was carried out on retrieved records (laboratory parameters) of the patients who were admitted at a tertiary care hospital, this included a total of 90 cases of anaemia between January 2020 - July 2021. These included records of patients with anaemia based on Vitamin B12 levels, LDH levels. Other various parameters like mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were also taken into consideration for descriptive statistics. **Results:** Liver disease was the most common cause of macrocytosis (40%). The other causes in decreasing order of frequency were megaloblastic anaemia (27.7%), hemolytic anemia (8%), drug induced (9%).When comparisons of various complete blood count parameters between megaloblastic and non-megaloblastic group was done with LDH having significance (p=0.001) in megaloblastic group and .011 in non-megaloblastic group. **Conclusions:** Evaluation of Methyl malonic acid and homocysteine levels, is sensitive, but expensive and scarcely available. The overall performance which is measured using Area under the Curve indicates that a contribution of LDH and MCV are good at differentiating megaloblastic Vs. Non megaloblastic causes of macrocytic anemias, However a larger population should be studied to find variances between ethnicity, gender and age

KEYWORDS

Anemia, Macrocytosis, LDH, Mean corpuscular volume, Megaloblastic anemia

INTRODUCTION

In the age of calibrated automatic analysers, biochemical and hematological parameter evaluation form the yardstick to define macrocytic anemias. The size of red cells in relation to mean corpuscular volume (MCV) has been used to categorize anemia [(1)]. Mean corpuscular volume (MCV of 100 – 110fl) measures the average volume of a red blood corpuscle [(2)]. MCV is calculated by multiplying the percent hematocrit by ten divided by erythrocyte count.[(2)] Macrocytic anemias can be categorized as megaloblastic and non-megaloblastic. Whereas the non-megaloblastic moiety results from a variety of processes, the megaloblastic form is caused by impaired DNA synthesis brought on by folate and/or vitamin B12 deficiency[(3)] .

Differentiating the two types of macrocytic anemias becomes imperative to establish early management which differs in the two categories. Synthesis of RBC nucleic acids requires folate and vitamin B12; without DNA and RNA there is ineffective erythropoiesis and nuclear cytoplasmic asynchrony. [(3)] With the introduction of automated cell coulters, there has been an increase in the detection of macrocytosis, ranging from 1.7 to 3.6 percent [(4)]. The most sensitive method of detecting isolated macrocytosis is to use an automated blood cell counter [(4)]. A peripheral blood smear examination will reveal undetectable macrocytosis in up to 33% of all patients with an MCV greater than 100, as determined by automated cell counting [(4)]. Though predominantly seen in megaloblastic anemias, Macrocytic erythrocytes are linked to several pathological conditions, including certain drugs. One can differentiate between megaloblastic and non-megaloblastic macrocytosis with the aid of systematic evaluation. Securing seum vitamin and metabolite levels is challenging; a combination of blood and biochemical markers, particularly CBC and serum LDH combined with LFT, may be able to assist in the diagnosis of megaloblastic anaemia. Anemia and neurological problems are the typical signs of vitamin B12 insufficiency. An unusual observation of increased liver function has been observed in vitamin B12 deficiency. When treated with vitamin B12 the liver enzymes reverted to normal within a month.

MATERIALS & METHODS

A retrospective study was carried out on retrieved records (laboratory parameters) of the patients who were admitted at a tertiary care hospital, this included a total of 90 cases of anaemia between January 2020 - July 2021. Patients who consulted and were admitted in the hospital during the study's duration had their (EDTA) anticoagulated blood samples obtained and run via the Sysmex XN-1000 (6 parts haematology automatic cell counter). Macrocytic anemias were categorized as megaloblastic anemia or non-megaloblastic anemia based on haemoglobin, MCV>100 and Vitamin B12 levels as follows: Megaloblastic anemia : Vitamin B12 (<187 pg/ml),[(2)] Non-megaloblastic, macrocytic anemia : Vit B12 :=/>187 pg/ml.[(2)] LFT and LDH levels were recorded in MS excel 2019, SPSS(Statistical package for social sciences) version 23 was used for statistical analysis.

RESULTS

The minimum age at presentation was 18 years and maximum age was 75 years. 25 cases had megaloblastic anemia a female preponderance and belonged predominantly in the 31-40 age group(Figure 2).

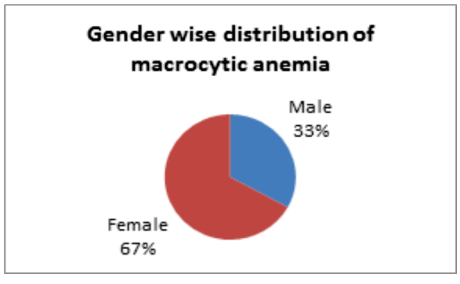


Figure 1: Gender wise distribution of macrocytic anemia

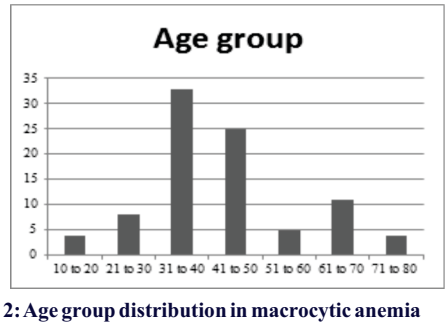


Figure 2: Age group distribution in macrocytic anemia

The research comprised 90 instances of macrocytosis. Non-megaloblastic causes include liver diseases and alcoholic abuse (40%),

Drugs(10%), hemolytic anemia (8.8%), chronic myeloid leukemia (3.3%), Acute myeloid leukemia(2.2%), Myelodysplastic syndrome (1.1%), Chronic lymphocytic leukemia(1.1%),Chronic kidney disease (4.4%). (Table 1)

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

	No of cases	Percentage
Liver disease	36	40%
Megaloblastic	25	27.7%
Hemolytic	8	8.8%
Drug induced	9	10%
Renal disease	4	4.4%
AML	2	2.2%
CML	1	1.11%
CLL	1	1.11%
MDS	1	1.11%

AML: Acute myeloid leukemia, CML: Chronic myeloid leukemia, CLL: Chronic lymphoid leukemia, MDS: Myelodysplastic syndrome.

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

	Megaloblastic		Non megaloblastic	
Parameters	Mean	STD	Mean	STD
HB	7.22	2.72	7.7	3.2
TLC	9.64	16.90	17.50	41.9
PLT	150.88	80.24	135.6	95.3
RBC	1.89	79683	2.12	9833
HCT	21.25	8.08	23.29	10.1
MCV	114.48	9.70	112.4	11.8
MCH	39.08	4.41	38.5	10.09
LDH	1744.40	1934.3	633.7	1097.9

HB: Hemoglobin, TLC: Total leukocyte count, PLT: Platelet count, RBC: Red blood cell, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, LDH:Lactate dehydrogenase

Table 3 comparisons of various complete blood count parameters between megaloblastic and non-megaloblastic group

Parameters	T value	P value	95% Confidence Interval of the Difference	
			Lower	Upper
HB	-0.662 -0.714	0.510 0.478	-1.9763 -1.8781	0.989 0.891
TLC	-0.904 -1.231	0.369 0.222	-25.159 -20.563	9.435 4.839
PLT	0.703 0.755	0.434 0.453	-27.922 -25.289	58.482 55.849
RBC	-1.059 -1.155	0.293 0.253	-0.677 -0.643	0.206 0.172
HCT	-0.893 -0.981	0.374 0.331	-6.582 -6.207	2.501 2.126
MCV	0.745 0.809	0.458 0.422	-3.334 -2.954	7.328 6.949
MCH	0.235 0.315	0.814 0.754	-3.687 -2.635	4.677 3.625
LDH	3.351 2.696	0.001 0.011	451.383 269.946	1769.9 1951.3

HB: Hemoglobin, TLC: Total leukocyte count, PLT: Platelet count, RBC: Red blood cell, HCT: Hematocrit, MCV: Mean corpuscular volume,MCH: Mean corpuscular hemoglobin, LDH:Lactate dehydrogenase

On comparison of various blood parameters, haemoglobin and total leukocyte count were found to be lower in megalolastic anemia with statistically significantly higher serum LDH levels, while other parameters failed to show statistical significant.

Table 4: Percentage of cases in megaloblastic and non-megaloblastic group under different MCV values

MCV range	Megaloblastic	Non megaloblastic
	Percentage	Percentage
100.1–110	36%	61.5%
110.1–120	36%	21.5%
120.1–130	20%	7.7%
130.1–140	8%	4.6%
140.1–150	0%	4.6%

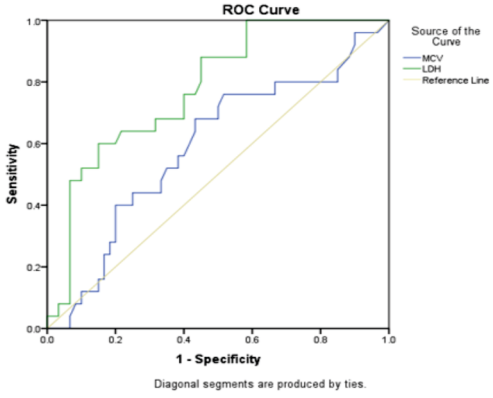
Majority of the cases were in the group of 100.1-110 group with a high number (61.5%) in non-megaloblastic group. In megaloblastic group, majority of the cases were in the MCV range of 100.1-120 with 18 cases. Three cases had MCV ranging in 140.1 to 150 from the non-megaloblastic group and no cases in the same MCV range of megaloblastic group. Taking a cut off MCV value of 110 it was noted that 64% of megaloblastic anemia had MCV > 110.

When LDH values were correlated with megaloblastic and non megaloblastic anemia, megaloblastic anemia had 9 cases with <300 LDH values and 36 cases in non megaloblastic anemia.

Table 5 Percentage of cases in megaloblastic and non – megaloblastic group under different serum LDH values

LDH group	Megaloblastic	Non-megaloblastic	Total
<300	9	36	45
301-600	1	6	7
601-900	0	8	8
901-1200	2	2	4
1201-1500	0	2	2
1501-1800	1	1	2
1801-2100	2	1	3
2101-2400	2	0	2
2401-2700	4	0	4
2701-3000	1	0	1
>300	3	4	7
Total	25	60	85

Area under the curve of LDH is 0.779 Which is better than that of MCV. From the chart it is inferred that when MCV and LDH is combined together there is a better predictive ability in discrimination of megaloblastic and non-megaloblastic anemias.



The cut off value for MCV is 109.75, derived using Youden's index. 68% sensitivity and 56.67% specificity. The cut off derived using Youden's index for LDH is 97.5. 60% sensitivity and 85% specificity.

DISCUSSION

Wintrobe recognised the importance of morphologic classification of anemia in 1934. Macrocytic, normocytic, simple microcytic, and hypochromic microcytic anaemias are some of his classifications.[5]

There have been a number of published surveys on macrocytosis. These papers documented relatively few patients, and there were wide variations in how macrocytosis was defined (Mcphedran et al[6] used MCV>115fl, Unnikrishnan et al used MCV>95fl[7] and some have used values ranging from 105 to 115 fl.)

Megaloblastic anaemia, caused by pernicious anaemia, was found to be the most frequent cause of macrocytic anaemia in Wintrobe's study.[5]The other causes, were cirrhosis, leukemia, myelodysplasia and aplasia.[5] Various western and Indian studies have different etiologies and demographic profiles. Drug therapy, Vitamin B12 and folate deficiencies, and alcohol misuse have all been linked to macrocytosis. In present study liver diseases and alcoholic abuse (40%) was the most common cause of macrocytosis. Using data from electronic cell

counts, Hattersley conducted a survey of 120 patients in 1964 and concluded that liver cirrhosis was the most frequent cause of macrocytosis.[8] More than 50% of the patients in his series had an MCV of 100-104.5 fl. Several macrocytosis studies are listed.

Table 6: Causes of macrocytosis in various studies.

Name of the Study	Place	Year	No of cases	MVC criteria used	Most common cause
Mahmoud MY et al 9	London	1996	124	>95fl	Megaloblastic anaemia (26.6%)
Savage DG et al 10	Colorado	2000	300	≥100fl	Drug induced (37%)
Unnikrishnan V et al 7	Puducherry	2008	60	>95fl	Megaloblastic anaemia (38.4%)
Aarthi Kannan et al 11	Varanasi	2015	100	≥100fl	Primary bone marrow disorders (46%)
Present study	Karnataka	2021	90	>100fl	Liver disease

Most patients with megaloblastic erythropoiesis and primary bone marrow abnormalities were 70 years of age or older, according to Savage et al,[10] whereas the mean age at presentation in Aarthi Kannan [11] was between 20 and 40 years of age. In our study mean age was 31 to 40 age group. The lower mean age at presentation in megaloblastic anaemia may be due to increased demand during growth spurts and adolescence. In our study, which was identical to Khanduri U, Sharma A, and Aarthi Kannan's, there was a female preponderance in megaloblastic anaemia.[11]

The mean hemoglobin value was ranging from 7.220 with 2.7263 standard deviation in megaloblastic. The degree of macrocytosis grew in direct proportion to the severity of the anaemia. However, neither our study nor those of Unnikrishnan V et al[7] or Aarthi Kannan[11] found such a large rise.

Majority of the cases were in 110-120fl category done by Aarthi Kannan.[11] Showed majority of megaloblastic anemias in 111-120 fl. When comparisons of various complete blood count parameters between megaloblastic and non-megaloblastic group was done with LDH having significance (p=0.001) in megaloblastic group and .011 in non-megaloblastic group, while other parameters failed to show statistical significant.

Table 7: Cut off value for MCV

	Sensitivity	Specificity
>101.15	96	8.333333
>109.75	68	56.66667
>115.7	44	73.33333
>121.45	24	81.66667
>126.6	12	88.33333
>130.6	8	90

From above table the cut off for MCV is 109.75. The cut off is decided using Youden's index. If MCV is more than 109.75 it indicates that the subject belongs to megaloblastic anemia group. This cut off has 68% sensitivity and 56.67% specificity.

When using the MCV value of 109.75 the sensitivity was 68% and specificity was 56.6% which did not parallel with the study conducted by Aarthi Kannan et al[11], who concluded that MCV was >120fl, the specificity for diagnosing the case as megaloblastic was 98.6%, in our study the specificity ranged from 81% to 90%. If the MCV value was more than >121fl the chances of it not being non megaloblastic remains higher.

Table 8: Cut off value for LDH

	Sensitivity	Specificity
>677	60	81.66667
>867.5	60	83.33333
>997.5	60	85
>1105	52	85
>1260	52	86.66667
>1409.5	52	88.33333
>1862.5	44	93.33333
>4164.5	8	96.66667

The cut off decided using Youden's index for LDH is 997.5. If LDH is more than 997.5 it indicates that the subject belongs to megaloblastic anemia group. This cut off has 60% sensitivity and 85% specificity. Even though LDH has better predictive ability, MCV outperforms it in terms of sensitivity. The MCV cut off is more sensitive (ie. is good in detecting g megaloblastic anemia), LDH cut off is more specific (i.e. good in detecting non megaloblastic anemia), the overall performance which is measured using Area under the Curve indicates that MCV combined with LDH has a better detection rate. In the study done by Aarthi Kannan et al[11] found a weak positive correlation between MCV and serum LDH, which was significant.

There has been studies reported that serum LDH levels reduces after treatment.[12] However in our studies we were not able to follow up the cases due to the COVID pandemic and many patients in our centers were from distant places.

An unusual observation of vitamin B12 deficiency with deranged liver function has been observed. When treated with vitamin B12 the liver enzymes reverted to normal within a month.[13] As there was no follow up of the cases available these findings were not correlated.

Limitations

A larger sample size with follow up including clinical history and treatment history is required to generalize the study.

CONCLUSION

Evaluation of Methyl malonic acid and homocysteine levels, is sensitive, but expensive and scarcely available. The overall performance which is measured using Area under the Curve indicates that a contribution of LDH and MCV are good at differentiating megaloblastic Vs. Non megaloblastic causes of macrocytic anemias, However a larger population should be studied to find variances between ethnicity, gender and age. An exclusive or standardised approach of assessment is impossible due to the variety and complexity of the causes of macrocytic anaemia. The investigative approach needs to be personalised for each patient, emphasising the clinical presentation.

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