International Journal of Research in Medical Sciences
Gaikwad AL et al. Int J Res Med Sci. 2018 Sep;6(9):3051-3056
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20183643

Original Research Article

Utility of serum lactate dehydrogenase in the diagnosis of megaloblastic anemia

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Received: 29 June 2018
Accepted: 27 July 2018

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ABSTRACT

Background: Megaloblastc anemia corresponds to severe macrocytic anemia with hypersegmented neutrophils and very high serum Lactate Dehydrogenase (LDH). The present study was undertaken to evaluate the utility of serum LDH and chloroform inhibited serum LDH in the diagnosis of megaloblastic anemia and to observe if this can be used to differentiate megaloblastic anemia from iron deficiency anemia and hemolytic anemia.
Methods: The present study was carried out on 75 patients of anemia categorised on bone marrow examination (into megaloblastic and non-megaloblastic anaemia) to evaluate the efficacy of total serum LDH levels and LDH isoenzyme pattern in the diagnosis of megaloblastic anemia. About 25 healthy adults were taken as controls.
Results: In megaloblastic anemia, total serum LDH level was found to be increased to about nineteen folds and in hemolytic anemia it was found to increased four folds as compared to normal. On statistical analysis this increased total serum LDH level in megaloblastic anemia and hemolytic anemia as compared to control group was found to be significant.In the present study serum LDH level above 3000IU/L was associated with megaloblastic anemia and serum LDH level below 900IU/L was suggestive of iron deficiency anemia. The chloroform inhibition test was less than 25% in megaloblastic anemia and more than 25% in hemolytic anemia and these differences were found to be statistically significant ($t=9.62$, $df=49$, $p<0.001$).
Conclusions: Total serum LDH levels more than 3000IU/L are diagnostic of megaloblastic anemia. Reversed LDH isoenzyme pattern ($LDH_1>LDH_2$) by chloroform inhibition test is an adjuvant in the diagnosis where total serum LDH levels are between 451-3000IU/L and can also differentiate megaloblastic anemia from hemolytic anemia.

Keywords: Iron deficiency anemia, Hemolytic anemia, Megaloblastc anemia, Serum LDH

INTRODUCTION

Anemia is a major global health problem, especially in developing countries.[1] Although somewhat less common than iron deficiency anemia, megaloblastic anemia constitutes a considerable health problem in developing countries like India. Highest incidence has been reported from India and Africa ranging from 50-71%. Because of generally excellent response to treatment, these anemias are of great clinical importance.

Macrocytes, macro-ovalocytes and hypersegmented neutrophils on peripheral blood smear provide a supportive evidence in the diagnosis of megaloblastic anemia. But for the confirmation of diagnosis of megaloblastic anemia, bone marrow examination is

required which is an invasive procedure and may not be available at peripheral centres.[2]

There are a large number of causes of megaloblastic anemia. The most frequent are disorders resulting in vitamin B_{12} or folate deficiency. The diagnostic process includes blood count, peripheral smear examination, serum vitamin B_{12} assay, red cell folate assay, serum folate assay and other useful investigations like serum/plasma methylmalonic acid (MMA), plasma total homocysteine (tHCYS) and serum holo-transcobalamin II assay. Most of these specialized investigations are expensive and require special equipment's, materials and expertise which may not be available everywhere.[3]

Lactate dehydrogenase (LDH) is a true intracellular enzyme found in many body tissues particularly heart, liver, skeletal muscles, kidney and red blood cells.[4]

LDH have five different isoenzymes LDH_1 to LDH_5.[5] Normally in serum, the concentrations of LDH isoenzymes are $LDH_2 > LDH_1 > LDH_3 > LDH_4 > LDH_5$.[6] Gross elevation of serum LDH in megaloblastic anemia was first reported in 1955 by Hess B et al.[7]Since then number of workers documented the role of serum LDH in megaloblastic anemia. Serum LDH estimation can be used as a screening test for the diagnosis of megaloblastic anemia before performing a bone marrow aspiration.[8]

Winston R et al, Jaswal T et al had shown a characteristics reversed LDH isoenzyme pattern i.e. $LDH_1 > LDH_2$ in megaloblastic anemia.[9,2]

LDH isoenzymes can be separated and estimated quantitatively by different electrophoretic methods.[10] Besides electrophoresis the preponderance of fast moving isoenzymes LDH_1 and LDH_2 can be demonstrated by other method such as Chloroform inhibition test as described by Warburton.[11]

With this background, the present study was undertaken to evaluate the utility of serum lactate dehydrogenase and chloroform inhibited serum LDH in the diagnosis of megaloblastic anemia and to observe if this can be used to differentiate megaloblastic anemia from iron deficiency anemia and hemolytic anemia.

METHODS

A prospective cross-sectional study was carried out for a duration of two years, in Department of Pathology, in a Rural Medical College and Hospital. Prior permission was taken from Institutional Ethics Committee. Total 34 controls and 76 study subjects were studied.

Control group

Included 34 healthy adults, who were randomly selected donors from blood bank, having normal hemoglobin concentration (i.e. 15±2gm/dL in males, 13.5±1.5gm/dL in females).[12]

Study group

All subjects with clinical diagnosis of anemia and whose blood sample were sent for pathological analysis were included. Clinical details including history and findings of clinical examination were recorded in pre approved proforma. Patients with myelodysplastic syndrome, aplastic anemia, leukemia, pregnant and lactating women were excluded.

In all the study subjects and controls, routine hematological investigations including CBC by automated cell coulter, K-21 (Transasia, Sismax), peripheral smear was done and total serum LDH levels was estimated using (P-L) kit manufactured by CREST biosystem. Chloroform inhibition test to study LDH isoenzyme pattern was studied as done by Warburton F et al.[11] Results were expressed in percentage inhibition of total serum LDH level. Investigations like reticulocyte count, sickling test, Hb electrophoresis, serum iron, serum total iron binding capacity (TIBC) and bone marrow aspiration were done wherever required for diagnosis. On the basis of all these investigations the study group was sub divided into four groups as under-

- Group I- Megaloblastic anemia
- Group II- Iron deficiency anemia
- Group III-Hemolytic Anemia
- Group IV- Mixed anemia showing megaloblastic anemia associated with other anaemias.

-IVA- cases of iron deficiency anemia with megaloblastic anemia showing a dimorphic picture on peripheral smear and presence of micronormoblasts and megaloblasts in bone marrow.
-IVB- cases of hemolytic anemia with megaloblastic anemia, showing anisopoikilocytosis, fragmented red cells on peripheral smear and megaloblast in bone marrow.

Statistical analysis

The data was tabulated and analyzed using STATA- 8 statistical software.

RESULTS

The salient observations of the present study are given in subsequent tables.

The control group comprising of 34 healthy persons, provided the normal serum LDH level. Out of the 76 study group cases, 53.95% had megaloblastic anemia, 18.42% had iron deficiency anemia, 14.47% cases had mixed anemia and13.16% cases had hemolytic anemia (Table 1).

Table 1: Distribution of controls and study subjects according to anemia.

Groups	No. of subjects	%
Control	34	100
Study groups		
Group I (Megaloblastic)	41	53.95
Group II (Iron deficiency)	14	18.42
Group III (Hemolytic)	10	13.16
Group IV (Mixed)	11	14.47
Total	76	100

The maximum [29 (70.73%)] cases of megaloblastic anemia were in the age group 11-40 years. Most of the cases of iron deficiency anemia [7 (50%)] and hemolytic anemia [4 (40%)] were in the age group 21-30 years. Mixed anemia had maximum cases [4 (36.36%)] in the age group 31-40 years (Table 2).

Male preponderance was seen in megaloblastic, hemolytic and mixed anemias, whereas female preponderance was seen in iron deficiency anemia (Table 3).

Table 2: Age-wise distribution of study subjects and controls.

Age groups (in completed years)	Study subjects				Controls
	Group I (Megaloblastic)	Group II (Iron deficiency)	Group III (Hemolytic)	Group IV (Mixed)	
1-10	1 (02.43)	1 (07.14)	2 (20)	0 (0)	0 (0)
11-20	9 (21.95)	2 (14.29)	3 (30)	2 (18.18)	6 (17.65)
21-30	12 (29.27)	7 (50.00)	4 (40)	2 (18.18)	18 (52.94)
31-40	8 (19.51)	3 (21.43)	1 (10)	4 (36.36)	9 (26.47)
41-50	5 (12.20)	1 (07.14)	0 (0)	2 (18.18)	1 (02.94)
51-60	2 (04.88)	0 (0)	0 (0)	0 (0)	0 (0)
>60	4 (09.76)	0 (0)	0 (0)	1 (09.09)	0 (0)
Total	41 (100)	14 (100)	10 (100)	11 (100)	34 (100)

Table 3: Sex-wise distribution of study subjects and controls.

Groups	Study subjects		Total
	Male	Female	
Control	17 (50.00)	17 (50.00)	34 (100)
Study groups			
Group I (Megaloblastic)	26 (63.41)	15 (36.59)	41 (100)
Group II (Iron deficiency)	5 (35.71)	9 (64.29)	14 (100)
Group III (Hemolytic)	6 (60.00)	4 (40.00)	10 (100)
Group IV (Mixed)	6 (54.55)	5 (45.45)	11 (100)
Total	43 (56.58)	33 (43.42)	76 (100)

Table 4: Total serum LDH level in study subjects and controls.

Groups	Number of study subjects	Total Serum LDH level in IU/L	
		Range	Mean ± SD
Control	34	242-402	328.06±45.14
Study groups			
Group I (Megaloblastic)	41	1488-10760	6061.32±2613.24
Group II (Iron deficiency)	14	260-782	467.64±158.57
Group III (Hemolytic)	10	986-2220	1563.3±421.09
Group IV (Mixed)	11	1236-6542	4020.73±2025.06

The increase in serum LDH level was significantly more in megaloblastic anemia followed by hemolytic anemia and iron deficiency anemia. In megaloblastic anemia, total serum LDH level was found to be increased to about nineteen folds and in hemolytic anemia it was found to increased four folds as compared to normal. On statistical analysis this increased total serum LDH level in megaloblastic anemia and hemolytic anemia as compared to control group was found to be significant (t=12.77,

df=73, p<0.001 and t=17.25, df=42, p<0.001) respectively.

Similarly, the increased serum LDH level in megaloblastic anemia was found to be statistically significant as compared to the values in iron deficiency anemia and in hemolytic anemia. (t=7.96, df=53, p<0.001 and t=5.39, df=49, p<0.001). The difference of serum LDH level between group II (iron deficiency anemia) and group III (hemolytic anemia) were also statistically significant (t=8.95, df=22, p<0.001) (Table 4).

Presuming that the increase in serum LDH in mixed anemia was due to megaloblastosis, it was excluded from further analysis.

On categorizing the cases according to different ranges of total serum LDH level, the control group cases i.e. 100% showed total serum LDH level below 450IU/L. In the study group, maximum cases [33 (80.49%)] of megaloblastic anemia showed total serum LDH level above 3000IU/L. None of the patient with iron deficiency anemia showed such a high serum LDH level, all were below 900IU/L. All cases of hemolytic anemia showed serum LDH level above 900IU/L but below 3000IU/L. Thus, it was evident that serum LDH level above 3000IU/L was associated with megaloblastic anemia and serum LDH level below 900IU/L was suggestive of iron deficiency anemia in the present study. Whereas, cases showing serum LDH level between 901-3000IU/L require further differentiation for its typing (Table 5).

Table 5: Distribution of study groups and controls within different ranges of total serum LDH level.

Groups	Serum LDH level in IU/L				
	≤450	451-900	901-3000	>3000	Total
Control	34	0	0	0	34
Study group					
Group I (Megaloblastic)	0	0	8	33	41
Group II (Iron deficiency)	7	7	0	0	14
Group III (Hemolytic)	0	0	10	0	10
Group IV (Mixed)	0	0	3	8	1

Table 6: Result of serum LDH level and Chloroform inhibition test.

Result	Types of anemia		
	Group I (Megaloblastic)	Group III (Hemolytic)	Group IV (Mixed)
Number of patients	41	10	11
Range of total serum LDH level	1488-10760	986-2220	1236-6542
Mean of total serum LDH level	6061.32±2613.24	1563.3±421.09	4020±2025.06
Range of percentage chloroform inhibition	2-24.66%	28.09-47.27%	2-22%
Mean of percentage chloroform inhibition	11.34±4.72%	33.37±5.81%	14.05±6.03%

Thus, we can conclude that serum LDH level above 3000IU/L could be diagnostic of megaloblastic anemia. Chloroform inhibition test was done in all the anemic patients, with total serum LDH level >900IU/L.

The chloroform inhibition test was less than 25% in megaloblastic anemia and more than 25% in hemolytic anemia and these differences were found to be statistically significant (t=9.62, df=49, p<0.001).

Further, maximum [39 (95.12%)] cases of megaloblastic anemia showed the chloroform inhibition test less than 20% whereas in hemolytic anemia maximum [7 (70%)] cases showed chloroform inhibition above 30%. Therefore, chloroform inhibition below 20% always favors megaloblastic anemia (Table 6).

DISCUSSION

Megaloblastic anemia is a fairly common condition in India. It requires a bone marrow aspiration for its diagnosis which is an invasive, painful procedure requiring aseptic conditions and laboratory facilities for interpretation.[2] Its non-availability at the peripheral centres leads to a great deal of interest in other serum markers. In the present study, Megaloblastic anemia had the highest proportion of cases (53.95%) followed by Iron deficiency anemia (18.42%), Mixed anemia (14.47) and Hemolytic anemia (13.16%). Khattak A et al, reported the proportion of iron deficiency anemia as highest with 32.69%, followed by megaloblastic anemia with 18.75%, whereas hemolytic anemia and mixed anemia had an equal proportion of 13.46% each.[13]

Ghazali A et al, reported the proportion of iron deficiency anemia as 10.37%, followed by megaloblastic anemia, mixed anemia and hemolytic anemia as 9.09%, 2.87% and 1.44% respectively.[14]

The highest proportion of megaloblastic anemia in the present study may be because, majority of patients were lactovegetarian. The average Indian vegetarian diet is deficient in cobalamin.[15]

The peak age incidence for megaloblastic anemia was found in the age group 11- 40 years. Pandya H et al found the Incidence of megaloblastic anemia highest in the age between 40 and 49 years.[16]

In the present study, male preponderance was observed in megaloblastic anemia. Similar finding was observed by Daljinder Kaur et al, Pandya et al.[17,16] Other studies observed female preponderance in megaloblastic anemia.[18,19]

In the present study, the mean serum LDH level was found to be highest in megaloblastic anemia with LDH activity showing 19 times increase as compared to control.

Kannan et al, reported the serum LDH, 54 times the normal value.[19] Stein D et al, found that the serum LDH level was significantly elevated, 1.3 to 24 times in megaloblastic anemia than normal.[20] Hess B et al, found increased serum LDH activity 5 to 21 times the normal in megaloblastic anemia.[7]

In present study hemolytic anemia showed a 4 fold increase in serum LDH level as compared to normal. This difference was found to be statistically significant ($t=17.25$, $df=42$, $p<0.001$). This finding was similar to those obtained in other studies carried out by Gronvall C et al and Emerson P et al.[21,22] Jaswal T et al**,** found the mean serum LDH level in hemolytic anemia as 2043IU/L.[2] The possible cause for increase in serum LDH levels in megaloblastic anemia was probably the markedly increased inflow of LDH molecules into the blood stream due to increased rate of destruction of red blood cells and slower elimination of LDH due to vitamin B_{12} deficiency. Similar pathophysiology could be responsible for hemolytic anemia.[21]

In the present study, the serum LDH level in iron deficiency anemia was almost within the normal range. It was found similar with the studies carried out by Emerson P et al, Winston R et al, Carmel R et al and Stein I et al.[20,22,9,23]

The group IV (Mixed anemia) comprising of megaloblastic anemia with either hemolytic or iron deficiency anemia also showed a marked increase in serum LDH level. As it was due to megaloblastosis, it was excluded from further analysis.

In the present study, it was found that Maximum number of megaloblastic cases [33 (80.49%)] had values more than 3000IU/L, all cases of hemolytic anemia (100%) had values between 901 to 3000IU/L and all cases of iron deficiency anemia (100%) showed serum LDH level below 900IU/L. Values above 3000IU/L were observed only in patients of megaloblastic anemia (Group I) or when it was associated with other types of anemia (Group IV). Gronvall et al and Jaswal et al had observed that serum LDH level more than 3000 IU/L were of diagnostic importance in megaloblastic anemia.[21,2]

Thus, serum LDH level could be used as a simple, less invasive test for providing an important clue in the diagnosis of megaloblastic anemia, when the values are more than 3000IU/L. The demonstration of reverse isoenzyme pattern, $LDH_1>LDH_2$ is a useful adjunct in the diagnosis of megaloblastic anemia. It is ideally demonstrated by electrophoretic separation and subsequent scanning. However, in peripheral centres, where this is not feasible, chloroform inhibition test can be done. Chloroform inhibits LDH_1 activity and the test is done on serum showing a raised serum LDH of over 900IU/L or on the red cells. A simple chloroform inhibition test made on serum gives a good indication of this reversed pattern.

In the present study, it was observed that the Chloroform inhibition was always less than 25% in megaloblastic anemia and values showing 20% or less inhibition were diagnostic of megaloblastic erythropoiesis. In hemolytic anemia, Chloroform inhibition was always more than 25% similar findings were obtained by Winston et al and Jaswal et al.[2,9]

CONCLUSION

Total Serum LDH and reverse LDH pattern can be used to diagnose megaloblastic anemia before doing a Bone-marrow examination.

ACKNOWLEDGEMENTS

Authors would like to thank Dr. Rasika Gadkari, Dr. Sonali Wankhede, Dr. Amol Gaikwad and the technical staff of Pathology department, SRTR Medical College, Ambajogai.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee of SRTR. Medical College, Ambajogai, Maharashtra, India

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Cite this article as: Gaikwad AL, Jadhav DS. Utility of serum lactate dehydrogenase in the diagnosis of megaloblastic anemia. Int J Res Med Sci 2018;6:3051-6.