

## Original article

# Study of correlation between hemoglobin, ferritin and lactate dehydrogenase in SARS-Covid 19 positive patients at tertiary care hospital

<sup>1</sup>Nadia N Ali , <sup>2</sup>Rahul Choudhari , <sup>3</sup>Mahsh Phad , <sup>4</sup> Santosh G Varma , <sup>5</sup>Kailas Gadekar\*

<sup>1</sup>Senior Resident, <sup>2,3,5</sup>Assistant Professor , <sup>4</sup>Head and Professor ,  
Department of Biochemistry, B J Government Medical College, Pune  
Corresponding author \*



## Abstract

**Introduction:** Coronavirus disease-19 (COVID-19) has been regarded as an infective-inflammatory disease, affecting multi-organs. Anemia reduces oxygen delivery to the tissue and may thus play an important role in the development of multi-organ failure. Therefore, it is crucial to understand the relation between anemia and iron metabolism in COVID-19 infection.

**Objective:** 1. Quantify the mean levels of hemoglobin, ferritin and LDH of iron metabolism in COVID-19 patients. 2. To correlate level of hemoglobin, ferritin and LDH of iron metabolism in COVID 19 patients.

**Material and Method:** Cross-sectional retrospective studies of 60 subjects i.e. 30 patients with COVID-19 disease and 30 covid negative subjects for a period of 1 month from 1/10/2020 to 31/10/2020.

**Result:** Correlation between Hb and Ferritin was found to be -0.53 i.e. negative correlation, decrease in hemoglobin cause increase in ferritin level. This correlation is statistically significant. Correlation between Ferritin and LDH was found to be 0.44 i.e. positive correlation, increase in LDH is associated with increase in ferritin level. This correlation is statistically significant.

**Conclusion:** In the present study, we investigated the correlation between hemoglobin, ferritin and LDH in covid 19 patients. We found that: It is found that there is correlation between decrease in serum hemoglobin and increase ferritin and Ldh level reason being 1) Increase hemolysis is associated with increase in LDH level. 2) The increased retention and storage of iron within ferritin in macrophages contribute to the characteristic fall in serum iron concentrations and an increase in serum ferritin concentrations, observed in an acute phase response.

**Keywords:** COVID-19; hemoglobin, Ferritin, Lactate dehydrogenase

## Introduction:

The current pandemic of corona virus disease 2019 (COVID-19) originally emerged from China, but has since then infected millions of patients worldwide. This condition is associated with high morbidity, leading to significant strain on healthcare infrastructure and resources. The associated fatality rate is also higher than other respiratory viral infections. Hence, it is necessary to urgently identify reliable predictors of disease severity and mortality for careful allocation of healthcare resources and to enable earlier clinical intervention and monitoring to improve clinical outcomes. <sup>1</sup>The clinical characteristics of this pandemic disease may be complicated by the onset of a severe

form of intestinal pneumonia in 10-15% of infected patients, which may then progress towards acute respiratory distress syndrome (ARDS) and eventually in multi-organ failure (MOF) and death.<sup>2</sup>

A study by Wenzhong, et al. could provide the missing link in the understanding of the pathogenesis of COVID-19. Through conserved domain analysis, homology modeling, and molecular docking; they compared the biological roles of certain proteins of the novel corona virus. Their results showed that these proteins, binding to porphyrin, could attack the 1-beta chain of hemoglobin to dissociate iron and form porphyrin (hemoglobin consists of hem and globin; hem is composed of iron and porphyrin).<sup>3, 5</sup> This Fe<sup>2+</sup> ions fail to carry O<sub>2</sub> and CO<sub>2</sub>. Due to the lack of O<sub>2</sub>/CO<sub>2</sub> exchange in the lung, CO<sub>2</sub> damages lung tissue along with the toxic ion of Fe<sup>2+</sup>. To combat the increased Fe<sup>2+</sup> concentration, the release of high serum ferritin level as a part of immunity system causes a chain reaction with an appreciable change in its values.<sup>4, 6</sup> LDH (lactate dehydrogenase) is a reliable marker of hemolysis. More interestingly, LDH figures increase of a two-three fold in worst scenarios, paralleling hemoglobin decrease.<sup>4, 7</sup> The aim of this study is to see the correlation between decrease hemoglobin, increase ferritin and LDH as a part of hemolysis in covid infected patients.

**Aim:** Study to observe the correlation between hemoglobin, ferritin and Lactate dehydrogenase in pathogenesis of SARS-Covid 19 infection.

**Objective:** 1. Quantify the mean levels of hemoglobin, ferritin and LDH of iron metabolism in COVID-19 patients.  
2. To correlate level of hemoglobin, ferritin and LDH of iron metabolism in COVID 19 patients.

**Material and Method:** Cross-sectional retrospective studies of 60 subjects i.e. 30 patients with COVID-19 disease and 30 covid negative subjects randomly selected between age group 20- 80 years were done for a period of 1 month from 1/10/2020 to 31/10/2020.

#### **Inclusion criteria**

**Cases:** Consists of 30 patients resulted positive for Covid 19 by Real Time-Polymerase Chain Reaction from the nasopharyngeal swab and their sample collected immediately after admission to prevent drug interference.

**Control:** 30 covid negative subjects which were in close contact with the covid positive patient.

#### **Exclusion criteria-**

- comorbid patients .
- Patients having any diagnosed hemolytic disorder.
- Not in a condition to give valid consent.
- Children below 20 years of age.

#### **Facilities and Equipments:**

- All the required facilities and equipment were available in the department of Biochemistry. The study did not involve any harm to the patients.
- Hemoglobin estimation was done on automated cell counter machine by electrical impedance method. Serum Ferritin levels were estimated by ECLIA method on e411 machine. Serum LDH level was estimated by colorimetry method on XL 640.

**Results:**

All the calculations were done using Microsoft Office Excel 2013 and statistical analysis was done using IBM SPSS Statistic version 20. P-value less than 0.05 ( $p < 0.05$ ) was considered to be statistically significant(S). P-value of less than 0.001 ( $p < 0.001$ ) was considered to be statistically highly significant (HS). P-value more than 0.05 ( $p > 0.05$ ) was considered to be statistically non- significant.

Table1. Mean Hemoglobin, LDH and ferritin Comparison between Cases and the Control Group.

Parameter	Cases(mean+/-SD)	Control(mean+/-SD)	P-value
Hb level	7.86+/-0.16	13.58+/-0.266	0.08
LDH	2252.93+/-293.87	558.3+/-43.78	0.05
Ferritin	1926.94+/-283.57	563.0+/-109.89	0.5

Hemoglobin value was compared between the cases and control and it come out to be 0.08, which is non-significant. Similarly LDH and Ferritin level was also compared between the cases and the control which came out to be 0.05 i.e. statistically significant and 0.5 which is again non-significant.

Table 2: Showing correlation between hemoglobin, LDH and Ferritin in Cases.

Parameter	r value
Between Hb and LDH	-0.73
Between Hb and Ferritin	-0.53
Between Ferritin and LDH	0.44

Figure 1: Scatter diagram showing negative correlation ( $r = -0.73$ ) between LDH and Hb level of Cases.

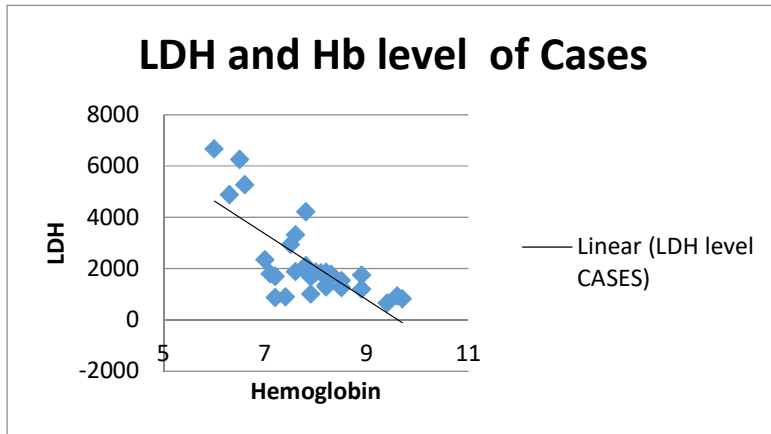


Figure 2: Scatter diagram showing positive correlation ( $r = 0.44$ ) between LDH and Ferritin level of Cases.

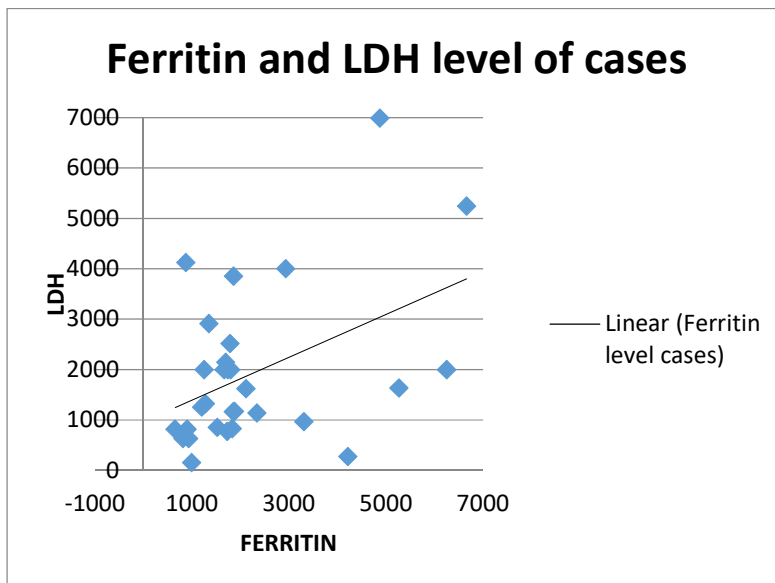
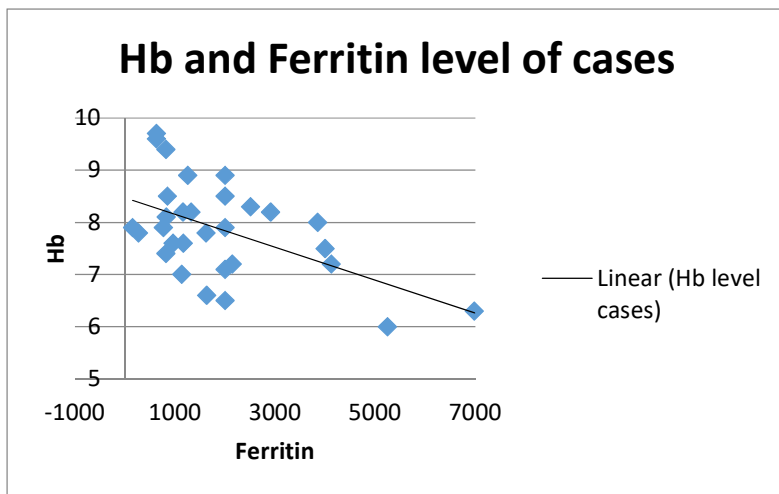


Figure 3: Scatter diagram showing negative correlation ( $r = -0.53$ ) between Hb and Ferritin level of Cases.



## DISCUSSION

The present study was carried out on 30 covid 19 positive patients and 30 covid negative subjects who were in close contact with the covid positive patient. In the patient group pooled mean hemoglobin level was 7.86 g/dl, and that of control group mean hemoglobin level is 13.58 g/dl. It is found that there is no significant difference in the mean hemoglobin level between the two groups ( $p=0.08$ ).

### Factors that cause low hemoglobin in covid 19 patients are as follows:

1. There is remarkable similarity between the distant amino acid sequence of SARS-CoV-2 spike glycoprotein cytoplasmic tail and the hepcidin protein. Corona viruses recognize host receptors using their spike proteins. They then bind the cell membrane and enter host cytoplasm; by using host furins and proteases. The found hepcidin mimicry by the virus would take place through this complex mechanism. Hepcidin is the master regulator of iron metabolism, interacting with ferroportin to favor iron entrance inside the cells. In case of hepcidin-like activity of SARS-CoV-2 a significant iron dysmetabolism may occur.<sup>4</sup>
2. Hepcidin is also an acute-phase protein. Chronic inflammation causes sustained hepcidin overexpression which causes iron to be locked up in macrophages through the blockage of ferroportin. This reduces iron availability to the erythron, which can lead to anaemia. Thus, iron, inflammation and anaemia are linked through the action of hepcidin.<sup>15</sup>
3. Hepatocytes are the primary source of hepcidin. Liver has a crucial role in the regulation of iron absorption by the gut. Viral infections that disrupt liver function can be accompanied by changes in iron homeostasis, and iron loading of this organ can exacerbate chronic viral disease.<sup>15</sup>
4. Hemoglobin level also decreases with older age, higher proportion of comorbid illness and severity.

In the patient group pooled mean LDH level was 2252.93 U/L, and that of control group mean hemoglobin level is 558.3 U/L. It is found that there is no significant difference in the mean hemoglobin level between the two groups ( $p=0.05$ ).

**Factors that cause high LDH level in covid 19 patients are as follows:**<sup>10</sup>

1. Severe infections may cause cytokine-mediated tissue damage and LDH release.
2. LDH is present in lung tissue (isozyme 3). Patients with severe COVID-19 infections can be expected to release greater amounts of LDH in the circulation. This cycle leads to severe form of interstitial pneumonia, often evolving into acute respiratory distress syndrome, which is the hallmark of the disease.
3. LDH levels are elevated in thrombotic microangiopathy, which is associated with renal failure and myocardial injury and is often seen in patients of covid 19.

In the patient group pooled mean Ferritin level was 1926.94 micro g/L, and that of control group mean hemoglobin level is 563.0 micro g/L. It is found that there is no significant difference in the mean hemoglobin level between the two groups (p=0.5).

**Factors that cause high ferritin in covid 19 patients are:**<sup>7</sup>

- 1) Proinflammatory cytokines such as interleukin-1  $\beta$  (IL-1  $\beta$ ), tumor necrosis factor- $\alpha$  (TNF-  $\alpha$ ), and IL-6 may increase ferritin synthesis early in inflammation.
- 2) The cellular damage derived from inflammation can promote the leakage of intracellular ferritin, thus elevating serum ferritin.
- 3) In acidosis, the microvascular environment and increased production of reactive oxygen species (ROS) might liberate iron from ferritin. This unliganded iron that can participate in Haber-Weiss and Fenton reactions, thus creating hydroxyl radicals, causing further cellular damage leading to worsening tissue injury, thus causing a vicious cycle of inflammation.

To find out the correlation between the hemoglobin, ferritin and LDH correlation analysis was done.

1. Correlation between Hb and LDH was found to be -0.73 i.e. negative correlation, decrease in hemoglobin cause increase in LDH level. This correlation is statistically significant.

Cause of this correlation is: LDH is physiologically measurable in serum due to physiological cellular turnover. Its 5 isoenzymes are present. In particular, LDH-1 and LDH-2 isoenzymes are expressed in RBC. In the hemolytic conditions, LDH (mainly isoenzymes 1 and 2) is often increased and it is useful biomarker of peripheral destruction driven anemia.<sup>14</sup>

Cause of this correlation is: The innate immune system control over iron metabolism as a response to viral infections. The innate immune system will react by: 1) decreasing the bioavailability of iron. 2) By limiting the replication of the virus during the acute phase of infection. In these conditions, through interleukin-6 and Toll-like-receptor-4 dependent pathways, the levels of hepcidin-the master regulator of iron homeostasis- could increase and block the activity of the transporter ferroportin (carries iron out of the cells). There will be decrease in the amount of iron absorbed from the diet, causing cellular sequestration of iron. Intracellular iron sequestration will lead to an upregulation of cytosolic ferritin to prevent iron-mediated free radical damage. The increased retention and storage of iron within ferritin in macrophages contribute to the characteristic fall in serum iron concentrations and an increase in serum ferritin concentration.

2. Correlation between Hb and Ferritin was found to be -0.53 i.e. negative correlation, decrease in hemoglobin cause increase in ferritin level. This correlation is statistically significant. These conditions are mainly observed in an acute

phase response. It will result in diminished iron availability for erythropoiesis and as a result further aggravation of anemia.<sup>6</sup>

3. Correlation between Ferritin and LDH was found to be 0.44 i.e. positive correlation, increase in LDH is associated with increase in ferritin level. This correlation is statistically significant.

Causes of this correlation are: LDH and Ferritin both being general indicator of acute or chronic tissue damage, is considered to be an inflammatory marker. LDH has been described to be increased during acute and severe lung damage, and elevated LDH values has been found in other interstitial lung infections.<sup>13</sup> Ferritin on other hand play a critical role in inflammation by contributing to the development of a cytokine storm. According to Shoenfeld et al., the clinical picture of the critical cases of COVID-19 resembles macrophage activating syndrome, which is commonly associated with high levels of ferritin or even a cytokine storm. H-chain of the ferritin could be important in activating macrophages to increase the secretion of inflammatory cytokines observed in COVID-19 patients.<sup>6</sup>

### **Conclusion**

In the present study, we investigated the correlation between hemoglobin, ferritin and LDH in covid 19 patients. We found that:

1. There is no significant difference in mean hemoglobin level between the cases and the control, though it was found that in viral infections there is sequestration of hemoglobin in cells through hepcidin mediated mechanism.
2. There is no significant difference in mean Ferritin level between the cases and the control, though high ferritin in covid positive patient is due to proinflammatory cytokines mediated increase ferritin synthesis and due to leakage of intracellular ferritin.
3. There is significant difference in mean LDH level between the cases and the control, through cytokine-mediated tissue damage.
4. It is found that there is correlation between decrease in serum hemoglobin and increase ferritin and Ldh level reason being 1) Increase hemolysis is associated with increase in LDH level. 2) The increased retention and storage of iron within ferritin in macrophages contribute to the characteristic fall in serum iron concentrations and an increase in serum ferritin concentrations, observed in an acute phase response.

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For any images presented appropriate consent has been obtained from the subjects: NA

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