Ministry of Higher Education

and Scientific Research

University of Diyala

College of Medicine

Quantitative measurement of LDH in anemia

Submitted to the Council of the College of Medicine, Diyala University, In Partial Fulfillment of Requirements for the bachelor's degree in medicine and general surgery.

Submitted by

Abdulla Adnan Alwan

Supervised by

Asst. Lec Wassan Saher Hassan

2022-2023

ABSTRACT

Background: The World Health Organization defines anemia as a hemoglobin concentration of less than 13 g/dL in men and less than 12 g/dL in women. Anaemia is a condition in which the number of red blood cells or the haemoglobin concentration within them is lower than normal. The most common causes of anaemia include nutritional deficiencies, particularly iron deficiency, though deficiencies in folate, vitamins B12 and A are also important causes; haemoglobinopathies; and infectious diseases, such as malaria, tuberculosis, HIV and parasitic infections.

Method: This study is cross sectional study, was carried out for four months in a local medical laboratory. Samples of patients with the clinical diagnosis of anemia were collected without regard to the type of anemia. The samples were taken from the patients who have done the test as requested by their doctors or as they were willing to be part of the study. We collected data regarding their age, gender and the serum level of LDH.

Results: 18 patients were enrolled in this study, 38.8 % of them were males (7) and 61.2% were females (11), with mean age of 21.8 years. Their mean level of LDH was 380 U/L.

Conclusion: It was found that the serum LDH can help in the diagnosis and differentiation of types of the anemia.

INTRODUCTION

Anemia is a major global health problem, especially in developing countries.[1] 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.[2]

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.[3]

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.[4] 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.[5] Iron deficiency anemia can be distinguished from other causes of microcytic and hypochromic anemia using iron studies[5] and a defined set of tests are used to distinguish megaloblastic from non-megaloblastic anemias.[4]

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.[6] The term hemolysis refers to the destruction of the red blood cells (RBC) ,releasing intra-erythrocyte content to the extracellular compartment and accounts for a wide range of laboratory and clinical conditions, both physiological and pathological.[7] In this process not only hemoglobin is released, other components of the erythrocyte cytoplasm such as potassium, lactate dehydrogenase (LDH), or neuro-specific enolase (NSE) among other components.[8]

At one time, the diagnosis of a deficiency of vitamin B12 or folate was 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.[9] One such candidate is serum Lactate Dehydrogenase (LDH).

Aim of study

To find if the LDH level can be used to help in the diagnosis and differentiation of anemia and if it can be used before further investigations.

Material and method

This study is cross sectional study, was carried out for four months in a local medical laboratory. Samples of patients with the clinical diagnosis of anemia were collected without regard to the type of anemia.

The samples were taken from the patients who have done the test as requested by their doctors or as they were willing to be part of the study.

A total of 18 patients of 7 males and 11 females were included in this study. We collected data regarding their age, gender and the serum level of LDH in the blood. The LDH was measured by IU/L.

Any patient who have anemia was eligible for the study. The consent of the patient was taken, and the confidentiality of patients was preserved.

In all the study subjects, routine hematological investigations were done as the samples were taken from venous blood and carried out in EDTA vials to measure the level of LDH in the blood. The samples were mixed with a working reagent in a tube and then were put in a spectrometer for several minutes. The difference of absorbance was calculated, and the results were displayed.

Results:

A sample of 18 patient was collected with mean age of 21.8 years. 38.8 % of them were males (7) and 61.2% were females (11).

The results presented in relation with their age and gender as below:

Table 1: LDH levels measured in U/L in relation with age.

Age (Years)	(5-10)	(11-20)	(21-30)	(31-40)
	467	369	358	483
	301	278	480	411
		307	348	285
		256	466	
		486	298	
		439	396	
AV	384	356	391	393
SD	83	84	65	82

Table 2: average of LDH with standard deviation in relation with age.

Age (Years)	LDH(SD)
(5-10)	384±83
(11-20)	356± 84
(21-30)	391±65
(31-40)	393±82

Table 3: LDH levels measured in U/L in relation with gender

	Female (17-40)	LDH	Male (17-40)	LDH
	21	358	39	483
	24	480	28	348
	19	278	35	411
	19	307	32	285
	27	466	25	298
	18	486	25	396
	24	420	17	439
	11	369		
	12	256		
	8	467		
	9	301		
AVERAGE		381		380
SD		83		68

Table 4: average of LDH with standard deviation in relation with gender.

GENDER	LDH
MALE	380±68
FEMALE	381±83

Figure 1: The frequency of LDH with the age in a chart.

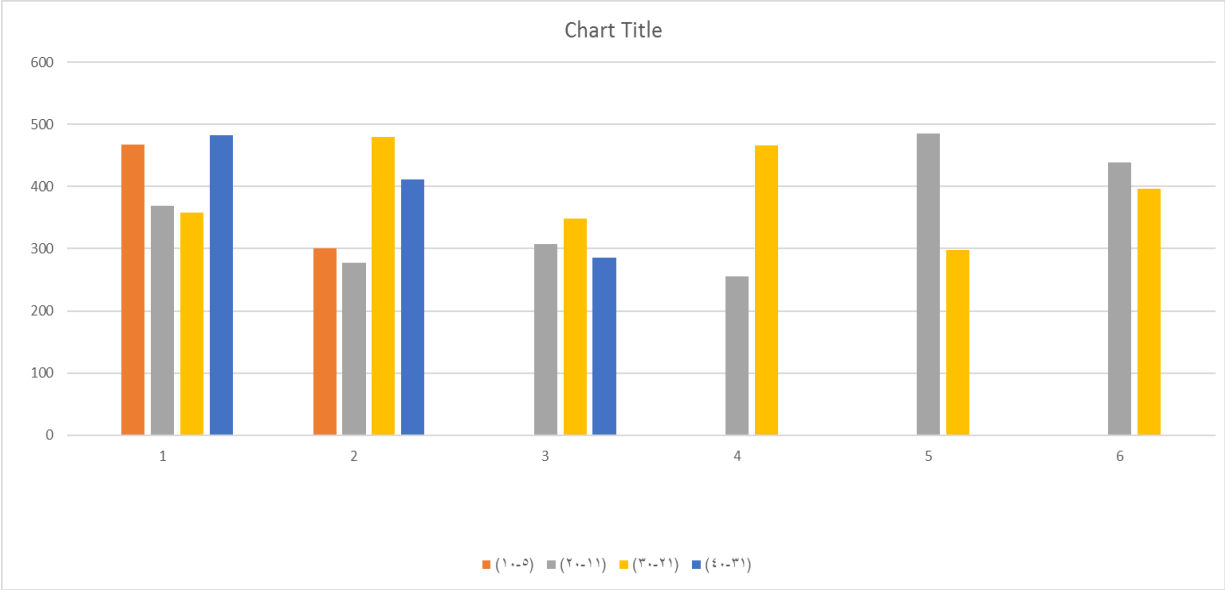
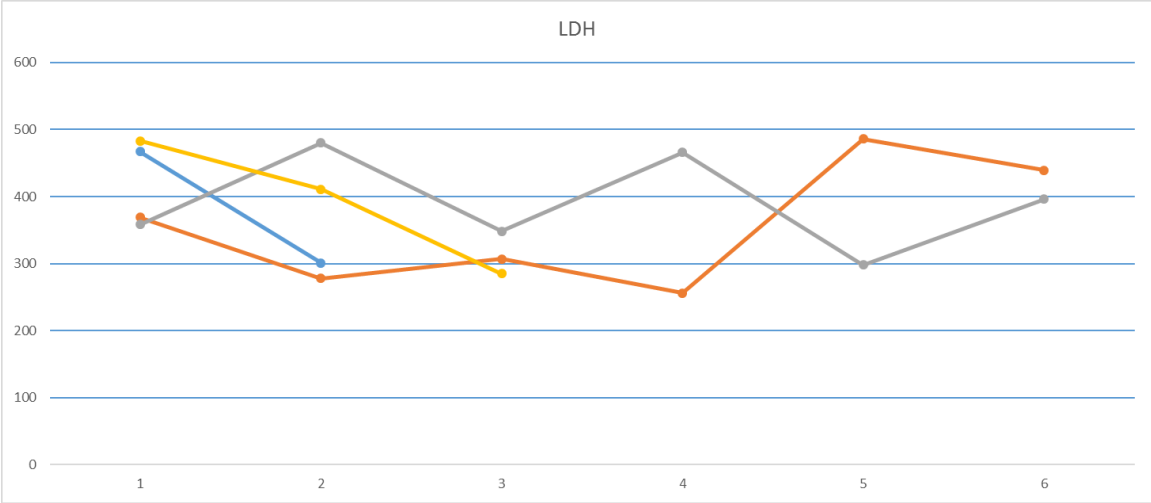


Figure 2: The frequency of LDH with the age in graphic lines.



Discussion

LDH is physiologically measurable in serum due to physiological cellular turnover and 5 isoenzymes are present.[10] 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,[11] 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.[12]

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]

Research by Galila et al. showed that patients with beta thalassemia major had significantly higher levels of LDH, when compared to patients with sickle cell disease and beta thalassemia intermedia.[15] Hemolytic anemia in patients with thalassemia major, often causes an increase in LDH levels, which is a marker of intravascular hemolysis.[16] Some studies suggest that the increase in LDH levels in thalassemia major is due to IE.[17] Hemolysis accompanied by IE in thalassemia major lead to anaerobic glycolysis, which is the cause of increased LDH levels in thalassemia major.[18]

Besides vitamin C supplements intake, there are several factors that can affect LDH levels, such as alcohol intake, intense physical activity, tumors,

and drugs (anesthesia, aspirin, narcotics, and procainamide).[19,20,21,22] Intense physical activity can increase LDH levels by 30- 50%, where serum LDH is often used as an indicator of muscle damage after resistance exercise and may indicate the status of muscle cell membranes.[20,23]

Hypoxia causes increase in the production of Epo from the kidneys, whose function is to increase the surviving erythroid progenitor and its proliferation.[24] However, due to impaired erythroid cell maturation by Gdf11 and Activin A, IE in thalassemia major will then experience amplification.[25] The differences in Activin A levels between thalassemia major and non-thalassemia are significant.[26]

In this study it was found that the mean LDH level for male was 380 ± 68 U/L while for female was 381 ± 81 U/L. 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.[27] The findings of present study are similar to that observed by Gaikwad and Kadhav,[28] 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.

In this study there was female predominance, with similar findings observed by Magnani et al & Kannan et al.[29,30]

Conclusion:

It was found that the serum LDH can help in the diagnosis and differentiation of types of the anemia and as a marker before further investigations such as bone marrow aspiration.

References:

1. Milman N. Anemia-still a major health problem in many parts of the world. Ann Hematol. 2011;90(4):369-77.

2. de Benoist B, McLean E, Egil I, Cogswell M (Eds.). Worldwide prevalence of anaemia 1993–2005 - WHO Global Database on Anaemia. WHO-Centers for Disease Control and Prevention Atlanta

3. Kotecha PV. Nutritional anemia in young children with focus on Asia and India. Indian J Community Med. 2011; 36:8-16. https://doi.org/10.4103/0970-0218.80786

4. Nagao T and Hirokawa M. Diagnosis and treatment of macrocytic anemias in adults. J Gen Fam Med. 2017; 18:200-224. https://doi.org/10.1002/jgf2.31

5. Jimenez K, Kulnigg-Dabsch S and Gasche C. Management of Iron Deficiency Anemia. Gastroenterol Hepatol (NY). 2015; 11:241-250.

6. Sasidharan PK. B12 deficiency in India. Arch Med Health Sci 2017; 5:261-268.

https://doi.org/10.4103/amhs.amhs_121_17

7. Hess B and Gehm E. Lactic acid dehydrogenase in the human blood. Klin Wochenschr. 1955; 33:91-93. https://doi.org/10.1007/BF01473548

8. Chaudhari S and Bindu S. Correlation of Lactate Dehydrogenase in Megaloblastic Anemia. International Journal of Current Medical And Applied Sciences. 2015;

9. Snow CF. Laboratory Diagnosis of Vitamin B12 and Folate Deficiency: A Guide for the Primary Care Physician. Arch Intern Med. 1999; 159:1289-1298.

https://doi.org/10.1001/archinte.159.12.1289

10. Barcellini W and Fattizzo B. Clinical Applications of Hemolytic Markers in the Differential Diagnosis and Management of Hemolytic Anemia. Dis Markers. 2015; 635-670. https://doi.org/10.1155/2015/635670

11. Nagababu E, Gulyani S, Earley CJ, Cutler RG, Mattson MP and Rifkind JM. Iron-deficiency anaemia enhances red blood cell oxidative stress. Free Radic Res. 2008; 42(9):824-829. https://doi.org/10.1080/10715760802459879

12. Heller P and Venger N. Problems in the differentiation of the megaloblastic anemias. Med Clin N Amer. 1962; 46: 121-138. https://doi.org/10.1016/S0025-7125(16)33752-X

13. Khattak AL, Hussain T, Muhammad A. Types of anemia in patients with hemoglobin less than 10g/dL. Pak Armed Forces Med J. 2007;57(1):39- 42.

14. Al-Ghazaly J, Al-Selwi AH, Abdullah M, Al-Jahafi AK, Al-Dubai W, Al-Hashdi A. Pattern of hematological diseases diagnosed by bone marrow examination In Yemen: A developing country experience. Clinical Lab Hematol. 2006;28:376-81.

15. Mokhtar GM, Adly AAM, Alfy MSE, Tawfik LM, Khairy AT. N-terminal natriuretic peptide and ventilationperfusion lung scan in sickle cell disease and thalassemia patients with pulmonary hypertension. Hemoglobin. 2010;34(1):78–94.

16. Kato GJ, McGowan V, Machado RF, Little JA, Taylor 6th J, Morris CR, et al. Lactate dehydrogenase as a biomarker of hemolysis-associated nitric oxide resistance, priapism, leg ulceration, pulmonary hypertension, and death in patients with sickle cell disease. Blood. 2006;107(6):2279–85.

17. Toren A, Or R, Kapelushnik J, Chividalli G, Aku M, Slavin S, et al. Normalization of serum lactic dehydrogenase in β-thalassemia patients following bone marrow transplantation. Am J Hematol. 1996;51(2):166–7.

18. Musharraf SG, Iqbal A, Ansari SH, Parveen S, Khan IA, Siddiqui AJ. β-thalassemia patients revealed a significant change of untargeted metabolites in comparison to healthy individuals. Sci Rep. 2017;7:1–10

19. Imaki M, Miyoshi T, Yoshimura T, Tanada S, Matsumoto K. Relationship between the activity of serum lactate dehydrogenase(LDH) and serum vitamin C in Japanese men and women in their middle and old age. J Japanese Assoc Rural Med. 1988;37(2):87–91.

20. Sepulveda J. Challenges in routine clinical chemistry analysis. Proteins and enzymes. [Internet]. First Edit. Accurate Results in the Clinical Laboratory: A Guide to Error Detection and Correction. Elsevier Inc.; 2013. 131–148.

21. Acierno SP, Aguayo P, Albanese CT, Algren DA, Alon US, Alonso MH, et al. Ashcraft's pediatric surgery 5th edition. In: Holcomb GW, Murphy JP, Ostlie DJBT-APS (Fifth E, editors. Philadelphia: W.B. Saunders; 2010. p. v–xiv. Available from: http://www. sciencedirect.com/science/article/pii/ B9781416061274000823

22. Evans R. Illustrated orthopedic physical assessment, 3rd edition. Elsevier. 2009. 1200 p.

23. Rodrigues BM, Dantas E, de Salles BF, Miranda H, Koch AJ, Willardson JM, Simão R. Creatine kinase and lactate dehydrogenase responses after upperbody resistance exercise with different rest intervals. J Strength Cond Res. 2010;24(6):1657-62. doi: 10.1519/ JSC.0b013e3181d8e6b1.

24. Liang R, Ghaffari S. Advances in understanding the mechanisms of erythropoiesis in homeostasis and disease. Br J Haematol. 2016;174(5):661–73.

25. Camaschella C, Nai A. Ineffective erythropoiesis and regulation of iron status in iron loading anaemias. Br J Haematol. 2016;172(4):512–23.

26. Voskaridou E, Ntanasis-Stathopoulos I, Christoulas D, Dimopoulou M, Komninaka V, Repa K, et al. Activin-A is elevated in patients with thalassemia major and double heterozygous sickle cell/beta-thalassemia and correlates with markers of hemolysis and bone mineral density. Ann Hematol. 2019;98(7):1583– 92.

27. Gronvall C. On the serum activity of lactic acid dehydrogenase and phosphohexoseisomerase in pernicious and hemolytic anemias. Scand J Clin Lab Invest.1961; 13: 29-36. https://doi.org/10.3109/00365516109137245

28. Gaikwad AL and Jadhav DS. Utility of serum lactate dehydrogenase in the diagnosis of megaloblastic anemia. Int J Res Med Sci. 2018; 6:3051-3056. https://doi.org/10.18203/2320-6012.ijrms20183643

29. Magnani KK, Sikarwar S, Rawat N. Prevalence of megaloblastic anemia in people of Gwalior Chambal region. Inter J Med Heal Res. 2017;3(8):09-10.

30. 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.