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ORIGINAL RESEARCH

Lactate dehydrogenase as an indicator of liver tumors

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***ABSTRACT*:**

Background: *Lactate dehydrogenase (LDH, L-lactate, NAD+ oxidoreductase, EC1.1.1.27) is a family of at least six NAD + -dependent isoenzymes (LD1-LD5 and LD6/LDX). It is recognized as being one of the most common enzymes in nature. It belongs to the class of oxidoreductases and it is characteristic for the final stage of anaerobic glycolysis.* ***Aim of the study:*** *To evaluate the efficacy of lactate dehydrogenase as an indicator of liver tumors.* ***Materials and methods:*** *The present study was performed in the Department of Biochemistry, Kidwai Institute of Oncology, Bengaluru, Karnataka, India. For the study, a total of 100 patients were selected. The age of the patients ranged from 18 to 40 years. Briefly, 20 µL of each patient serum was mixed first with 1000 µL of buffer (pH 7.35) supplied by the manufacturing company and incubated for 1-5 min at 25 °C. A volume of 250 µL of substrate that composed of nicotinamide-adenine dinucleotide (0.75 mmol/L) and sodium azide (0.095%) was added to the mixture. After mixing, the absorbance at 340 nm was read by UV-visible spectrophotometer (CECiL, CE 1021, England) after 1 min. CK levels were assayedaccording to Humazym M-test as described by the instructions of the produced company.* ***Results:*** *It was observed that number of male patients was 45 and number of female patients was 55. The mean age of the participating patients was 31.35 years. High LDH level are observed in large number in females (30 patients) than in males (24 patients). Increased LDH levels in blood could not be indicator for liver diseases only, but it may be resulted from damage in muscular tissues. 30 females and 24 males who possessed high level of LDH were investigated for CK, as monitor for muscular injury. Additionally, 6 male patients and 9 female patients showed increased levels of LDH and CK in their blood which means they may sever from muscular damage.* ***Conclusion:*** *From the results of the present study, this can be concluded that LDH can be used as a good biomarker for diagnosis of liver, muscular and even cancer diseases. There is little variable between males and females in the elevated values of LDH. Patients with normal values of CK and high level of LDH suggested to have unidentified cancer disease, except liver cancer, and for that other specific tests are required.*

Keywords: *LDH, liver enzyme, liver disease, Lactate dehydrogenase*

Introduction:

The liver is a large, complex organ that is well designed for its central role in carbohydrate, protein and fat metabolism. It is the site where waste products of metabolism are detoxified through processes such as amino acid deamination, which produces urea. In conjunction with the spleen it is involved in the destruction of spent red blood cells and the reclamation of their constituents. It is responsible for synthesizing and secreting bile and synthesizing lipoproteins and plasma proteins, including clotting factors.[1, 2] It maintains a stable blood glucose level by taking up and storing glucose as glycogen (glycogenesis), breaking this down to glucose when needed (glycogenolysis) and forming glucose from noncarbohydrate sources such as amino acids (gluconeogenesis).[3] Lactate dehydrogenase (LDH, L-lactate, NAD+ oxidoreductase, EC1.1.1.27) is a family of at least six NAD + -dependent isoenzymes (LD1-LD5 and LD6/LDX). It is recognized as being one of the most common enzymes in nature. It belongs to the class of oxidoreductases and it is characteristic for the final stage of anaerobic glycolysis. LDH catalyzes the reversible conversion of pyruvate to lactate with the concomitant oxidation/reduction of NADH to NAD+. [4] The unique role of lactate dehydrogenase is particularly evident under limited oxygen conditions, when oxidation of NADH in the respiratory chain is not possible. The reduction of pyruvate catalyzed by LDH allows the regeneration of NAD+ molecules, which are needed for the continuous generation of ATP to maintain glycolysis. [5] Therefore, under hypoxia, the reduction of pyruvate to lactate allows cells with high glycolytic activity to survive an anaerobic episode. However, since the use of lactate for further metabolic processes can only take place after it is converted back to pyruvate, lactate production is a dead end for the cells' metabolism. [6] Hence, the present study was conducted to evaluate the efficacy of lactate dehydrogenase as an indicator of liver diseases.

Materials and methods:

The present study was performed in the Department of Biochemistry, Kidwai Institute of Oncology, Bengaluru, Karnataka, India. Ethical clearance for the study protocol was obtained prior to starting the study. For the study, a total of 100 patients were selected. The age of the patients ranged from 18 to 40 years. The clinical examination by physician confirmed that all patients showed signs of abnormal liver functions. Thus, liver function tests (LFTs) were suggested for those patients. Serum of all patients was collected with all due precautions and LDH levels were measured by using liquid UV method that modified based on the recommendations of the Scandinavian Committee on Enzymes. Briefly, 20 µL of each patient serum was mixed first with 1000 µL of buffer (pH 7.35) supplied by the manufacturing company and incubated for 1-5 min at 25 °C. A volume of 250 µL of substrate that composed of nicotinamide-adenine dinucleotide (0.75 mmol/L) and sodium azide (0.095%) was added to the mixture. After mixing, the absorbance at 340 nm was read by UV-visible spectrophotometer (CECiL, CE 1021, England) after 1 min. CK levels were assayed according to Humazym M-test as described by the instructions of the produced company. Data of all biochemical tests were expressed as mean ± SD.

The statistical analysis of the data was done using SPSS version 11.0 for windows. Chi-square and Student's t-test were used for checking the significance of the data. A p-value of 0.05 and lesser was defined to be statistical significant.

Results:

Table 1 shows the demographic data of the patients. It was observed that number of male patients was 45 and number of female patients was 55. The mean age of the participating patients was 31.35 years. Table 2 shows LDH level in patients. The values of LDH in males and females were variable. From 100 patients, 22 females and 19 males showed normal levels of LDH. High LDH level are observed in large number in females (30 patients) than in males (24 patients). Increased LDH levels in blood could not be indicator for liver diseases only, but it may be resulted from damage in muscular tissues. 30 females and 24 males who possessed high level of LDH were investigated for CK, as monitor for muscular injury. Additionally, 6 male patients and 9 female patients showed increased levels of LDH and CK in their blood which means they may sever from muscular damage (Table 3).

Table 1: Demographic data

Variables	Number
Total number of participating patients	100
Number of males	45
Number of females	55
Mean age (years)	31.35

Table 2: LDH level in patients

LDH level	Number of patients (Male)	Number of patients (Female)
Low	2	3
Normal	19	22
High	24	30
Total	45	55

Table 3: CK level in patients with high LDH level

CK level	Number of patients (Male)	Number of patients (Female)
Normal	18	21
High	6	9
Total	24	30

Discussion:

In the present study, a total of 100 patients were included. 45 were males and 55 were females. The mean age of the patients was 31.25 years. LDH level was seen to be high in 54 patients (30 females and 24 males). Patients with high LDH were checked for CK level, which is indicator of muscular damage. It was increased in 15 patients (9 females and 6 males). The results of the study were compared with previous studies form the literature. Kotoh K et al [7]evaluated the amounts of lactate dehydrogenase (LDH) in hepatocytes, since its production increases under low oxygen concentrations. Histological examination was performed in 7 patients with ALF. All 7 patients underwent a liver biopsy during the acute phase of ALF, and 4 of them underwent a second biopsy during the recovery phase. The obtained samples were immunohistochemically stained with anti-LDH5 and anti-CD-68 antibodies. As controls, we examined samples from patients with acute hepatitis, chronic hepatitis and liver cirrhosis. The production of LDH by hepatocytes and the number of CD-68 positive macrophages were markedly increased at the acute phase of ALF, and both of these effects abruptly decreased during the recovery phase. By

contrast, most of the samples from the patients with chronic hepatitis and acute hepatitis showed slightly any increase in LDH staining. In cirrhotic patients, partially elevated LDH production was observed mainly around the central vein, but the staining intensity was less compared to that in ALF patients. Our findings indicate that hepatic hypoxic conditions exist in ALF at the acute phase and seem to closely correlate with macrophage overactivation in the liver. They speculated that microcirculatory disturbance may be a key process in the development and progression of ALF. Kotoh K et al [8] hypothesized that the production of serum lactate dehydrogenase (LDH) might increase in the liver under hypoxic conditions and could be an indicator to discriminate between conservative survivors and fatal patients at an early stage. To confirm this hypothesis, they developed a new parameter with serum alanine aminotransferase (ALT) and LDH: the ALT-LDH index = serum ALT/(serum LDH - median of normal LDH range). They analyzed retrospectively 33 patients suffering acute liver injury (serum ALT more than 1000 U/L or prothrombin time expressed as international normalized ratio over 1.5 at admission) and evaluated the prognostic value of the ALT-LDH index, comparing data from the first 5 days of hospitalization with the Model for End-Stage Liver Disease (MELD) score. Patients whose symptoms had appeared more than 10 days before admission were excluded from this study. Among those included, 17 were conservative survivors, 9 underwent liver transplantation (LT) and 7 died waiting for LT. They found a rapid increase in the ALT-LDH index in conservative survivors but not in fatal patients. While the prognostic sensitivity and specificity of the ALT-LDH index was low on admission, at day 3 they were superior to the results of MELD. They concluded that ALT-LDH index was useful to predict the prognosis of the patients with acute liver injury and should be helpful to begin preparation for LT soon after admission.

Li MX et al [9] evaluated that lactate dehydrogenase is a prognostic indicator in patients with hepatocellular carcinoma treated by sorafenib. A total of 119 HBV-related HCC patients treated by sorafenib from a Chinese center were included into the study. They were categorized into 2 groups according to the cut-off value of pre-treatment LDH, which was determined by the time dependent receiver operating characteristics (ROC) curve for the overall survival. The prognostic value of LDH was evaluated. The relationships between LDH and other clinicopathological factors were also assessed. The cut-off value was 221 U/L. With a median follow up of 15 (range, 3-73) months, 91 patients reached the endpoint. Multivariate analysis proved that pre-treatment serum LDH level was an independent prognostic factor for both overall survival (OS) and progression-free survival (PFS). For patients whose pre-treatment LDH $\geq$ 221 U/L, increased LDH value after 3 months of sorafenib treatment predicted inferior OS and PFS. And patients with elevated pre-treatment LDH level predisposed to be featured with lower serum albumin, presence of macroscopic vascular invasion, advanced Child-Pugh class, advanced T category, higher AFP, and higher serum total bilirubin. They concluded that serum LDH level was a potentially prognostic factor in HCC patients treated by sorafenib in HBV endemic area. More relevant studies with reasonable study design are needed to further strengthen its prognostic value. AL-Janabi et al [10] determined lactate dehydrogenase (LDH) level as an indicator of liver, muscular or cancer diseases in patients of more than 40 years of age. Ninety-one patients (43 females and 48 males) had been tested for LDH and liver function tests (LFTs). Creatine kinase (CK) levels were measurement only in patients who had high levels of LDH. As an indicator for liver diseases, high levels of LDH and one or more of LFTs, especially alkaline phosphatase, had been observed in 12 patients (8 females and 4 males). For muscular damage, measurement of CK in patients with elevated levels of LDH and normal levels of LFT revealed that CK values elevated in three males and one female. Whereas high LDH levels, as an indicator for cancer

diseases, were found in three males and one female who had normal values of LFTs and CK. They concluded that LDH can be regarded as a good biomarker for diagnosis of liver, muscular and cancer diseases. There is little variable between males and females in the elevated value of LDH. Patients who had high values of LDH, although they had normal levels of LFTs and CK are proposed to have unidentified cancer disease.

Conclusion:

From the results of the present study, this can be concluded that LDH can be used as a good biomarker for diagnosis of liver, muscular and even cancer diseases. There is little variable between males and females in the elevated values of LDH. Patients with normal values of CK and high level of LDH suggested to have unidentified cancer disease, except liver cancer, and for that other specific tests are required.

References:

1. Green RM, Flamm S. AGA technical review on the evaluation of liver chemistry tests. Gastroenterology 2002;123(4):1367-84.
2. Burkitt HG, Young B, Heath JW. Wheater's functional histology: a text and colour atlas. 3rd ed. Edinburgh: Churchill Livingston, 1993.
3. Worobetz L, Hilsden R, Shaffer E, Simon J Pare P, Scully L, et al. The liver. In Thomson BR, Shaffer EA, editors. First Principles of Gastroenterology. 2nd ed. University of Toronto Press: Toronto; 1994.
4. Krieg AF, Rosenblum LJ, Henry JB. Lactate dehydrogenase isoenzymes a comparison of pyruvate-to-lactate and lactate-to-pyruvate assays. Clin Chem. 1967;13(3):196–203.
5. Argiles A, Mourad G, Mion C, Atkins RC, Haiech J. Two-dimensional gel electrophoresis of urinary proteins in kidney diseases. Contrib Nephrol. 1990;83:1–8.
6. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–674.
7. Kotoh K, Kato M, Kohjima M, et al. Lactate dehydrogenase production in hepatocytes is increased at an early stage of acute liver failure. Exp Ther Med. 2011;2(2):195-199. doi:10.3892/etm.2011.197
8. Kotoh K, Enjoji M, Kato M, Kohjima M, Nakamuta M, Takayanagi R. A new parameter using serum lactate dehydrogenase and alanine aminotransferase level is useful for predicting the prognosis of patients at an early stage of acute liver injury: a retrospective study. Comp Hepatol. 2008;7:6. Published 2008 Aug 14. doi:10.1186/1476-5926-7-6
9. Li MX, Zhao H, Bi XY, et al. Lactate dehydrogenase is a prognostic indicator in patients with hepatocellular carcinoma treated by sorafenib: results from the real life practice in HBV endemic area. Oncotarget. 2016;7(52):86630-86647. doi:10.18632/oncotarget.13428
10. AL-Janabi, Ali & Ali, Zahraa & Noree, Zahraa. (2015). Lactate dehydrogenase as an indicator of liver, muscular and cancer diseases. Journal of Coastal Life Medicine. 3. 10.12980/JCLM.3.2015J5-54.