Effect of COVID-19 on Hemoglobin: Theories and Recommended Drugs

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ABSTRACT

COVID-19 has affected over 110 million patients worldwide till February 2021. Scientists seek to understand the pathophysiology and proposed several theories. COVID-19 severe clinical phenomena occurred due to cytokine storms that cause hypochlorous acid (HOCl) production. HOCl has several harmful effects on hemoglobin, like heme degradation and iron overload toxicity. There are two theories about the attack of COVID-19 on Hemoglobin. One widely shared hypothesis based on the docking model, recommended that SARS-CoV-2 proteins ORF1ab and ORF3a bind to hemoglobin protein, changing heme to ORF10, resulting in the breakdown of the heme into iron and Protoporphyrin IX (PPIX), causing the loss of hemoglobin function and iron toxicity. While other experimental study of patients with COVID-19 and acute respiratory distress syndrome (ARDS) patients is compared in the objection theory. The results showed that COVID-19 patients were not showing any hemolytic anemia or altering the hemoglobin-oxygen dissociation curve and hence, COVID-19 has no role in the removal of iron and oxygen delivery. In addition, there is a hypothesis that hemoglobin structure plays a role in the physiopathology of COVID-19 disease. In children a high fetal hemoglobin levels leading to a decrease in the mortality rate with COVID-19. Another hypothesis linked between the blood grouping and the risk of developing COVID-19 infection. O group persons have a lower chance for infection. From all the previous studies, many drugs formulated like fetal hemoglobin inducers, Iron chelators, and Lactoferrin represents options for the treatment of COVID-19. The present review is an attempt to describe some hypotheses related to the Hemoglobin theory. In addition, there is information on several novel treatments for COVID-19 therapy.

INTRODUCTION

COVID-19 has shaken the world for the last months; An acute respiratory syndrome caused by Coronavirus SARS-CoV-2 spread fast and leading to rapid deterioration in the respiratory functions and may lead to death. In addition to the negative impacts on the economy and people's social life 1. A specific hallmark involving COVID-19, the rapid health deterioration due to multi-system injuries. The hypothesis assumed that the generation of excess reactive oxygen species leads to hypoxemia, further cell stress, and heme destruction 2. Oxygen supplementation fails in critical cases due to the severe hypoxia caused by the virus. The prognosis and pathophysiology of COVID-19 are not the same as the rest of the Coronavirus family and is still poorly understood 3. About 80% of COVID-19 cases suffer from mild to moderate symptoms, while 20% with severe
symptoms. The mortality rate in COVID-19 patients is up to 50 percentage, due to accompanied risk factors as aging and non-communicable diseases. Diagnosis of COVID-19 is related to the decreased Hemoglobin, leukocytes-neutrophil ratio, elevated D-dimer, and ferritin enzyme. The violent immune response of the human body against the COVID-19 virus causes the release of "cytokines storm," resulting in ROS production's cascade leading to hypoxia ⁴⁻⁵.

Various hypotheses about the relation between the COVID-19 and the 1-beta Hemoglobin chain were assault. Theoretical research relies on the ability of COVID-19 proteins with porphyrin to form a conserved domain and release free iron leading to a drop in the affinity of Hemoglobin binding to oxygen and interfere with the Hemoglobin anabolism. ⁶

The experimental research argues the link between COVID-19 and Hemoglobin clinical laboratory results from 21 patients positive to COVID-19 to 21 patients with acute respiratory distress syndrome (ARDS) but without COVID-19.⁷ Many studies and clinical trials linked the presence of Fetal Hemoglobin (HF), change of blood grouping, iron toxicity, mortality, and morbidity rate of COVID-19 ⁸, which lead to hypotheses created to approve these theories using HF inducer and iron chelator for treatment.

The present review is an effort to describe some hypotheses and pathophysiology of COVID-19 related to the Hemoglobin theory. In addition, there is information on several novel treatments for COVID-19 therapy.

**Genomic organization of COVID-19**

Coronaviruses consist of two types of protein, which are structural and nonstructural. Structural proteins constitute a significant part consists of four types of a Spike (S), an envelope (E), a membrane (M), and a nucleocapsid (N). Nonstructural proteins (nsp) are minor parts occupied two-thirds of the genome, consist of two open reading frames ORF1a and ORF1b encoded into 16 types (nsp1-nsp16), each protein of them plays a critical role in viral metabolism and interaction with the host machinery **Figure 1.** ⁹

![Figure 1. Genomic organization of COVID-19.](image-url)

**Pathophysiology of COVID-19**

SARS-CoV-2 is a positive sense RNA strand, nucleocapsid, and spikes protein. The virus enters the host cell by spike protein through angiotensin-converting enzyme two receptors (ACE2), located on the lower respiratory epithelium, releasing the nucleocapsid for mRNA transcription and the hijacking of the host cell producing viral proteins ¹⁰.

In a mild to moderate viral infection, WBCs, especially neutrophils, produce myeloperoxidase enzymes releasing inflammatory cytokines, generating Reactive Oxygen Species (ROS) like (H₂O₂), (O₂⁻), and (HOCI) as scavengers for viral proteins ¹¹⁻¹². However, in severe cases, HOCI is heavily produced due to the cytokine storm, beating oxygen at the heme-binding site causing Hemoglobin degradation and Iron (Fe²⁺) release; free iron undergoes Fenton reaction producing another reactive hydroxyl (•OH) ¹³.

Another critical regulator is nitric oxide; upon depletion, vasoconstriction occurs, leading to a negative impact on the pulmonary and peripheral circulations. Producing excess ROS followed by an increase in hemoglobin degradation rate leads to hypoxia ¹⁴.

Linking these mechanisms (ROS, cytokine storm, myeloperoxidase, nitric oxide depletion) might beat the hypoxia cascade that occurs by COVID-19 ² as in **Figure 2.**

In the early stage of COVID-19 infection, infected cells produce cytokines to influence leukocytes, neutrophils, and macrophages. Neutrophils produce myeloperoxidase enzymes to generate the reactive oxygen species (•O, •OH, H₂O₂, HOCI) act as a scavenger against the viral protein ¹⁵. As a result, the overproduction of reactive oxygen molecules leads to oxidative stress, activation of caspase-3 inducing apoptosis; and reactive oxygen species are converting to more stable H₂O₂ ¹⁶.

The antimicrobial activity of Neutrophils is through Myeloperoxidase enzymes, followed by HOCI production to fight the virus. However, in severe cases, hemeprotein destruction and free iron ions are released by interaction with HOCI and O- radical. ¹⁷

Decrease in peripheral oxygen saturation is one of the hallmarks of COVID-19, with a reduction of about 50-70% without symptoms due to Hemoglobin's alteration at the alveolar membrane by the HOCI-MPO ¹⁸.

Hemoglobin is a tetramer of four heme groups attached to globin subunits, and it carries O₂/C₀₂. Hb exists in two forms, reduced oxygenated (Hb-oxy) and deoxygenated. HOCI binds to Hb-oxy, leading to Hemoglobin disruption and the release of free iron, and the formation of metHemoglobin and Ferryl Hb (Hb-Fe₂⁺O). The oxygen affinity will decrease, eventually, tissue hypoxia. Early oxygen supplementation might help in Hb reoxygenation, but in late cases, due to increased ROS, oxygen supplement may make it worth ¹⁹⁻²⁰.
Figure 2. Neutrophil MPO-generated oxidants and their potential role in COVID-19. At normal condition, neutrophils produce MPO enzyme & ROS particles as reduced O$_2$ which converted by superoxide dismutase to generate H$_2$O$_2$ as a scavenger for antimicrobials. A secondary more potent scavenger HOCI is produced by myeloperoxidase from H$_2$O$_2$ with chloride ions from blood. In severe COVID-19 cases, MPO is produced in excess forming a lot of HOCl particles. HOCl cause oxidative stress leading to heme destruction, vasoconstriction & hypoxia. In blue arrows heme destruction hypothesis, which result in formation of Free iron & OH leads to hypoxia, blood clotting & DNA damage. In red arrows nitric oxide consumption hypothesis, which result in vasoconstriction and low oxygen saturation levels due to methemoglobinemia. In green arrows another hypothesis of nitric oxide consumption by formation of compound 1 & 2 by MPO and free iron result in toxic NO$^+$ metabolite.

Iron is an essential element for many metabolic processes like DNA synthesis, repairing, transcription of DNA, energy production, and drug detoxification. The excessive amount of free unbound Fe$^{2+}$ leads to increased toxic reactive oxygen species (ROS) formation by the Haber-Weiss and Fenton reactions. ROS formation alters lipid, nucleic acid, and protein functions, activation of acute/chronic inflammatory response leading to many complications 21 (Figure 3).

Clinically, severe hypoxemia COVID-19 patients have lower levels of serum iron in association with lymphocyte counts. The significantly low serum iron may contribute to possible T-cell dysfunction.22 Shah et al. found non-significant differences in transferrin saturation or serum ferritin levels between the non-severe and severe hypoxemia patient groups. Zhao and colleagues have reported that “the severity and mortality of the disease was closely correlated with serum iron levels”.23 The common presence of digestive symptoms in COVID-19 patients was explained in part by a link to hepcidin.24 In Bardon et al. pooled analysis, elevated LDH values were associated with a 6-fold increased odds of severe COVID-19 disease and >16-fold increase in odds of mortality.25

Theories of COVID-19 attack on Hemoglobin

The first theory was done by Liu and Li using a computational model, sequencing analysis, and molecular docking. It depends upon what's protein can form a conserved domain. Previously reported studies have proved that heme is a lipophilic protein that can't uptake by the liver unless binding with a protein transporter forming a conserved domain.6 Virus proteins of COVID-19 bind with heme or porphyrin binding or with heme oxygenase activity if they form a similar conserved domain.6

According to the study of Liu and Li COVID-19 Hemoglobin (Hb) metabolism will interfere with the
formed conserved domain and separate harmful irons in the blood, which leads to a significant increase in serum ferritin, albumin, ESR, LDH, and CRP, and decrease neutrophils.6

**Types of proteins that form a conserved domain with Hemoglobin**

The structural proteins of COVID-19 that form a conserved domain with porphyrin are E2 glycoprotein, Envelope protein, and Nucleocapsid phosphoprotein. Nonstructural proteins of COVID-19, which include a conserved domain, are ORF1ab, ORF3a, ORF7a, ORF8, and ORF10, which do not provide with the SWISS model6 are illustrated in Table 1 and Table 2.

There is a difference in the ability of COVID-19 proteins to form a conserved domain with porphyrin according to the number of amino acids and binding energy.

Table 1 illustrates conserved domains between structural proteins and nonstructural proteins of COVID-19 with porphyrin according to the number of amino acids bound to porphyrin and binding energy. E2 glycoprotein forming the most stable conserved domain in the structural proteins, ORF8 is the most stable conserved domain in the nonstructural proteins depending on the highest binding energy.

The possible mechanism of nonstructural proteins of COVID-19 that attack the heme ring and release porphyrin are ORF10, ORF3a, and orf1ab. Porphyrin is directed and binds with ORF8 by ORF6 and ORF10. Finally, ORF8 forms a stable complex with porphyrin.

There are two types of Hemoglobin, oxyhemoglobin, and deoxyhemoglobin; the role, conformation, and the sites of nsps are different in each one.6,33

Table 2 shows the mechanism of heme attack and release of porphyrin by nonstructural proteins of COVID-19. The first step, ORF10, ORF3a, and orf1ab, attack the heme ring and release the porphyrin free. Then porphyrin is directed and binds with ORF8 by ORF6 and ORF10. ORF8 forms a stable complex with porphyrin.6

The second theory found that there is no evidence between COVID-19 and Hemoglobin defect7 experimentally. When comparing the laboratory results of 21 patients with positive COVID-19 in ICU with 21 patients non-COVID-19 selected in the Acute Lung Harm Registry (ALIR) from various etiologies at UPMC. At the beginning of clinical studies, they record Hb, total bilirubin, venous blood gas values of the partial pressure of carbon dioxide (PvCO2), the partial pressure of oxygen (PvO2), pH, and venous oxygen saturation of Hemoglobin (SvO2) levels.

They found no Hb affinity variation to carry oxygen in positive COVID-19 patients, so no Hb value variation means no association between Hb and COVID-19.7,27. Patients with COVID-19 have a higher incidence rate of Thromboembolism 28 and no relation between it and Hb toxicity. No absorbance occurs to free heme when measured at the wavelength range.29

Hopp, Marie-Thérèse et al., were proposing a study of common pathways performed by superimposing two knowledge graphs, namely the "Heme KG" and the "COVID-19 KG", which contained information about theme-driven and COVID-19 pathophysiology on a molecular level.29

Several biomarkers from proinflammatory pathways (cytokines, such as TNF), the complement system (e.g., C3), the blood and coagulation system (e.g., ferritin, platelets), and organ-specific indicators (i.e., LDH and bilirubin) developed to support the evidently shared clinical22.

Four proteins involved in SARS-CoV-2 entry into host cells (the two viral proteins S protein and protein 7a, as well as the two human host cell proteins ACE2 and TMPRSS2) were suggested as potential heme-binding proteins in a second approach (out of six analysed COVID-19-related proteins). With the use of the web tool HeMoQuest and manual refining, the heme-binding potential of these proteins was determined, revealing two possible HBMs on the surface of protein 7a, three for S protein, five for ACE2, and ten motifs for TMPRSS2.29

Finally, a link between COVID-19 infection symptoms and the consequences of excess heme does not have to be relevant for every patient; but, in certain circumstances, it may correlate or even create a more severe illness development due to pre-existing hemolytic diseases or hemolysis-provoking events.29

**Role of Fetal Hemoglobin in COVID-19:**

Hemoglobin F (HBF) may be one of the main reasons for decreasing the prevalence of COVID-19 in pediatrics.30 The hypothesis based on the molecular docking study approved that SAR-COV2 attacks the Beta chain, causing a release of iron from porphyrin and iron toxicity.6 The pilot study results showed that patients with hemoglobinopathies like beta-thalassemia and sickle cell anemia had low mortality and fatality rates toward COVID-19.30

From the previous results of studies, some clinical trials started to use HBF inducers agent s in protocols for treatment COVID-19 like Hydroxyurea and Panobinostat 31. However, some literature and case studies approved that beta-thalassemia patients had a higher risk of COVID-19 complications due to iron overload and splenectomy32,34.

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Figure 3. The relationship between COVID-19 and iron homeostasis. In addition to the complications of the increase iron overload and treatment of iron toxicity.

Table 1. Conserved domains between structural proteins and nonstructural proteins of COVID-19 with porphyrin according to the number of amino acids bound to porphyrin and binding energy

<table>
<thead>
<tr>
<th>Types of COVID-19 proteins</th>
<th>Number of amino acids bound to porphyrin</th>
<th>Binding energy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structural proteins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2 glycoprotein</td>
<td>18 amino acids</td>
<td>219,317.76 kcal/mol</td>
</tr>
<tr>
<td>Nucleocapsid</td>
<td>22 amino acids</td>
<td>15,532,506.53 kcal/mol</td>
</tr>
<tr>
<td>Envelope protein</td>
<td>18 amino acids</td>
<td>219,317.76 kcal/mol</td>
</tr>
<tr>
<td><strong>Non-structural proteins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORF8</td>
<td>18 amino acids</td>
<td>12,804,859.25 kcal/mol</td>
</tr>
<tr>
<td>orf1ab</td>
<td>18 amino acids</td>
<td>561,571.10 kcal/mol</td>
</tr>
<tr>
<td>ORF7a</td>
<td>15 amino acids</td>
<td>37,123.79 kcal/mol</td>
</tr>
</tbody>
</table>

**Blood group type and COVID-19**

Here is no clear evidence between blood grouping and COVID-19. However, some studies have shown that blood type O individuals had a low risk of developing COVID-19, while blood group A was associated with a higher risk (35). Blood iron state studies reported that some serum iron indicators are lower in patients with blood group O than those with other blood groups (8). Increasing amounts of serum iron may contribute to complications associated with COVID-19, like inflammation and hypercoagulation (35).

**Prognostic role of Hemoglobin in COVID-19**

COVID-19 individuals with severe illness had considerably lower hemoglobin levels than those with milder forms, according to a meta-analysis of four relevant studies (36). In another study, it was discovered that a drop in Hb level following a pneumonia diagnosis might be a predictor of deteriorating pneumonia in 23 COVID-19 patients with pneumonia, admitted to the Kumamoto City Hospital, 23 COVID-19 patients with pneumonia, admitted to the Kumamoto City Hospital (37).
The study reported done by Anai M., et al. found that Hemoglobin concentration is not related to overall survival. Hemoglobin is affected by factors like red blood cell population, age, lactate dehydrogenase. Also adding the ratio of arterial partial oxygen pressure to inspired oxygen percentage. Anemia is a condition that affects the old and feeble, and it can have a detrimental impact on their health.  

World Health Organization has recently taken into consideration the possible contribution of blood changes within the COVID-19 course.  

**Iron chelators** own multiple pharmacological actions, such as chelating iron, inhibiting redox properties exerted by free iron, reduction of hepcidin. It acts as anti-ferritin by removing iron from its proteins and preventing iron from participating in Fenton reactions, leading to inhibition of hydroxyl radical production, ROS formation, and production ferroptosis. Furthermore, it induces, lowering of glutathione and change of glutathione peroxidase 4 (GPX4), which act as a ROS regulator.  

Accordingly, iron toxicity patients get treated with iron chelators like deferasirox, deferoxamine, and deferiprone. And it's a potential therapeutic option in COVID-19. Iron chelation, with deferiprone or deferoxamine, has been proposed in COVID-19, also in view of its demonstrated efficacy in other viral infections.  

**Cures work** potentially useful interventions in hypoxia-based pulmonary hypertension and vasoconstriction may be highlighted recommendation for treatment in literature: Nitroglycerine-based drugs; Prostanoids; Calcium channel blockers; high concentration/high flow oxygen at low pressure; Vadenosine; Acetazolamide; Sildenafil.  

Mitochondrial organelles play a role in innate and adaptive immunity in viral infections. Hospitalized COVID-19 patients showed an increased lactate level, a marker for mitochondrial degeneration. Specific mitochondria-targeted antioxidants (based on triphenylphosphonium cation) as Curcuminoids, Polydatin, Ubiquinol, Nicotinamide-mononucleotide may improve mitochondrial biogenesis/ function.  

SARS-CoV2 attacks endothelial cells, iron metabolism, and erythrocytes which may lead to a series of changes within the COVID-19 course.  

**Table 2. The mechanism of heme attack and release of porphyrin by nonstructural proteins of COVID-19**

<table>
<thead>
<tr>
<th>Type of nsps</th>
<th>Oxyhemoglobin</th>
<th>Deoxyhemoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORF1ab</td>
<td>Configuration change in alpha and beta chain when attack both of them. Form a stable complex with porphyrin.</td>
<td>Configuration changes in globin protein when attacking both alpha chain. Form a stable complex with porphyrin.</td>
</tr>
<tr>
<td>ORF3a</td>
<td>Attack beta chain and reveal heme</td>
<td>Drive 2 alpha chain to attack beta chain and reveal heme</td>
</tr>
<tr>
<td>ORF10</td>
<td>Attach with a beta chain to release its iron atom to form porphyrin.</td>
<td>Attach with 1- beta chain to release its iron atom to form porphyrin.</td>
</tr>
</tbody>
</table>

Lf also owns the ability to obstruct viral entry by binding to a host’s viral or cell surface receptors, or both. Like, bind to Angiotensin-converting enzyme 2 (ACE II) receptor by adhesion molecules called heparan sulfate proteoglycans (HSPGs) 46,47, so it is possible to use LF for preventing entry of COVID-19 to a cell of humans.  

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of coagulopathies. In COVID-19 patients are treated with anticoagulants as heparins. Anticoagulants are showing a multi-organ beneficial effect and become essential in pharmaceutical treatment. 59

**Ozone** may be the function in the treatment of COVID-19 patients. Ozone has many biochemical effects. 60,61 These effects including (increase GSH activity and heme oxygenase pathway; Elevation of IL10 and decrease of proinflammatory cytokines and TNFα; Transient increase of ROS and consequent NrF2/ARE pathway activation; Improved mitochondrial oxidative phosphorylation; 2,3- diphosphoglycerate increase with better hemoglobin functionality; Improved erythrocyte rheology and NO elevation).

**Polyphenols** own several epigenetic activities through the NrF2-ARE pathway. 62 Polyphenols have direct antiviral action documented in a few studies. Curcuminoids, anthocyanins, and catechins proved to benefit processes in COVID-19. Polyphenols are effective in inflammasome activation interleukin storm and SARS-CoV-2 attack to bone marrow 63.

**Melatonin** exhibits a proven protective role against hypoxia, ferroptosis, and hemoglobin denaturation. 64

**Vitamin D** showed an epigenetic action on a few antioxidant systems and modulate immune system. 55

**Rifkind and Heim** refer to the central role of zinc in the immune system. It helps in respiratory infections and activity on hemoglobin O2 affinity as well. 61

**Horowitz et al.** register quick relief from COVID-19 pneumonia-related dyspnea in two cases after multiple doses of GSH. 62

**Ascorbic acid** constitutes a significant role in Intensive Care Unites due to its immune, anti-infective, and anti-cytokine storm activity. Ascorbic acid can increase NO production by improving heme iron/cell redox balance. 63

Clinically, **dexamethasone and other corticosteroids** are among the very few widely used treatments and diagnoses for COVID-19 64, which significantly correlates with the course of the disease. The severity of the disease is associated with the serum levels of **hepcidin** and **ferritin**. Serum Ferritin is an indicator of inflammation and infection. 65

**CONCLUSION**

This review highlights the genomic organization of the COVID-19 virus, and the Pathophysiology of SARS-CoV-2 discusses the mechanism of the COVID-19 virus attack on the cell and hemoglobin. Moreover, Scientists have not found the proper mechanism of COVID-19 that creates them to interconnect between theoretical modeling and limitation of clinical studies. The virus can attack cells via interaction with the highly expressed ACE2 receptor in many organs and tissues. The study also includes details on medicine-based medications and other adjuvant treatments for haemoglobin malfunction that are currently being tested in numerous COVID-19 patients.

**Conflict of interest**

The authors declare that there is no conflict of interest regarding the publication of this paper.

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