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Review Article

An unorthodox pathophysiology of severe cases of COVID-19 the weak heme hypothesis

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Abstract: Important amount of severe cases is the main concern in COVID-19 pandemic. It could be the running cause of the burn out of the health system in many countries. The aim of this paper is to suggest a pathophysiologic hypothesis to explain the main characteristics of severe cases of COVID-19 and its underlying conditions. In fact, the clinical and biological picture of severe cases of COVID-19 can easily be explained by free heme toxicity exceeding the endogenous antioxidant systems. Severe cases of COVID-19 are comparable to acute porphyria. On the other hand, the geographical distribution of severe cases of COVID-19 is directly associated to how fresh or polluted the air is. Finally, the relatively low rate of severe cases of COVID-19 could be explained by the presence of an unstable hemoglobin variant highly sensitive to the intrinsic conditions resulting from the acute pneumonia secondary to SARS-CoV2 infection. The combination of air pollution and free heme toxicity, resulting from the interaction between an unstable hemoglobin variant and SARS-CoV2 infection, seems to be the best scheme to explain clinical and biological manifestations in severe COVID-19. The arguments to support this hypothesis are detailed. We also propose some strategies to verify the concordance of our hypothesis with the reality and the implications it could have, if verified, either for scientists and decision makers.

Keywords: Severe COVID-19, air pollution, free heme toxicity, antioxidant systems

Introduction

The main concern in COVID-19 pandemic is the sudden jump number of severe cases. It exceeds the capacities of health system in most countries. The situation is very serious to the extent that it requires a general lockdown of almost half of the world's population [1].

Serious or severe cases of COVID-19 are diversely defined. The most common definition was a severe pneumonia with hypoxemia (SpO_2 < 90%), or critical acute respiratory distress syndrome (ARDS), possibly associated with shock, encephalopathy, myocardial injury, heart failure, coagulation dysfunction or acute kidney injury. These cases require hospitalization and can lead to death [2].

The main aim of this paper is to suggest a pathophysiologic scheme to explain the main characteristics of severe cases of COVID-19 and its underlying conditions. It also proposes some strategies to verify the concordance of our hypothesis with reality and its possible implications.

Geographic distribution, "the weight of the ecology"

Many articles described geographic disparities regarding the rate of severe cases of COVID-19. Case fatality rates are very important in industrial areas with high air pollution [3, 4]. Epidemic's outspread also correlated well with air pollution indexes [5, 6]. The situation also improved after some weeks of lockdown in parallel to significant decrease observed in rates of many air pollutants. NASA (National Aeronautics and Space Administration) and ESA (European Space Agency) released evidence which suggests that environmental quality improved and emission of NO_2 reduced up to 30% [1].

It has been established that air pollution is an important risk factor for cardiovascular diseases, systemic inflammation and prothrombotic state. In a recent review, Rajagopalan et al. reported that fine particulate matter air pollution (PM2.5) was the most important environmental risk factor contributing to global cardiovascular mortality and disability. They emphasized the central role of oxidative stress in PM-mediated effects. In fact, oxidative stress, occurring in the lung and/or systemically, may initiate many secondary processes. A high level of reactive oxygen species (ROS) and reactive nitrogen species (RNS) has been demonstrated after long term exposition to PM (superoxide, peroxynitrite). ROS and RNS act as site-specific mediators of cell signaling and as central regulators of inflammation. There is also a depletion of low molecular weight antioxidants resulting from excessive consumption by oxidative stress or a genetic predisposition illustrated by polymorphisms of antioxidant genes [7].

Long term air pollution exposition seems to be a major prooxidant condition and therefore enhances prevalence of comorbidities thought to be the bed of severe COVID-19 especially degenerative and cardiovascular diseases [7].

Clinical and biological characteristics of severe COVID-19, "the hemolysis-like syndrome"

In our personal experience, the clinical picture of these patients is dominated by an acute respiratory failure with very important hypoxemia and carbon dioxide retention (high $PaCO_2$ and high serum bicarbonates). We also observed a high rate of hypernatremia and hyperchloremia. This contrasts with the results of recent meta-analysis including five studies with a total sample size of 1415 COVID-19 patients where authors reported low sodium rate and normal chloride [8]. In a recent meta-analysis including 21 studies with 3377 patients and 33 laboratory parameters, Henry et al. reported that patients with severe and fatal COVID-19 had significantly increased white blood cell (WBC) count, and decreased lymphocyte and platelet counts compared to non-severe disease and survivors. Biomarkers of inflammation, cardiac and muscle injury, liver and kidney function and coagulation measures were significantly elevated in patients with both severe and fatal COVID-19. Interleukins 6 (IL-6) and 10 (IL-10) and serum ferritin were strong discriminators for severe disease [9]. Thus, the symptoms of severe forms of COVID-19 can be grouped as follows:

- Hemolysis: decrease of hemoglobin, increase in bilirubin, serum ferritin and lactate dehydrogenase;

- Immune dysregulation: increase of WBC especially neutrophil, decrease in lymphocyte, eosinophil, high erythrocyte sedimentation rate, high CRP, high PCT, high IL-6, High IL-8, High IL-10, High IL-2R;

- Coagulation disturbances: low platelets, high prothrombin time, high D-dimer;

- Other organs impairment:

○ Liver: low albumin, high alanine aminotransferase, aspartate aminotransferase, total bilirubin, lactate dehydrogenase,

○ Muscles: high creatine kinase, high lactate dehydrogenase, high myoglobin,

○ Heart: high creatine kinase MB, high cardiac troponine I,

○ Kidney: high BUN & creatinine.

Many literatures exist in relation to the pathophysiology and consequences of hemolysis reported interesting findings that fit point by point to characteristics of severe COVID-19. Kumar et al. published a global review in 2005 about free heme toxicity [10]. All the features of severe COVID-19 are reported in the description of free heme toxicity. These effects are mediated by iron dependent oxidative stress and pro-inflammatory effect of heme. Heme-induced oxidative stress is responsible of microangiopathic hemolytic anemia, vasculitis-like syndrome and hepatic cells apoptosis. It also provokes acute kidney injuries and neurologic injuries. Heme-induced cytotoxicity to cardiac cells is associated with complete loss of cell integrity, sarcolemmal damage and release of cytosolic enzymes.

Heme induces systemic inflammation by the activation of endothelial cells which express number of important cytokines (ICAM-1: intercellular adhesion molecules 1, VCAM-1: vascular cell adhesion molecule 1 and *E*-selectin:

endothelial leukocyte adhesion molecules) that recruit leukocytes especially neutrophils (neutrophil chemotaxis and cytoskeleton reorganization).

In recent years, several studies identified the role of plasma-free heme in altering immune cell differentiation as well as activity and migration of effector cell. In a recent review (2018), Zhong et al. reported several novel mechanisms of immunological alterations and severe complications secondary to hemolysis [11]. Plasma-free heme induces neutrophil extracellular traps (NETs) formation through reactive oxygen species signaling. NETs are composed of chromatin and neutrophil granule proteins (mesh-like scaffolds). They act as a physical support, with high local concentrations of antimicrobials, to prevent spread of microbes [11]. NETs; however, may play negative role in autoimmune and inflammatory diseases. In-vitro and in-vivo studies also showed that NETs were associated with thrombosis [11].

Based on autopsy results, Barnes et al. hypothesized that NETs may contribute to organ damage and mortality in COVID-19 [12]. They discussed prior reports linking aberrant NETs formation to pulmonary diseases, thrombosis, mucous secretions in the airways, and cytokine production [12]. This fact could explain the biphasic evolution of lung impairment in COVID-19.

In a recent report from Teheran, Faraji et al. reported that particulate matter (PM10: aerodynamic diameter ≤ 10 μm) induced hemolytic responses. Hemolysis was significantly intensified by increased PM concentrations ($P < 0.001$) [13].

The central role of sensitive hemoglobin variant, "the weak heme hypothesis"

As shown in the previous paragraph, evidences of heme loss in severe COVID-19 combined with the possibility of direct induction of hemolysis by particulate matter (PM10) can easily make the link between air pollution and severity of SARS-CoV2 infection. Otherwise, peroxynitrite produced after a long-term exposition to air pollutants reacts with hemoglobin to produce methemoglobin, which readily releases heme [14]. This reaction is amplified by high CO_2 concentrations [15, 16]. This situation is frequent in the early phase of corona virus induced pneumonia, just after respiratory muscles exhaustion. Then a high proportion of methemoglobin can be the result of long-term exposition to air pollution and a significant hypercapnia. Additionally, it has been observed that peroxynitrite induced post transcriptional modifications in hemoglobin chains [17]. In this study, the authors identified peroxynitrite-induced post-translational modifications (PTMs) in human hemoglobin (Nitration on α-Tyr-24, α-Tyr-42 and β-Tyr-130). They also characterized oxidation of all three methionine residues (α-Met-32, α-Met-76, and β-Met-55) to the sulfoxide. They also found oxidation of cysteine on α-Cys-104 to sulfinic acid and on α-Cys-104, β-Cys-93, and β-Cys-112 to sulfonic acid. These modifications in the quaternary structure of hemoglobin can increase the instability of the tetramer and then facilitate the release of the hemin.

This "direct link hypothesis" does not seem probable because of the small rate of severe cases despite the same exposition to air pollutants. In fact, the proportion of severe cases is around 15-20% in almost series. Supplementary factors related to heme physiology seem of central role.

It is well known that human hemoglobin is subject to genetic polymorphism and to date we are aware of more than 1818 hemoglobin variants with different characteristics regarding O_2 affinity, Bohr effect, and behavior in extreme pH conditions [18].

In our hypothesis, the combined effects of met oxidation of the heme and PTM of hemoglobin chain secondary to peroxynitrite (resulting from air pollution) and the presence of hemoglobin variant highly sensitive to such alterations is responsible of massive heme loss. The free heme toxicity overwhelms the antioxidant system and results in the clinical and biological manifestations of severe COVID-19.

Many arguments support our hypothesis:

- Epidemiologic facts:

○ The frequency of hemoglobin variant is very important in some countries with high prevalence of severe COVID-19 cases. The most interesting examples are Italy (234 variants),

France (58 variants), UK (107 variants), Spain (80 variants), USA (98 variants), Iran (43 variants), Turkey (83 variants), China (127 variants), Russia (21 variants), India (87 variants) [18].

○ The controversy about the effect of smoking as risk factor of severe COVID-19. In fact, smoking appears without any effect or sometimes protective in a meta-analysis [19]. Whereas a most recent meta-analysis found an OR of ongoing smoking at 1.98 (Fixed effect model, 95% CI: 1.29-3.05) [20]. The result of this meta-analysis was heavily influenced by one study and after removing the study from analysis association between active smoking and severe Covid-19 was found to be non-significant [20]. This could be due to the action of CO on the stability of the heme in some hemoglobin variant (protective effect) and its action promoting met oxydation in some other variant (deleterious effect).

- Pathophysiologic facts:

○ There are many reports supporting a major role of some species of Prevotella in the pathogenesis of severe pulmonary infection with SARS-CoV2 [21, 22]. A previous study of 2002 showed that porphyrins and inorganic iron are essentials for the growth of Prevotella intermedia [23]. The high rate of free heme or free hemin in severe COVID-19 seems to be the ideal condition to the development of such Prevotella species.

- Therapeutic facts:

○ Some treatments used in COVID-19 with variable efficiency, i.e. Hydroxychloroquine [24], are well known to their heme stabilization and interception proprieties [25].

○ In a study, the authors reported a patient with COVID-19 who after being treated with recombinant human erythropoietin (rhEPO) due to a severe anemia exhibited primarily unexplainable rapid symptoms relief and viral regression [26]. This observation is supplementary evidence that the reuse of the important amount of free iron in severe COVID-19 and its incorporation into red blood cells can stop the oxidative stress and then improve the outcome.

"Weak heme hypothesis" verification tools

The simple verification of the presence of hemolysis in severe COVID-19 cases can answer the question about the validity of our hypothesis. The work up of such research is easy and based on the dosage of haptoglobin, the search of red blood cells ghost (schizocytes) and in some variants of hemoglobin the highlighting of Heinz bodies. Then, specific investigations could be used to search for hemoglobin variants such as hemoglobin electrophoresis.

In the limits of our bibliographic research, we found only one paper that mentioned haptoglobin dosage [27]. The association between COVID-19 and hemolytic anemia is discussed as fortuitous and haptoglobin dosage is decreased in almost all patients. A generalization of such hemolysis workup should certify that the association is not fortuitous.

The possible implications of our hypothesis

The loss of heme by weak or unstable hemoglobin in conditions of high $PaCO_2$ and oxidative stress due to air pollution seems to be the cornerstone in the pathophysiology of severe COVID-19. Then, the first question to answer is: Is there a real value to vaccine in these conditions?

In fact, the vaccine is specific to one pathogen and even if we can prepare a vaccine against SARS-CoV2, the underlying conditions are still present if another pathogen responsible of severe pneumonia occurs. A high rate of contagion is sufficient to mimic the COVID-19 pandemic in higher proportion. It seems more efficient to target the underlying conditions than the pathogens.

The second issue is the timing of stopping lockdown. In our hypothesis, the decrease of severe COVID-19 is mainly due to the improvement of air pollution parameters especially the PM and NO_2 rates. A return to the anterior state of high pollution will result in a relapse of critical cases of COVID-19. A serious thinking about the ecological concern should be the prior condition to any resumption of economic activity.

Our hypothesis allows to target at high risk population by the work up, in the early stages of the

disease, of hemolysis investigations as mentioned upper.

Finally, our hypothesis could be used as a platform for other diseases with the same pathophysiological process. Metabolic syndrome and its associated disorders as obstructive sleep apnea are suspected for a long time to be the result of air pollution and red blood cells dysfunction [15] Preeclampsia seems to involve the same free heme toxicity [28] with an implication of air pollution [29]. The implication of fetal hemoglobin is highly suspected.

Conclusion

The hypothesis that severe COVID-19 results from the combination of oxidative stress secondary to air pollution and a sensitive heme secondary to an hemoglobin variant, can explain the whole clinical and biological picture through the free heme toxicity. Many arguments presented to support this hypothesis. Its verification is under investigation and its implications are listed.

Disclosure of conflict of interest

None.

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