

# Diagnostic Role of Lactate Dehydrogenase and Bilirubin Level in Megaloblastic Anemia

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**Abstract:** ***Objective:** To study the change in LDH and bilirubin levels in cases of megaloblastic anemia and to correlate it with severity of vitamin b12 deficiency. **Method:** A case control study was done on 200 people with 100 cases having megaloblastic anemia and 100 normal healthy controls during a span of 1 year. Required biochemical studies were done and data was tabulated in predesigned proforma and assessed. The continues variables were presented as means and compared by unpaired t test. Correlation was assessed by Pearson correlation coefficient. **Results:** The mean LDH level in cases (5076.1 u/l) was very significantly (p value <0.0001) higher than controls (443.6 u/l) and mean bilirubin level was also very significantly higher in cases than controls. On correlation analysis, the levels of both LDH correlated with severity of anemia. **Conclusion:** Since, LDH and bilirubin both are significantly raised in cases of megaloblastic anemia and also their levels correlates well with the severity of anemia, so they can be used as indirect markers of diagnosis and severity.*

**Keywords:** megaloblastic anemia, lactate dehydrogenase, bilirubin, vit B12

## 1. Introduction

Anemia is the most prevalent hematological disorder worldwide, affecting 1.62 billion people (i.e. 24.8% of world population) spanning both developing and developed countries with the highest prevalence being in pre-school age children and the group with highest number of individuals affected being non-pregnant women.[1]

Megaloblastic anemia constitutes an important group. It can be caused by a myriad of reasons, such as:

- Vitamin B12 or folate deficiency- dietary or malabsorptive.
- Anti-folate drugs e.g. methotrexate.
- Leukemia.
- Myelodysplastic syndrome.
- Drugs interfering with DNA synthesis e.g. hydroxyurea, azathioprine, 6-mercaptopurine.

Vitamin B12 (cobalamin) deficiency is a major reversible cause of megaloblastic anemia. Cobalamin is a cofactor for the enzyme methionine synthase, whose deficiency causes ineffective DNA replication and cell maturation leading to the formation of megaloblastic cells in the bone marrow. Daily cobalamin requirements of the body are 1-3 ug whereas the body stores are about 2-3 mg, so the body stores are sufficient for 3-4 years even if the supplies are completely cut-off. Cobalamin is synthesized only by microbes. The only source for humans is animal products such as meat, fish and dairy products.

If cobalamin deficiency is left untreated then it can also cause disabling neurological complications due to ineffective myelination even in the absence of anemia.[2]

The major neurological effects include symmetrical peripheral neuropathy, posterior spinal cord involvement (mainly of cervical and thoracic region) and cerebral white

matter abnormalities leading to paresthesia, muscle weakness, ataxia, dementia, depression, psychosis and cognitive impairment. Autonomic nervous system involvement may cause impotence, postural hypotension and incontinence, all of which can cause significant morbidity.[2]

The risk of developing neurological complications increases with reducing level of vitamin B12 in blood. Also, the anemia responds very well with appropriate replacement therapy, abutting the need for blood transfusion in most cases. Thus, it is highly necessary to diagnose the condition early and to start timely management.

Presently available tools for confirming the diagnosis, after getting a suspicious finding in a complete hemogram and peripheral blood smear are:

- Serum vitamin B12 level estimation.
- Serum methyl Malonic acid level estimation.
- Serum homocysteine level estimation.
- Serum trans-cobalamin 2 level estimation.
- Serum folate levels.
- Bone marrow aspiration

All these biochemical methods bear high cost on the patient's pocket and require an expensive setup which is usually not feasible in the under-resourced peripheral parts of the developing countries. So, we studied some commonly available biochemical parameters and their correlation with megaloblastic anemia.

## 2. Method & Materials

### Study design

This was a case control study done on patients admitting in the general medicine specialty of Government Medical College, Kota, Rajasthan, a tertiary level health care facility in the region. The study was conducted between march 2019

to march 2020 after prior permission from Institute' Ethical Committee.

### Case definition

100 cases of megaloblastic anemia were selected on the basis of following:

### Inclusion criteria

- 1) Hemoglobin (Hb) level < 10 mg/dl.
- 2) Serum Vitamin B12 level < 200 ng/l, measured by Competitive Binding Luminescence Assay (CBLA).

Or

Serum folate level <2 ug/l, measured by Automated ELISA

### Exclusion criteria:

- 1) Age < 18yrs or >70 yrs.
- 2) Patients having chronic kidney disease (CKD), chronic liver disease (CLD), acute myocardial infarction (MI).
- 3) Patients having concomitant other causes of anemia like hemolytic anemia, leukemia, myelodysplastic syndrome.

**Control definition:** 100 normal healthy adults were selected who had come for routine medical checkups.

Total serum bilirubin and serum LDH levels of all these cases were measured from the institute's central laboratory. The results were recorded systematically in pre-designed pro-forma and evaluated statistically.

**Statistical analysis:** Categorical variables were presented as proportions and continuous variables as means. Continuous variables were compared using unpaired t test. Correlation between variables was assessed by Pearson coefficient. Results were considered significant if p value was <0.05.

## 3. Results

The demographic details of the sample population are:

**Table 1:** Demographic details

|             | Cases (n=100) | Controls (n=100) |
|-------------|---------------|------------------|
| Age (years) |               |                  |
| 18-30       | 50            | 29               |
| 31-50       | 28            | 52               |
| 51-70       | 24            | 19               |
| Mean        | 37.4          | 39.01            |
| Sex         |               |                  |
| Males       | 54            | 65               |
| Females     | 46            | 35               |

The average levels of various markers in cases and controls was as computed and compared by unpaired t test.

**Table 2:** Average distribution of various laboratory markers in cases and controls. LDH (lactate dehydrogenase), Hb (hemoglobin), MCV (mean corpuscular volume), TLC (total leukocyte count), T. Bilirubin (total bilirubin).

|                                  | Cases (n=100) | Controls (n=100) | P value |
|----------------------------------|---------------|------------------|---------|
| LDH(u/l)                         | 5076          | 423              | <0.0001 |
| T. Bilirubin (mg/dl)             | 1.93          | 0.58             | <0.0001 |
| Hb (g/dl)                        | 4.90          | 13.1             | <0.0001 |
| MCV (fl)                         | 111           | 88               | <0.0001 |
| TLC (% 10 <sup>3</sup> /ul)      | 4.01          | 6.87             | <0.0001 |
| Platelets (*10 <sup>5</sup> /ul) | 104.5         | 203.18           | <0.0001 |

Statistical correlation was assessed between various markers using Pearson correlation coefficient.

**Table 3:** Correlation between HB, MCV and vit b12 level with LDH and bilirubin levels

| Correlation between | R value | P value (one tailed) |
|---------------------|---------|----------------------|
| LDH & Hb            | -0.27   | 0.02                 |
| LDH & MCV           | 0.02    | 0.42                 |
| LDH & Vit B12       | 0.10    | 0.23                 |
| Bilirubin & Hb      | -0.09   | 0.25                 |
| Bilirubin & MCV     | -0.12   | 0.20                 |
| Bilirubin & Vit B12 | 0.13    | 0.17                 |

## 4. Discussion

LDH is an enzyme which reversibly catalyzes the conversion of pyruvate to lactate along with the conversion of NADH to NAD<sup>+</sup>. Pyruvate (the end product of glycolysis) is converted to lactate in oxygen deficient conditions and the reverse reaction occurs during the Cori cycle in liver. It is a tetrameric protein made up of 2 types of subunits (M and H) which forms 5 iso-forms having similar enzymatic activity but stored in different tissues. LDH-1(4H) in heart and RBCs, LDH-2(3H1M) in reticuloendothelial system, LDH-3(2H2M) in lungs, LDH-4(1H3M) in kidneys and pancreas, LDH-5(4M) in liver, skeletal muscles. LDH-2 is usually the abundant form in plasma but an LDH-1 level more than LDH-2 ("flipped pattern") was earlier used to diagnose myocardial infarction.[4]

In the present study, people of all age groups were present with mean age of cases being 37.4 years while the mean age of controls being 39.01 years.

The average hemoglobin level in cases is 4.9 g/dl while 13.1 g/dl in controls. This shows that most of the cases had severe anemia, which is because the fact that cases were selected from pool of in-patients, which are usually admitted if there is a need of blood transfusion i.e. either having severe anemia or cardiovascular instability.

The mean MCV level in cases was 111 fl while in controls was 88fl. But 12 cases had a normal MCV but the LDH level was still significantly raised (6715.3 u/l) in them also.

The average total leukocyte count in cases is on the lower limit of normal range while that of controls is well within the normal range. Similarly, the average platelet count in the cases is 1.05 lakhs/ml while in the controls was 2.03 lakhs/ml. This is consistent with the fact that vit b12 or folate deficiency causes reduction in all cell lines due to impaired DNA synthesis. But the reduction in the other cell lines is usually modest.

Previous literature showed that LDH level is highly raised in the plasma of megaloblastic anemia patients.

**Table 4:** Earlier studies regarding LDH levels in megaloblastic anemia

| Year | Author                    | Results   |
|------|---------------------------|---|
| 1959 | Ruben Gordin et al        | With corticosteroid therapy, in addition to complete hematological remission, a decrease in the LDH activity was observed in megaloblastic anemia cases, while the serum B12 values were still low. The normalization of the LD activity is thus independent of the kind of therapy but is due directly to the change of the bone marrow from megaloblastic to normoblastic.[5] |
| 1964 | Norman Anderssen          | Mean LDH value in megaloblastic anemia was 3,800 units, while the mean value among controls was 257 units.[6]   |
| 1966 | C.F McCarthy et al        | LDH level in patients with marked megaloblastosis were markedly raised.[7]  |
| 2000 | T.S Jaswal et al          | Total LDH levels >3000 IU/L are diagnostic of megaloblastic anemia and in those with levels between 451-3000 IU/L, flipped (LDH <sub>1</sub> > LDH <sub>2</sub> ) pattern is suggestive of megaloblastic anemia.[8]   |
| 2015 | S. Chaudhari et al        | Marked increase in serum LDH up to 5 times of the upper serum limit with mean value of 2396.04 IU/L in megaloblastic anemia.[9]   |
| 2018 | Amrapali L. Gaikwad et al | Total serum LDH >3000 IU/L and reversed LDH pattern can be used to diagnose megaloblastic anemia.[10]   |

In our study mean LDH level was found to be significantly high in the cases with megaloblastic anemia i.e. 5076.14 u/l as compared to 443.6 u/l in controls.

Bilirubin, another indirect marker of hemolysis is formed from the catabolism of heme released from the lysed RBCs. But bilirubin is also a non-specific compound which can be raised in a multitude of other hepatic and post hepatic disorders. To our knowledge there are no studies regarding bilirubin levels in megaloblastic anemia. Upon extensive literature search only few case reports were found.

**Table 5:** Literature regarding bilirubin level in megaloblastic anemia

| Year | Author                | Results   |
|------|-----------------------|---|
| 2007 | Khanduri U et al      | Indirect bilirubinemia was found in 32% of patients in whom liver function tests were done.[11]           |
| 2012 | Sowjanya dasari et al | Reported a case of recurrent indirect hyperbilirubinemia diagnosed as having severe B12 deficiency.[12]   |
| 2013 | Suartcha et al        | Reported a case of combined cobalamin and iron deficiency anemia with elevated total serum bilirubin.[13] |
| 2015 | Nilgun Eroglu et al   | Ascribed B12 deficiency as one of the causes of neonatal hyperbilirubinemia in his study.[14]             |

In our study, mean bilirubin level was higher (1.93 mg/dl) in cases as compared to controls (0.3mg/dl). But unlike LDH, the rise in bilirubin level is modest.

The rise of both LDH and bilirubin can be ascribed to ineffective erythropoiesis leading to the lysis of megaloblasts in the bone marrow as evidenced by the normalization of LDH level in plasma with conversion of

cells from megaloblastic to normoblastic by corticosteroid therapy in Ruben gordin's study.[5]

On correlation analysis using pearson coefficient, only hemoglobin levels correlated negatively with LDH levels and that too with low strength (<0.3). this may be due to a smaller sample size and narrow range of anemia severity as most of the cases were severe.

## 5. Conclusion

Thus, it can be concluded that LDH and Bilirubin both are significantly raised in cases of megaloblastic anemia. Also, the level of LDH correlates with severity of anemia. Thus, they can be used as supportive markers for diagnosis of megaloblastic anemia and probably for severity of anemia. But more studies with larger sample size are required to establish correlation between these markers and severity of anemia and vit b12 deficiency.

## 6. Declarations

**Funding:** none

**Conflicts of interest:** none

**Ethics approval:** prior approval from institute's ethics committee.

**Consent to participate:** informed consent was taken from all the participants included in the study.

**Consent for publication:** not applicable.

**Availability of data and material:** all data generated or/and analyzed in this study is available from corresponding author on reasonable request.

**Author's contribution:** all the authors contributed equally to the design of research, analysis of results and writing of the manuscript.

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