# Clinico-Pathological Association of Haemoglobin At The Time of Presentation: A Predictive Tool of Survivorship in COVID-19

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## ABSTRACT

***Objective:*** To determine the clinico-pathological association of haemoglobin at the time of presentation as a predictor of survivorship in patients suffering from COVID-19.

***Study Design***: Prospective observational study.

***Place and Duration of Study***: Combined Military Hospital Hyderabad Pakistan, from Mar to Sep 2020.

***Methodology***: Two hundred and four patients who were symptomatic and PCR positive for COVID-19 were included in the study. Informed consent was taken from patients and approval from the institutional ethics committee was obtained. Haemoglobin values were analyzed using SPSS version 22.

***Results***: In our study, 186 (91.2%) patients survived the disease and 18 (8.8%) patients died. The mortality rate was high in patients who presented with low haemoglobin levels at time of presentation.

***Conclusion***: This study concluded that hemoglobin level at time of presentation could be a predictor of survivorship.

**Keywords**: Anemia, COVID-19, Haemoglobin, Haemoglobinopathies.

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## INTRODUCTION

The novel corona virus (COVID-19) causes an infectious acute respiratory disease. The disease mainly affects lungs leading to respiratory insufficiency but in recent studies a multi organ involvement has been reported including cardiovascular thrombotic events, together with pulmonary embolism, disseminated intravascular coagulation (DIC) and liver/myocardial/renal failures.[1] An endothelial cell causing viral injury, contributes to the blood and vascular complications.[2] The virus is a RNA virus with high homology to bat corona virus.[3] There are various pathways of haemoglobinopathies leading to hypoxia which may have potential pathophysiological role.[4]

The patients who have anemia when encounter attack by the virus, respond differently from those with normal haemoglobin levels. Considering the fact that COVID-19 virus attacks haemoglobin molecule causing haemolysis; in a person with already reduced haemoglobin levels, the effect of the disease would be different compared to a person with normal haemoglobin levels. This study was planned to determine the relationship between haemoglobin levels and symptomatology and how a patient would combat the disease. Low haemoglobin at presentation can be a predicting factor for increased mortality and morbidity. In this study, we aimed to determine whether a person with low haemoglobin at time of presentation faces same morbidity as normal counterparts or not.

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## METHODOLOGY

This was a prospective observational study, carried out at Combined Military Hospital, Hyderabad from March to September 2020. Patients of COVID-19 were recruited after informed consent through universal sampling method.

**Inclusion Criteria**: All of the newly diagnosed cases of COVID-19 with positive PCR were included in study.

**Exclusion Criteria**: Symptomatic patients with negative PCR and PCR positive neonates and children less than 16 years of age were excluded from the study.

A total of 204 patients were included in the study, which was carried out after taking approval from Institutional Ethical Review Board. All the chosen patients had a detailed history, physical examination and complete basic diagnostic tests. None of the patient had a known history of blood disorder. For the entire baseline blood picture, blood samples were drawn in EDTA tubes, within two hours of admission and run immediately. They were analyzed on Sysmex Kx21. The primary endpoint in the study was defined as a combination of admission to the ICU, requirement of mechanical ventilation or death.

All the data was analyzed using Statistical Package for Social Sciences (SPSS) version-22. Quantitative variables were measured as mean ± SD while frequencies and percentages were calculated for qualitative variables. Paired sample t-test was applied between pre-treatment and post-treatment haemoglobin levels. Comparison of haemoglobin levels at the time of admission and the last in-hospital haemoglobin, in both genders were carried out by applying independent sample t-test. The *p*-value of ≤0.05 was considered statistically significant.

## RESULTS

In our targeted population, there were 65 (31.9%) females while 139 (68.1%) males. The mean age of presentation was 53.3 ± 8 years (ranging from 18-86 years). A total of 117 (57.35%) patients were from rural background. Mean haemoglobin level of patients before treatment was 12.23 ± 1.34 g/dl while mean haemoglobin level of patients after treatment was 13.10 ± 1.26 g/dl and the result was statistically significant ($p<0.001$) (Table-I). The mean days of hospital admission were 9.38 ± 4.70 days.

**Table-I: Comparison of pre-treatment and post-treatment haemoglobin levels.**

| Parameter | Pre-treatment | Post-treatment | *p*-value |
|---|---|---|---|
| Haemoglobin level (g/dl) | 12.23 ± 1.34 | 13.10 ± 1.26 | <0.001 |

Out of 204 patients included in our study, 59 (28.92%) patients had haemoglobin below WHO defined parameters for age and gender (males ≥13 g/dl and females ≥12 g/dl) at time of presentation while 145 (71.08%) had it within normal levels. Out of those 59 cases who presented with low haemoglobin, 18 died. Mortality cases were shifted to Intensive care for intubation. In deceased patients 11 (61%) were males and 7 (39%) were females. The haemoglobin levels at the time of admission and last in-hospital haemoglobin in both genders were shown in the Table-II.

**Table-II: Comparison of haemoglobin levels at the time of admission and the last in-hospital haemoglobin in both genders.**

| Male Patients | | | | |
|---|---|---|---|---|
| **Parameter** | | **Haemoglobin Level at the Time of Admission** | **Last in-Hospital Haemoglobin Level** | ***p*-value** |
| Haemoglobin level (g/dl) | Recovered (n=128) | 12.61 ± 1.08 | 13.43 ± 1.00 | <0.001 |
| | Dead (n=11) | 10.37 ± 2.52 | 11.52 ± 2.81 | 0.324 |
| **Female Patients** | | | | |
| Haemoglobin level(g/dl) | Recovered (n=58) | 11.86 ± 1.04 | 12.80 ± 0.83 | <0.001 |
| | Dead (n=7) | 11.27 ± 1.93 | 11.92 ± 1.97 | 0.5446 |

## DISCUSSION

According to the literature, about two mechanisms of injury including; interaction of virus with haem molecule via CD 147 and CD 26 located on RBCs and ferroportin blockage are caused by hepcidin-mimetic action of a viral spike protein.[5] Virus attacks the beta chain of haem molecule in RBC via CD 147 and CD 26.[6,7] It is also possible that bone marrow erythroblasts may be attacked by the viruses as the larger dimension and cytoplasmic nuclear material of the precursors would facilitate virus replication and interaction with the hemoglobin.[8,9] In some studies, it was postulated about the virus associated haemoglobin denaturation leading to oxygen deprivation and multi organ failure. Recent studies, based on preliminary computational and genetic sequencing researches, have shown that hemoglobin dysfunction and tissue iron overload caused the dreadful damage in multiple organs.[10-14] The progressively decreasing hemoglobin level may lead to a sideroblastic-like anemia pattern, with myelodysplastic features on peripheral blood smear.[15-20]

Wenzhong and Hualan carried out a study on COVID-19 affected patients in China in 2020.[11] They suggested that COVID-19 attacked the 1 Beta chain of hemoglobin and captures the porphyrin to inhibit heme metablolism. They mentioned that the COVID-19 infection initiated an immune response in the body that led to genesis of immune antibodies after recovery from the illness. The likely mechanism involved the binding of viral protein with porphyrins. The cytokine storm was so dangerous that it might be fatal in some patients. The anatomical features found in dead COVID-19 infected patients were markedly different from other SARS patients.[21] The important findings was the presence of too much mucus in the tissues due to abundance of mucin proteins that causes firm adherence of cells. The authors documented that plasma cells were mainly found in connective tissues of intrinsic membrane in digestive and respiratory tract. Being the antibody secreting cells, they synthesized and stored immunoglobulins (antibodies) contributing to humoral immune responses. The presence of IgM and IgG antibody depend on the state of infectivity and recovery. The authors mentioned that an infected person

with COVID-19 pneumonia had greater amount of IgM in blood which gradually reduces during treatment and recovery process while the amount of IgG increases depicting the body immunity and resistance to COVID-19. The authors stated that the ACE2 receptors on plasma cells might be involved in Spike-ACE2 infection pathway which convinced them to speculate the involvement of plasma cells during infection and recovery of patients. The authors suggested that these plasma cells secreted various antibodies which were evident by release of viral proteins in infected patients. The viral proteins like ORF1ab, ORF3a and ORF10 were made in plasma cells which ultimately attacked the hemoglobin and heme. They further suggested that the viral proteins might be found outside the cell through secretory protein pathways.

Zhang and Guangsheng mentioned that the COVID-19 infected patients showed lower levels of hemoglobin as compared to healthy population.[17] They observed that out of 99 patients who reported in Jin Yin-tan Hospital China, hemoglo-bin was below the normal range in 51% of COVID-19 patients. They further observed that the hemoglobin level of severe patients with COVID-19 pneumonia was lower in 41 patients, although the difference was not marked (12.2 g/L (11.1–12.8) vs 13.05 g/L (12.0–14.0), $p$=0.20). They mentioned that out of 1099 pati-ents infected with COVID-19, the severe group pati-ents had lower hemoglobin level of 12.8 g/L (11.1–14.1) than that of 13.5 g/ L (12-14.8) in non-severe one ($p$<0.001). They found that critical ill patients who required admission in ICU, invasive ventilation and those who did not survive had more drastic reductions in hemoglobin as compared to patients who had mild to moderate illness. The authors had a comparative analysis with other researchers Zhou *et al*,[22] who observed that severe COVID-19 cases had significant reduction in levels of hemoglobin (12.5 g/L (9.7–14.4) vs 14.5 g/ L (11.1–16.2), $p$=0.002). The authors suggested that COVID-19 caused inflammatory changes that led to interference with erythropoiesis, resulting in a reduction in hemoglobin levels in affected patients. They suggested that long lifespan of erythrocytes and compensatory proliferation of erythrocytes due to pneumonia-associated hypoxia was reason for low incidence of anemia observed in COVID-19 patients. They recommended that researchers should pay attention to declining levels of hemoglobin.

Chowdhury and Anwar mentioned that hemoglobin levels were considerably reduced in patients of COVID-19 with severe illness.[20] This finding was supported by meta-analysis of available studies on COVID-19.[23] The authors mentioned that patients with other types of pneumonia had similar reduction in hemoglobin levels. The patients with milder form of COVID-19 illness had less reduction in hemoglobin which supported earlier studies.[10,15] Viral proteins invade the 1-B chain of hemoglobin causing dissociation of iron to form porphyrins. They suggested that deoxyhemoglobin is at higher risk of viral attack as compared to oxidized hemoglobin but the conclusive experimental evidence is still awaited. The authors recommended that future research should pay attention to the declining levels of hemoglobins in COVID-19 patients, as it was area of much significance in treatment and management of such patients especially those having severe form of COVID-19. The authors concluded that lower levels of hemoglobins were associated with drastic outcomes.

Cavezzi *et al* suggested that multiple viral attacks at different levels resulted in a cascade of biochemical reactions which are hallmark of COVID-19.[12]

Sharp and Ghodke carried out a retrospective study, on about 506 patients in India.[21] They mentioned that haemoglobin being oxygen transporter for the body required to be maintained in optimum level in order to compensate for the decreased oxygenation in the lungs. The authors found that COVID-19 patients were at increased risk of reduction in hemoglobin. Out of 506 patients in the study population, the authors found that 54.34% were non-anaemic, 26.28% of the patients were mildly anaemic, 12.64% of the patients were moderately anaemic and 6.72% were severely anaemic. The authors recommended that low haemoglobin concentration could be addressed by dietary and management protocols. They suggested that larger databased studies incorporating extensive clinical and pathological findings must be done to establish and confirm potential mechanism involving COVID-19 effects on hemoglobin.

Gille *et al* carried out a retrospective observational study that compared the blood gas analyses of 100 COVID-19 patients (from March-April 2020) to those of 100 non COVID-19 patients (from March-April 2019).[23] They also included a third group of 55 patients with carboxy-haemoglobin (HbCO) ≥8% (positive controls with altered haemoglobin affinity) in which P50 was corrected for body temperature, pH and PCO2. They found that patients were not statistically different for age or sex ratio in the COVID-19 and non COVID-19

groups. Median P50 at baseline was 26 (25.2-26.8) versus 25.9 (24-27.3), respectively ($p$=0.42). They found that it was lower in the high HbCO group: 22.5 (21.6-23.8) ($p$<0.0001). The authors observed that when considering the time course of P50, no significant difference was observed between COVID-19 and non COVID-19 groups, from 1-18 day. Median haemoglobin concentration at baseline was 14 g.dl-1 (12.6-15.2) in the COVID-19 group versus 13.2 (11.4-14.7) in the non COVID-19 group ($p$=0.006). Out of the 24 COVID-19 patients who had anemia, none exhibited obvious biological haemolysis. The authors concluded that there was no biological argument to support the hypothesis that SARS-CoV-2 could alter O2 binding to haemoglobin.

In a letter to the editor of Hematology Transfusion and Cell Therapy, Giuseppe Lippi from the University of Verona (Italy) and colleague reported that hemoglobin value was significantly lower in COVID-19 patients with severe disease, compared with those with milder forms.[13] A meta-analysis was done on the basis of that letter to the editor. The analysis included four studies which comprised of 1,210 COVID-19 patients (224 with severe disease; 18.5% comparable with our study showing 16.6% severe cases). The primary endpoint in that analysis was defined as a composite of admission to the ICU, need of mechanical ventilation or death that was also comparable to our study however we had lower mortality rate of 8% compared to 12.6% in international study.[14]

The mortality rate in our hospital was higher (8.8%), more than mortality rate observed in Pakistan (2.1%).[15] Considering the fact that we are in a third world country and a peripheral hospital set up with a majority rural catchment area, we encountered poor patients who had a trend of visiting hospital during advanced disease stages when lungs had been compromised. Another reason could be that our poor patients are usually malnourished with already low haemoglobin that may be worsening by the disease process. Our population under study consisted of most of the people living below the poverty line and having low haemoglobin. This can be a confounding factor in our study. However, our focus was on the fact that those presenting with low haemoglobin were not able to compete with the complications of the disease compared with those with normal levels of haemoglobin. Serum ferritin is usually used to determine the iron status of the patient but its role is also controversial considering the fact that it is an acute phase reactant that is usually raised in COVID-19 patients. Bone marrow iron is gold standard to determine iron status of the patient but it is usually not advised considering its invasive nature.

Another confounding factor in our study was previous history of any pulmonary or cardiac disease. This was a major limitation in our study. We usually dealt with patients who were entitled for free treatment in our hospital so we could search their previous histories as well. Nevertheless, this could not be applied to all the patients. However only 1 out of 16 deceased patients had previous ischemic heart disease. No previous record was available for 8 (50%) patients. However, 7 patients (43.75%) had no record of any cardiac or pulmonary disease.

Our study was unique because it was carried out in Pakistani/South Asian population(which is known to have a different epidemiology in regards to COVID-19) and discussed association of haemoglobin at time of presentation with mortality.

## LIMITATIONS OF STUDY

The study was conducted at a peripheral hospital where the patients were from a rural catchment area of Sindh. The patients were brought to hospital, mostly, in deteriorating condition, rather than early reporting. Our study was limited by the small sample size, predominantly male population and lack of availability of previous clinical documents.

## RECOMMENDATIONS

The existing scarce literature about COVID- 19 is major hindrance to reach definitive recommendations. The limited knowledge may lead to speculative elaborations. The findings of this study may be helpful in contribution to stimulate future studies, so to corroborate or disprove our original study.

Major scientific research advancement with extensive multi-center studies having higher number of study population with equal gender representation in future will be helpful in reaching definite recommendations.

## CONCLUSION

Patients presenting with SARS-CoV-2 illness should be thoroughly assessed and longitudinal monitoring of hemoglobin values must be done for better clinical outcomes. The data suggests that progressive decrease in the hemoglobin concentration may indicate deteriorating clinical picture.

**Conflict of Interest:** None.

**Authors' Contribution**

ISW: Conception interpretation of data, IK: Intellectual contribution, MA: Direct intellectual contribution, SF: Direct intellectual contribution, ZI: Design, analysis, SH: Intellectual contribution.

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