# Study of LDH Isoenzymes in Myocardial Infraction at Vidharbha Region (Central India)

**Rakesh Kumar Jha[1], Pradip Jain[2], Ankita Khandolkar[1], Archana Dhok[3]**

*[1]Tutor, Dept. of Biochemistry Datta Meghe Medical College, Nagpur, [2]Professor and HOD, Dept. of Biochemistry Datta Meghe Medical College, Nagpur, [3]Professor and HOD Dept. of Biochemistry Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences, Sawangi Meghe Wardha*

## Abstract

**Introduction:** A significant cause of morbidity and mortality worldwide is myocardial infarction (MI). Within the first few hours of the beginning of MI, the greatest risk of death occurs. Thus, for the effective treatment of patients with MI, early detection of cardiac ischemia is essential. Inappropriate diagnosis of patients with chest pain sometimes leads to improper admission, and vice versa, of patients without MI. Physical examination, precise ECG results and evaluation of cardiac biomarkers play an important role in the early diagnosis of acute ischemia, in addition to clinical history. In the present analysis, the cardiac biomarker lactate dehydrogenase released during a MI event is discussed in depth.

**Aim:** We conducted a cross sectional study to examine the different levels of LDH isoenzymes in myocardial infarction patients those admitted in cardiac care unit in SMHRC Nagpur.

**Material and Methods:** The present study included 100 subjects of age group 30-80 years. Patients admitted to the coronary care unit at SMHRC & AVBRH between January 2020 and June 2020 was considered eligible for the study. These guidelines ensured that sufficient blood samples were available and the criteria for diagnosis were consistent. Lactate dehydrogenase isoenzyme was fractionated by agarose gel electrophoresis as described by Cawley and Eberhard. The stained slides were scanned on Chromoscan at 520 mix and the percentages of the fractions were quantitated.

**Results:** The results obtained in the present study indicate that serum lactate dehydrogenase enzyme activity increases 4-5 folds in the first 24-72 hours after the onset of clinical infarction with a peak on the second day. It then declines gradually towards the normal within two weeks. Thus, determination of total serum LDH is valuable if patients present themselves for clinical check up even after a week from the day of infarct. Although the contribution of myocardium to total serum lactate dehydrogenase is large, its interpretation becomes difficult if the diseases of other organs such as liver, kidney, etc.

**Conclusion:** In conclusion, the present data strongly support the use of Lactate dehydrogenase for the diagnosis of MI. Such a strategy should boost both the precision and sensitivity for myocardial infarction diagnosis.

***Keywords****: Lactate Dehydrogenase, Myocardial Infarction, Coronary Artery Disease, Isoenzymes, ECG.*

**Corresponding author:**
**Mrs. Ankita Khandolkar**
Tutor Dept. of Biochemistry Datta Meghe Medical College, Nagpur.
Email.id. jhaarakesh1993@gmail.com
Mob No.90821 26696

## Introduction

Because of prolonged ischemia, myocardial infarction is known as myocardial cell death.[1] Myocardial cell death does not occur straight away after the beginning of myocardial ischemia, but occurs at 6 hour. The most frequent cause of myocardial infarction is, by far, atherosclerosis.

Myocardial infarction is typically associated with chest pain or discomfort, fatigue, sweating, nausea, vomiting, and arrhythmias. Common risk factors include old age, obesity, smoking, high blood pressure, hypercholesterolemia, and DM. Atherosclerosis, a gradual accumulation of cholesterol and fibrous tissue in plaques inside the arterial wall over decades, is by far the leading cause of MI.[2-5] Atherosclerotic plaques may, however, become unstable, burst, and produce a thrombus that occludes the artery. It contributes to thrombosis and complete vascular occlusion when a major plaque rupture occurs in the coronary arteries, ending with the incidence of MI. [6,7]

The leading cause of death worldwide is cardiovascular disease, responsible for an estimated 16.7 million deaths per year[8.] Coronary artery disease (CAD) incidence varies across various geographical areas across the globe. Pakistani people have the highest known rate of CAD among the South Asian populations, for example. According to careful figures, in 2002, almost 100,000 individuals in Pakistan suffered from acute myocardial infarction (AMI)[9.] Chest pain or discomfort, fatigue, sweating, nausea, vomiting, and arrhythmias are commonly presented with acute MI, often loss of consciousness and syncope. This happens with the abrupt disruption of the flow of coronary blood and is a life-threatening medical emergency requiring immediate management.[10, 11]

For better prognosis, rapid recognition of MI is necessary to initiate successful therapy. The new definition of MI diagnosis emphasises the importance of the 12 lead ECG and the evaluation of early cardiac biomarkers, since MI is often insufficient to diagnose the ECG itself. Kidneys, and. Because of its lack of cardiac tissue specificity, it is no longer used for the diagnosis of MI[12, 13]. LDH was used for the diagnosis of MI[14] in the year 1960. Finally, the WHO recommended the LDH panel for the diagnosis of MI in the year 1979.[15]

**Table 1: The five isoenzymes are found in different amounts in tissues throughout the body.[16]**

| Isoenzyme | Location |
|---|---|
| LDH I | Heart and red blood cells |
| LDH II | White blood cells. It is also found in heart and red blood cells, but in lesser amounts than LDH-1. |
| LDH III | lung tissue |
| LDH IV | white blood cells, kidney and pancreas cells, and lymph nodes |
| LDH V | liver and muscles of skeleton |

Serum LDH activity exceeds the normal range within 24 to 48 hours in a typical patient with acute myocardial infarction, reaches a peak elevation of two to tenfold within 3 to 6 days, and decreases to the normal range within 8 to 14 days.[17] Differences in substrate specificity for LDH isoenzymes were then used to develop serum a-hydroxybutyrate dehydrogenase (HBD; LDH isoenzyme1) assays, which were then used to develop serum a-hydroxybutyrate dehydrogenase (HBD; LDH isoenzyme1) assays. [18]

Accordingly, we undertook the following study to determine the sensitivity of Lactate dehydrogenase cardiacisoenzymes, forthe diagnosis of myocardial infarction.

## Material and Methods

Our study was carried out in the Biochemistry Department, DMMC&SMHRC Nagpur, from January 2020 to June 2020. The study was approved by Institutional Ethical Committee and informed consent was taken prior to the study. A total 100 subjects of age between 30-80 years were enrolled in this study. Out of 100 subjects, 50healthy normal as the same age group and 50were suffering myocardial infarction as a study group. hundred patients belonging to both the

sexes between the age group of 30 -80 years who had an attack of myocardial infarction diagnosed by W.H.O. criteria and admitted to the Intensive Cardiac Care Unit of Shalinitai Meghe Hospital Nagpur were selected for the present study.

**Inclusion criteria**

Inclusion criteria were age between 30-80 years both [Male and Female], diagnosed myocardial infarction and admitted to the Intensive Cardiac Care Unit of Shalinitai Meghe Hospital Nagpur.

**Exclusion criteria**

Patients were not included if they refused informedconsent, could not be sampled serially, or had bypass surgery,which precluded sampling throughout their hospital stay.

**Blood sample collection and processing**

Blood was collected in heparin bulbs by venepuncture soon after the acute episode of myocardial infarction. Haemolysis was avoided while separating the plasma which was immediately subjected to the enzyme assay. The blood samples were collected serially upto three days and a week from the day of infarction. Samples were processed in the clinical chemistry laboratory of Shalinitai Meghe Hospital Nagpur. LDH isoenzymes were measured within 24 h from serum samples stored at room temperature. Aliquots of thesamples were frozen at -70$^0$Cfor subsequent measurement of LDH.

**Biochemical analysis**

Lactate dehydrogenase isoenzymes were fractionated by agarose gel electrophoresis as described by Cawley and Eberhard.[19] The stained slides were scanned on Chromoscan at 520 mix and the percentages of the fractions were quantitated.The ratio of LDH1/LDH2 isoenzymes was determined in theclinical chemistry laboratory at Shalinitai Meghe Hospital by using agarose gel electrophoresis toseparate the LDH isoenzymes eletrophoretically and then quantifyingtheir activity by their NADPH fluorescence. Electrophoreticmethods have been reported to have variable accuracy[20].

## Result

**Table-2: Lactate dehydrogenase (LDH) isoenzymes levels in study and control group**

| Isoenzymes | Control Group (n=50) Mean ± SD | Study Group (n=50) Mean ± SD | P value |
|---|---|---|---|
| LDH 1 (U/L) | 295.8±65.8 | 1157±843 | P < 0.0001 |
| LDH 2 (U/L) | 22.5±5.82 | 92.64±83.12 | P < 0.0001 |
| LDH 3 (U/L) | 0.45±0.36 | 0.58±0.49 | P = 0.1338 |
| LDH 4 (U/L) | 0.52±0.39 | 0.78±0.62 | P = 0.0137 |
| LDH 5 (U/L) | 0.86±0.63 | 0.97±0.64 | P = 0.3385 |

LDH1 and LDH2 both are statistically significant as compared to other isoenzymes.

Table 2 shows the level of lactate dehydrogenase (LDH) isoenzymes in myocardial infraction patients. Hence in this study shows significantly increase the level of LDH 1 and LDH 2 in study group as compared to control group during the stroke and there is no significant difference in the level of LDH3, LDH4 and LDH5 in study group as compared to the control group.

**Table 3: Atypical Symptoms in Myocardial Infraction in Elderly Patients.**

| PERCENTILE OF PATIENTS WITH SYMPTOM | | | |
|---|---|---|---|
| **SYMPTOMS** | **Age 31-60 yrs** | **Age 61-80 yrs** | **Age >80 yrs** |
| Chest Pain | 80 | 77 | 40 |
| Shortness of Breath | 50 | 60 | 62 |
| Sweating | 50 | 25 | 20 |
| Syncope | 5 | 19 | 21 |
| Acute Confusion | 8 | 14 | 26 |
| Stroke | 6 | 18 | 22 |

Table 3 shows that the percentages of myocardial infraction symptoms in patients. The most common symptom is chest pain it's seen mostly in age criteria between 30-60 yrs, shortness of breath symptom seen in age group above 80 yrsother symptoms are also show in different percentile in different age criteria.

## Discussion

The findings obtained in the current study show that the activity of the serum lactic dehydrogenase enzyme increases by 4-5 folds in the first 24-72 hours after the onset of clinical infarction, with a peak on the second day. It then progressively decreases to normal within two weeks. Therefore, the assessment of total serum LDH is useful even after a week from the day of infarction if patients submit themselves for clinical check-up.While the proportion of myocardium to total serum lactic dehydrogenase is high, if other organ diseases such as liver, kidney, etc. coexist, its perception becomes difficult.

For laboratory diagnosis, the electrophoretic isolation of serum enzymes identified by tissue-based isoenzyme patterns is more specific. Agarose gel was found to have a distinct advantage over the others from the various supporting media such as paper, agar, starch gel, polyacrylamide used for gel electrophoresis, Both staining and separation are stronger and it is easier to scan on the densitometer.

**In 1973, Cohen et al.**[21] found that a 1:2 increase in LDH above 2.0 was observed in all patients with myocardial infarction. LDH 1:2 improvement in angina pectoris patients with evidence of myocardial infarction.

In the diagnosis of acute infarction, **Roe et al.**[22] researched the use of LDH isoenzymes in combination with CPK isoenzymes. They showed that the diagnosis was supported by an increase in LDH1:2 to more than 1.0. The increase in LDH isoenzymes occurred about 12 to 24 hours after an acute infarction in their study.

When they used LDH 1:2 in combination with CPK isoenzymes, **Wagner et al**[23] noted 90 percent sensitivity and 95 percent specificity. Although the LDH 1:2 was relatively specific and fragile, they concluded. They found that LDH1:2 typically increased within 24 to 48 hours of symptom onset. LDH 1:2 was particularly helpful in their study in patients whose infarction occurred several days prior to admission.

Our study shows that the determination of LDH isoenzymes is a useful addition to the enzyme diagnosis of myocardial infarction when performed by a rapid and sensitive process. The LDH 1:2 would help to validate the findings of the CK-MB in most cases.

## Conclusion:

The usefulness of complex enzyme reactions is becoming increasingly evident in clinical medicine. Patients suffering from the loss of different tissue cells

in their serum have increased enzymatic activity. When cell death happens, the enzymes usually present within the cells are released into the blood stream. As an aid in the diagnosis of myocardial infarction, the assessment of serum lactic dehydrogenase (LDH) has two advantages: first, there is an increased elevation for as long as six to eight days after the attack; second, there is a much less complex technical method than other enzyme tests. In particular, the determination of LDH, which is usually not important in the diagnosis of acute myocardial infarction, is useful when the electrocardiographic pattern masks recent changes in acute infarction.

Cardiac biomarker analysis has become the frontline diagnostic method for MI and has significantly allowed clinicians to quickly diagnose and prepare for timely treatment, thus greatly reducing the mortality rate. The study of a panel of markers for the diagnosis and prognosis of myocardial infarction would, however, track the future of cardiac biomarkers.

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**Ethical Clearance:** taken from institutional ethics committee

## References

1) Jennings RB and Reimer KA: Factors involved in salvaging ischemic myocardium: Effect of reperfusion of arterial blood. Circulation 68: I25-I36, 1983.
2) Davies MJ, Thomas AC. Plaque fissuring—The cause of acute myocardial infarction, sudden ischaemic death, and crescendo angina. British Heart Journal. 1985;53(4):363-373
3) Hort W. Pathology of acute myocardial infarction and the infarct vessel. European Heart Journal. 1985;6:5-9
4) Falk E. Coronary thrombosis: Pathogenesis and clinical manifestations. The American Journal of Cardiology. 1991;68(7):28B-35B
5) Kristensen SD, Lassen JF, Ravn HB. Pathophysiology of coronary thrombosis. Seminars in Interventional Cardiology. 2000;5(3):109-115
6) Davies M. The pathophysiology of acute coronary syndromes. Heart. 2000;83(3):361-366
7) Arbustini E, Dal Bello B, Morbini P, Burke AP, Bocciarelli M, Specchia G, Virmani R. Plaque erosion is a major substrate for coronary thrombosis in acute myocardial infarction. Heart. 1999;82(3):269-272
8) World Health Organization. The World Health Report 2003-Shaping the Future. Geneva, Switzerland: World Health Organization; 2003
9) Iqbal P. Hyperhomocysteinemia and coronary artery disease in Pakistan. The Journal of the Pakistan Medical Association. 2006;56(6):282-285
10) Lindsay J Jr, Pichard AD. Acute myocardial infarction with normal coronary arteries. The American Journal of Cardiology 1984;54:902-904
11) Osulal S, Bell GM, Hornung RS. Acute myocardial infarction in young adults: Causes and management. Postgraduate Medical Journal. 2002;78:27-30
12) Dolci A and Panteghini M: The exciting story of cardiac biomarkers: From retrospective detection to gold diagnostic standard for acute myocardial infarction and more. ClinChimActa 369: 179-187, 2006.
13) 13. Ladenson JH: A personal history of markers of myocyte injury (myocardial infarction). ClinChimActa 381: 3-8, 2007.
14) Lee TH and Goldman L: Serum enzyme assays in the diagnosis of acute myocardial infarction. Recommendations based on a quantitative analysis. Ann Intern Med 105: 221-233, 1986.
15) Task force: Nomenclature and criteria for diagnosis of ischemic heart disease. Report of the Joint International Society and Federation of Cardiology/World Health Organization task force on standardization of clinical nomenclature. Circulation 59: 607-609, 1979.
16) Hinkle J, Cheever K. Brunner &Suddarth's Handbook of Laboratory and Diagnostic Tests. 2nd Ed, Kindle. Philadelphia: Wolters Kluwer Health, Lippincott Williams & Wilkins; c2014. Lactate Dehydrogenase; p. 354.
17) Entman ML, Kaniike K, Goldstein MA, Nelson TE. Association of glycogenolysis with cardiac sarcoplasmic reticulum. J BiolChem 1976; 251: 3140±6
18) Elliot BA, Jepson EM, Wilkinson H. Serum alpha hydroxybutyrate dehydrogenase: a new test with improved speci®city for myocardial lesions. ClinSci 1962; 23: 305±16

19) Cawley, L. P. and Eberhard, L.: Simplified gel electrophoresis. Rapid technique applicable to the clinical laboratory, Amer. J. Clin. Path., 38: 539-547, 1562

20) Moses GC, Ross ML, Henderson AR. Ten electrophoretic methods compared with a selected method for quantifying lactate dehydrogenase isoenzymes in serum. ClinChem 1988;34:1885-90.

21) Cohen L, Dj or djevich J, Jacobsen S: The contribution of isoenzymes of serum lactic dehydrogenase (LDH) to the diagnosis of specific organ injury- with special reference to myocardial injury. Med Clin N Am 50: 193, 1966

22) Roe CR, Limbird LE, Wagner GS, Nerenberg ST: Combined isoenzymes analysis in the diagnosis of myocardial infarction: Application of electrophoretic methods for the detection and quantitation of the creatine phosphokinase MB isoenzymes. J Lab Clin Med 80: 577, 1972

23) Wagner GS, Roe CR, Limbird LE, Rosati RA, Wallace AG: The importance of identification of the myocardial specific isoenzymes of creatine phosphokinase (MB form) in the diagnosis of acute myocardial infarction. Circulation 47: 263, 1973