ORIGINAL ARTICLE ASIAN JOURNAL OF MEDICAL SCIENCES

Role of LDH levels in differentiating anemias

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Submission: 11-05-2021 Revision: 29-07-2021 Publication: 01-09-2021

ABSTRACT

Background: There is a need to differentiate megaloblastic anemia from mixed deficiency anemia as both require different management protocols. With the acquisition of more information about them, tests such as serum vitamin estimation and Schilling test, were found to have their limitations. Hence there is a need to search newer diagnostic candidates to differentiate between megaloblastic anemia and mixed deficiency anemia. **Aims and Objective:** The current study was undertaken to find usefulness of serum Lactate Dehydrogenase (LDH) in differentiating megaloblastic anemia from mixed deficiency anemia. **Materials and Methods:** 100 patients were included in the study. Blood smears were stained and analysed. Complete blood counts were performed. Bone marrow examination was done, where needed. Biochemical tests were performed for estimation of vitamin B12, Folate and for LDH. **Results:** Out of the 100 cases 51 were diagnosed as megaloblastic anemia and 49 were diagnosed as mixed deficiency anemia. The LDH levels were significantly higher in cases of megaloblastic anemia as compared to mixed deficiency anemia. **Conclusion:** Serum LDH levels can be used in differentiating megaloblastic anemia from mixed deficiency anemia.

Keyword: Lactate dehydrogenase; Megaloblastic anemia; Mixed deficiency anemia

Access this article online

Website: http://nepjol.info/index.php/AJMS

DOI: 10.3126/ajms.v12i9.37024

E-ISSN: 2091-0576

P-ISSN: 2467-9100

INTRODUCTION

Anaemia is defined by the World Health Organisation as haemoglobin (Hb) < 120 g/L in women and Hb < 130 g/L in men. Anaemia is a recognized public health problem throughout the world. Almost every fourth person on the earth is anaemic. Most of the burden of anemia is attributed to nutritional deficiencies.[1]

The term 'nutritional anemia' encompasses all pathological conditions in which the blood hemoglobin concentration drops to an abnormally low level, due to a deficiency in one or several nutrients. The main nutrients involved in the synthesis of hemoglobin are iron, folic acid, and vitamin B12. Iron deficiency is the commonest cause of nutritional anemia worldwide while folic acid and/or B 12 deficiency is less widespread and is often observed with iron deficiency.[2]

For differential diagnosis, it is useful to classify the type of anemia based on the red cell indices which is calculated from red blood cell count, hemoglobin concentration, and hematocrit. The mean corpuscular volume (MCV) is calculated from hematocrit (%) × 10/RBC count (106/μl), and macrocytic anemias are defined as MCV >100 fl.

The cause of macrocytic anemia is classified into one of the following categories, megaloblastic or nonmegaloblastic whereby megaloblastic anemia is caused by deficiency or impairment of utilization of vitamin B12 or folate.[3] The mean corpuscular Hb (MCH) and MCV distinguish macrocytic anemia from iron deficiency anemia, which is hypochromic and typically microcytic. Deficiencies of multiple nutrients or the use of certain medications can lead to a combination of iron deficiency anemia and macrocytosis, with resultant normocytic anemia.[4]

Iron deficiency anemia can be distinguished from other causes of microcytic and hypochromic anemia using iron studies[4] and a defined set of tests are used to distinguish megaloblastic from non megaloblastic anemias.[3]

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Due to various nutrient deficiency mixed deficiency anemias generally exhibit dyserythropoiesis which may lead to a hemolytic picture. There is intramedullary destruction of red blood cells along with low reticulocyte count.[5]

At one time, the diagnosis of a deficiency of vitamin B12 or folate was considered to be relatively straight forward. As knowledge has accumulated, the limitations of such tests as serum vitamin level measurements and the Schilling test have become apparent and hence need for newer tests.[6] One such candidate is serum Lactate Dehydrogenase (LDH).

Elevation of plasma lactate dehydrogenase concentration (LDH) is known to occur in patients with megaloblastic anaemia. Gross elevation of serum Lactate dehydrogenase (LDH) in megaloblastic anemia was first reported by Hess and Gehm back in 1955.[7] Still LDH could not be popularized owing to difficulty in assessment. However, with the emergence of newer technology simplifying these assessments, a new interest has generated in identifying the usefulness of Serum LDH levels for differentiation of megaloblastic anemia from other nutritional anemias but there is still a lack of consensus regarding its clinical usefulness in the primary diagnosis of megaloblastic anemia.[8-10]

Hence we carried out a study to compare the LDH levels in megaloblastic anemia and mixed nutritional deficiency anemias and to assess the differentiating role of Serum LDH levels as a low-cost early diagnostic modality for identification of megaloblastic anemia.

MATERIALS AND METHODS

This was a comparative study carried out over a period of 18 months. All cases of megaloblastic anemia and mixed nutritional deficiency anemia were included in the study. Proper ethical permission had been taken and patient consent was also taken. Blood samples were taken in EDTA vials for complete blood counts and in plain vials for folate and vitamin B 12 estimation and for iron studies. Blood smears were stained using Leishman stain to study general blood picture.

Biochemical tests were done by Chemiluminescence method on VITROS-5600. Ferritin assessment was done using Orthoclinical Diagnostics kit, LOT number-1960 while Serum Iron assessment used Orthoclinical Diagnostics kit, LOT number-50307. Total Iron Binding Capacity assessment was done by Orthoclinical Diagnostics kit, LOT number-36787. Folate and Vitamin B12 estimation was done using Orthoclinical Diagnostics kit, LOT number-1860. Serum LDH estimation was done by Orthoclinical Diagnostics kit, LOT number-6352-8555. Bone marrow aspiration, if needed, was done and slides stained by Giemsa stain.

Using the above mentioned test results patients were divided into two groups:

1) Mixed nutritional deficiency anemia- decreased levels of folate and/or vitamin B12 and deranged iron studies.
2) Megaloblastic anemia: decreased levels of folate and/or vitamin B 12 with normal iron profile.

RESULTS

The present study was carried out to ascertain the usefulness of LDH in differentiating megaloblastic anemia from mixed nutritional deficiency anemia. A total of 100 patients were included in the study with 51 patients diagnosed as megaloblastic anemia and 49 diagnosed as mixed deficiency anemia. Table 1 summarizes the baseline characteristics of all the 100 cases whereas Table 2 compares the hematological, clinical and biochemical parameters of the megaloblastic and mixed nutritional anemia group.

These 100 patients comprised of 57 males and 43 females. The mean age of presentation was 34.69 years, 35.96 years for megaloblastic anemia and 33.37 years for mixed nutritional anemia.

Forty-seven (92.2%) patients of megaloblastic anemia presented with tingling and numbness while 32 (71.4%) among mixed nutritional anemia presented with the same and this was statistically significant. However other presenting symptoms like fatigue, weakness, pallor and icterus did not show any statistically significant difference.

Among hematological parameters, mean hemoglobin and RBC count were 9.29 gm/dl and 2.69 million cells/dl respectively, without any statistically significant difference between the two groups. Mean values of mean corpuscular volume (MCV) for megaloblastic anemia and mixed deficiency anemia were 106.13 and 97.7 fl respectively and mean corpuscular hemoglobin (MCH) were 34.28 and 32 pg respectively. The differences were statistically significant. However the mean values of mean corpuscular hemoglobin concentration (MCHC) for megaloblastic and mixed deficiency anemia were 33.1 and 32.6 g/dl respectively and the difference was not statistically significant.

Among biochemical parameters, mean serum iron was 48.04 mcg/dl in the megaloblastic anemia group while it was 42.96 mcg/dl in the mixed nutritional anemia group, which is a significant difference. Mean TIBC was 258.0 mcg/dl in megaloblastic anemia cases while it as

Table 1: Baseline clinical characteristics of 100 anemia patients

S.No	Variables	Values
1.	Age	
	Mean ± SD	34.69±10.8
	Median (IQR)	31.00
2.	Sex	
	Males N(%)	57%
	Females N(%)	43%
3.	Fatigue N(%)	100 (100%)
4.	Weakness N (%)	99 (99%)
5.	Tingling N(%)	82 (82%)
6.	Pallor N(%)	100 (100%)
7.	Icterus N (%)	19 (19%)
8.	Type of anemia	
	Megaloblastic N(%)	51 (51%)
	Mixed N (%)	49 (49%)
9.	Hemoglobin	
	Mean ± SD	9.29±1.8
	Median (IQR)	9.75 (2.5)
10.	RBC count	
	Mean ± SD	2.69±0.7
	Median (IQR)	2.65 (0.8)
11.	Serum iron	
	Mean ± SD	45.55±12.6
	Median (IQR)	42 (17)
12.	Ferritin	
	Mean ± SD	198.33±41.5
	Median (IQR)	189 (28)
13.	TIBC	
	Mean ± SD	265.41±32.9
	Median (IQR)	270 (17)
14.	MCV	
	Mean ± SD	102.00±7.4
	Median (IQR)	101.8 (6.7)
15.	MCH	
	Mean ± SD	33.26±7.4
	Median (IQR)	33 (3)
16.	MCHC	
	Mean ± SD	32.86±2.3
	Median (IQR)	33.5 (2.6)
17.	Folic acid	
	Mean ± SD	4.89±5.6
	Median (IQR)	4.35 (3.2)
18.	Vitamin B12	
	Mean ± SD	294.40±195.8
	Median (IQR)	197.5 (301)
19.	Serum LDH	
	Mean ± SD	293.16±7.1
	Median (IQR)	275 (38)

Table 2: Comparison of clinical, hematological and biochemical characteristics of megaloblastic and mixed anemia

Variables	Megaloblastic N= 51	Mixed N= 49	P value
Age			
Mean ± SD	35.96±11.0	33.37±10.7	0.28
Median (IQR)	38 (18)	30 (17)	
Sex			
Males N (%)	27 (52.9%)	30 (61.2%)	0.114
Females N (%)	24 (47.1%)	19 (38.8%)	
Fatigue N (%)	51 (100%)	49 (100%)	
Weakness N (%)	50 (98%)	49 (100%)	0.51
Tingling N (%)	47 (92.2%)	32 (71.4%)	0.006
Pallor N (%)	51 (100%)	49 (100%)	
Icterus N (%)	12 (23.5%)	7 (14.3%)	0.103
Hemoglobin			
Mean ± SD	9.36±1.7	9.22±1.9	0.1
Median (IQR)	9.8 (2.5)	9.7 (2.5)	
RBC count			
Mean ± SD	2.76±0.7	2.63±0.7	0.4
Median (IQR)	2.7 (0.9)	2.6 (0.8)	
Serum iron			
Mean ± SD	48.04±9.7	42.96±14.6	<0.05
Median (IQR)	45 (14)	37 (15)	
Ferritin			
Mean ± SD	206.84±42.9	189.47±38.4	0.05
Median (IQR)	192 (25)	188 (29.5)	
TIBC			
Mean ± SD	258.0±40.6	272.53±21.6	<0.05
Median (IQR)	265 (18)	275 (11)	
MCV			
Mean ± SD	106.13±5.8	97.7±6.3	<0.05
Median (IQR)	105 (7.9)	100 (6.9)	
MCH			
Mean ± SD	34.28±3.5	32±3.3	0.004
Median (IQR)	34 (4)	33 (3)	
MCHC			
Mean ± SD	33.1±2.1	32.6±2.5	0.46
Median (IQR)	33.5 (2.5)	33.5 (2.9)	
Folic acid			
Mean ± SD	5.58±7.6	4.17±1.8	0.4
Median (IQR)	4.5 (3.2)	3.1 (3.1)	
Vitamin B12			
Mean ± SD	255.69±154.6	334.69±225.6	0.06
Median (IQR)	190 (199)	199 (355)	
Serum LDH			
Mean ± SD	320.86±95.3	264.3±14	<0.05
Median (IQR)	295 (34)	260 (10)	

272.53mcg/dl among mixed nutritional anemia cases, which was again a statistically significant difference.

LDH was also found to be significantly higher in cases of megaloblastic anemia as compared to mixed nutritional anemia. The mean LDH value in former group was 320.86±15 whereas in the latter group was 264.3 ± 14.

DISCUSSION

LDH is physiologically measurable in serum due to physiological cellular turnover and 5 isoenzymes are present.[11] Two isoenzymes of LDH, LDH-1 and LDH-2 are expressed in RBC. As decrease in red blood cell count is a distinct feature of anemia,[12] hence a relationship between LDH expression and anemia could be anticipated. According to Heller & Venger increased LDH activity combined with normal or slightly elevated transaminase values is typical of megaloblastic anemia.[13] The mean patient age for megaloblastic anemia was 35.96 years whereas for mixed nutritional anemia was 33.37 years and we had taken only adult patients for our study i.e. above 18 years of age. There was a slight male preponderance as far as gender was concerned. These findings were quite

similar to previous studies like the one by Pandya and Patel[14] where maximum patients were in the age group 40-49 years and an overall male preponderance was seen.

Among hematological parameters, no statistically significant difference between the two groups was observed for mean hemoglobin, DLC, RBC and MCHC, however, mean MCV and MCH of patients with megaloblastic anemia were significantly higher as compared to that of mixed nutritional deficiency anemia. According to a study done by Deepthi et al.,[15] on dimorphic anemia, due to concomitant iron deficiency and fragmentation of red blood cells MCV may be normal in nutritional anemia.

Serum vitamin B12 levels were significantly lower in megaloblastic anemia cases as compared to that in mixed nutritional deficiency anemia cases, however folate levels did not show any significant difference among the two groups. Cases with isolated folate deficiency were higher as compared to isolated B12 deficiency.

In present study, mean serum LDH levels were observed to be 321.78+96.08 IU/dl in megaloblastic anemia group as compared to 264.54+13.97 IU/dl in mixed nutritional deficiency anemia group. Statistically, mean values were significantly higher in megaloblastic anemia group as compared to that in mixed nutritional deficiency anemia group. As far as mean serum LDH levels in megaloblastic anemia are concerned, the findings in present study are similar to that observed in a number of previous studies (Table 3).

Mean serum LDH levels tend to be higher in cases of megaloblastic anemia. The increased LDH activity may be a result mainly of haemolysis, however it would require a much greater haemolytic activity than that found in megaloblastic anaemia.[16] The findings of present study are similar to that observed by Gaikwad and Kadhav [17] who observed mean serum LDH levels of megaloblastic anemia cases to be significantly higher as compared to that of hemolytic anemia and mixed anemia cases. They observed that though the mean LDH in mixed anemia cases were highly increased as compared to hemolytic anemia cases yet were lower as compared to megaloblastic anemia cases as observed in present study. These findings in turn suggest a more dominant role of serum B12 and folic acid levels in governing the LDH levels while iron deficiency seemed to play a regressive role.

Since our hospital caters to semi urban population of minority group, this study is first of its kind to be carried in such a population.

CONCLUSION

The present study aimed to utilize LDH as a discriminating marker between megaloblastic anemia and mixed nutritional deficiency anemia. It was found that serum LDH level

Table 3: Mean serum LDH levels in megaloblastic anemia cases in different contemporary studies and their comparison with present study

SN	Author (Year)	Sample size	Mean LDH levels in Megaloblastic anemia cases (IU/dl)	Mean LDH levels in comparative group, if any	Type of comparative group, if any
1.	Eivazi-Ziaei et al. (2007)[49]	29 Cases	423 IU/dl (Before intervention)	78.3 IU/dl (After intervention)	After intervention
2.	Chaudhari and Bindu (2015)[15]	50 Cases, 50 Controls	239.6 IU/dl	31.6 IU/dl	Healthy controls
3.	Gore et al. (2015)[50]	42 Cases	238.6 IU/dl	-	-
4.	Kannan et al. (2016)[51]	38 Cases	466.1 IU/dl	68.3 IU/dl	62 Macrocytic non-megaloblastic
5.	Sakhare et al. (2017)[52]	56 Vitamin B12 def. 23 FA def. megaloblastic anemia cases	384.4 IU/dl 406.6 IU/dl	-	-
6.	Gaikwad and Jadhav (2018)[53]	41 Cases 10 + 11 Controls	606.1 IU/dl	156.3 IU/dl 402.0 IU/dl	Hemolytic (n=10) Mixed deficiency (n=11)
7.	Present study (2018)	50 Megaloblastic 50 Mixed nutritional deficiency	321.78 IU/dl	264.54 IU/dl	Mixed nutritional deficiency

>277.5 IU/L can help in differentiating mixed nutritional deficiency and megaloblastic anemia with a high sensitivity as well as specificity (86% and 92% respectively). This can be useful for future studies and references.

ACKNOWLEDGEMENT

The authors would like to take this opportunity to thank the technical staff at Hospital Laboratory Services (HLS) where all the tests were carried out.

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Authors' contribution:
NZ- Conceptualization of study, statistical analysis, preparation of final draft; **RZU-** Result interpretation, manuscript preparation; **SI-** Coordination, manuscript preparation; **KT-** Coordination, manuscript preparation; **SRM-** Review of literature, coordination.

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Source of Funding: None, **Conflict of interest**: None.