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Could glutathione depletion be the Trojan horse of COVID-19 mortality?

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Abstract. – OBJECTIVE: Since the emergence of coronavirus disease (COVID-19), the death toll has been increasing daily. Many risk factors are associated with a high mortality rate in COVID-19. Establishment of a common pathway among these risk factors could improve our understanding of COVID-19 severity and mortality. This review aims at establishing this common pathway and its possible effect on COVID-19 mortality.

MATERIALS AND METHODS: The current review was executed in five consecutive stages starting from determining the risk factors of COVID-19 mortality and trying to find a common pathway among them depending on the available literature. This was followed by proposing a mechanism explaining how this common pathway could increase the mortality. Finally, its potential role in managing COVID-19 was proposed.

RESULTS: This review identified this common pathway to be a low baseline of reduced glutathione (i.e., GSH) level. In particular, this review provided an in-depth discussion regarding the pathophysiology by which COVID-19 leads to GSH depletion, tissue damage, and acute respiratory distress syndrome. In addition, the current review demonstrated how GSH depletion could result in failure of the immune system and rendering the end organs vulnerable to damage from the oxidative stress.

CONCLUSIONS: This preclinical study shows that GSH depletion may have a central role in COVID-19 mortality and pathophysiology. Therefore, elevating the GSH level in tissues may decrease the severity and mortality rates of COVID-19.

Key Words:
COVID-19, COVID-19 mortality, SARS-CoV-2, Glutathione, Antioxidant, Acute lung injury, Respiratory distress syndrome.

Introduction

The coronavirus disease (COVID-19) pandemic has been affecting the global population, with an ever-increasing death toll. No nation, population, age group, or sex has been spared. Tens of thousands of cases are being reported per day, and no standard treatment protocol has been established.

Perplexingly, COVID-19 is selective in its severity as some patients may develop mild or no symptoms, whereas others may develop severe symptoms and subsequently die. The reason for this phenomenon should be studied and elaborated to reduce COVID-19 mortality and/or morbidity.

The literature has revealed that mortality from COVID-19 is highly associated with the cytokine storm that may cause end-organ damage[1]. It was also found that the COVID-19 mortality rate is high in patients with different medical or disease conditions[2]. The current review aims to examine the literature regarding these risk factors in an attempt to find an association between these factors and the induction of a cytokine storm. This correlation would facilitate a better understanding of COVID-19 pathophysiology and answer pertinent questions concerning this disease mortality, complications, and prevention.

The current review was executed in five consecutive stages: (1) the literature involving COVID-19 mortality and patient demographics was reviewed to identify the risk factors associated with a higher mortality rate; (2) multiple hypotheses and assumptions suggesting a common pathophysiology among all these factors were proposed; (3) a literature review was conducted to challenge these hypotheses and assumptions to determine this common factor; (4) based on the literature, an explanation of this common factor's role in increasing the mortality rate and severity of COVID-19 was given; and (5) finally, a review of the literature was conducted to determine any potential role of this common factor in the management of COVID-19.

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Search Strategy and Selection Criteria

PubMed and Google Scholar search engines were used to search for articles related to the current review. Searched references were published from January 1960 to April 2020 by using the following terms either in solo or in combination: "COVID-19," "SARS-CoV-1 and SARS-CoV-2," "influenza, flu, H1N1, and H1N5," "glutathione," "N-acetylcysteine," "acute respiratory distress syndrome and acute lung injury," "acute inflammatory response syndrome," "cytokine storm," "myocarditis," "reactive oxygen species and reactive nitrogen species," "oxidative stress," "mortality," "HIV," "lymphocytes," "interleukin," "tumor necrosis factor," "interferon," "end-organ damage," "coronavirus," "antiviral and viral inhibition," "endothelial damage," "lymphopenia," "redox and reduction-oxidation," "randomized control trials and meta-analysis," "hospital stay and intensive care unit stay," "neutrophils," "macrophage," and "natural killer cells". Articles resulting from these searches and relevant references cited in those articles were reviewed. Articles published in English, Chinese, and Korean were included.

Risk Factors Associated with COVID-19 High Mortality Rate

Since the emergence of COVID-19, multiple risk factors have been identified to be associated with a high mortality rate. Different researchers[2-11] have reported significant risk factors, including age, hypertension, ischemic heart disease, diabetes, and chronic respiratory disease.

Other factors, including obesity, pre-existing malignancy, and smoking, were claimed as risk factors for COVID-19 mortality but with equivocal evidence[9,12-18]. Interestingly, the negative effect of non-steroidal anti-inflammatory medications on COVID-19 was not widely accepted and was termed inconclusive by many authors[19-21].

These heterogeneous risk factors could be associated with a common pathway that may cause a high incidence of COVID-19 mortality. Determining this pathway would enable a better understanding of the disease pathophysiology, which may eventually help decrease the mortality rate.

Once the risk factors were identified (as shown above), common pathways involving these risk factors were hypothesized. Each assumed hypothesis was tested based on the available medical literature to prove or disprove the mutual associations among COVID-19 risk factors. These hypotheses included the involvement of baseline (before the development of COVID-19) C-reactive protein levels, white cells counts, vitamin D3 levels, lymphocyte counts, total protein levels, albumin levels, frequency of multivitamin and nutrient supplement intakes, physical activity levels, and glutathione (i.e., GSH) levels. All these hypotheses were disproved, except that involving the GSH level.

In particular, a low baseline GSH level was the common factor among all patients included in the high-risk group for COVID-19 mortality. This association with low GSH levels is discussed in detail in the next section.

Glutathione and the Risk Factors for COVID-19 Mortality

In the current review and based on the published literature, all reported risk factors were identified to reduce the baseline GSH level.

Increasing age, which is one of the most noticeable risk factors for COVID-19 mortality, was associated with declined GSH levels[22-24]. This decline may be due to extensive GSH oxidation or a combination of extensive GSH oxidation and a decrease in the total pool of thiol[22,23]. The drop in the thiol pool was also noticed in a larger scale study in middle-aged and older community-living healthy subjects in Europe[25]. Under either circumstance, this will lead to a drop in the level of available GSH.

Hypertension was linked to altered GSH metabolism in which the ratio of reduced GSH to its oxidized form (GSSG) is altered[26,27].

Ischemic heart disease was associated with a decreased GSH level and GSH to GSSG ratio[28]. This impaired ratio was also demonstrated in patients with atherosclerosis, where it was found in subjects with early disease who are even clinically asymptomatic[29]. Moreover, GSH levels were also shown to be low in the offspring of patients with coronary artery disease[30]. It was shown that N-acetylcysteine administration (which is a precursor for GSH synthesis)[31,32] improved vasodilation in the coronary and peripheral vasculature[33].

Diabetes was associated with low GSH levels[22,34]. This was evident in patients with or without diabetes-related complications[22,35]. Different types of chronic lung diseases were also associated with low GSH levels in the lung or plasma[36-40]. This association was even found in infants[41].

Smoking and obesity were associated with low baseline GSH levels[39,42-44]. Even a lower body mass index was associated with a less oxidized

form of GSH in the circulation[45]. In addition, impairment of GSH homeostasis was found in patients with alcoholism, renal failure, malignancies, and chronic illnesses[46-55]. Finally, acetylsalicylic acid (one of the non-steroidal anti-inflammatory drugs) was found to induce GSH depletion when used[56].

Previously published literature confirms that the different risk factors associated with high mortality rates in COVID-19 have low baseline GSH levels or impaired GSH metabolism in common. Therefore, how could low GSH levels (absolute or relative) put these patients at risk for developing a severe/fatal form of the disease? This question will be thoroughly answered in the following sections. First, the development of a cytokine storm in COVID-19 patients and the subsequent depletion of GSH levels will be tracked. Then, the effect of GSH depletion on suppressing the innate immune system will be highlighted. Subsequently, the effect of GSH depletion on the reduction of the activity of the cellular antioxidant system, which would render the end-organ vulnerable to damage caused by reactive oxygen species, will be discussed. Finally, the potential role of GSH as an antiviral agent will be explained. A summary of the hypothesis is shown in Figure 1.

COVID-19 and Its Cytokine Storm

COVID-19 mortality is mainly caused by respiratory failure due to acute respiratory distress syndrome or myocarditis[2,57,58]. These deadly complications are mainly a result of the cytokine storm[2,59]. Until now, it is not clear why this happens in some patients and not in others; however, there is emerging evidence that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mainly affects lymphocytes, especially T lymphocytes, resulting in dysregulation of the immune system, lymphopenia, and the cytokine storm[58,60]. As of the time of writing this review, no definitive treatment is available for resolving the cytokine storm. Thus, prevention of this condition would be the cornerstone of management.

During the cytokine storm, many cytokines are elevated, particularly interleukin 1 (B and RA), interleukin 6, and tumor necrosis factor α, which are very potent pro-inflammatory compounds[58,61,62]. This elevation is proportional to the

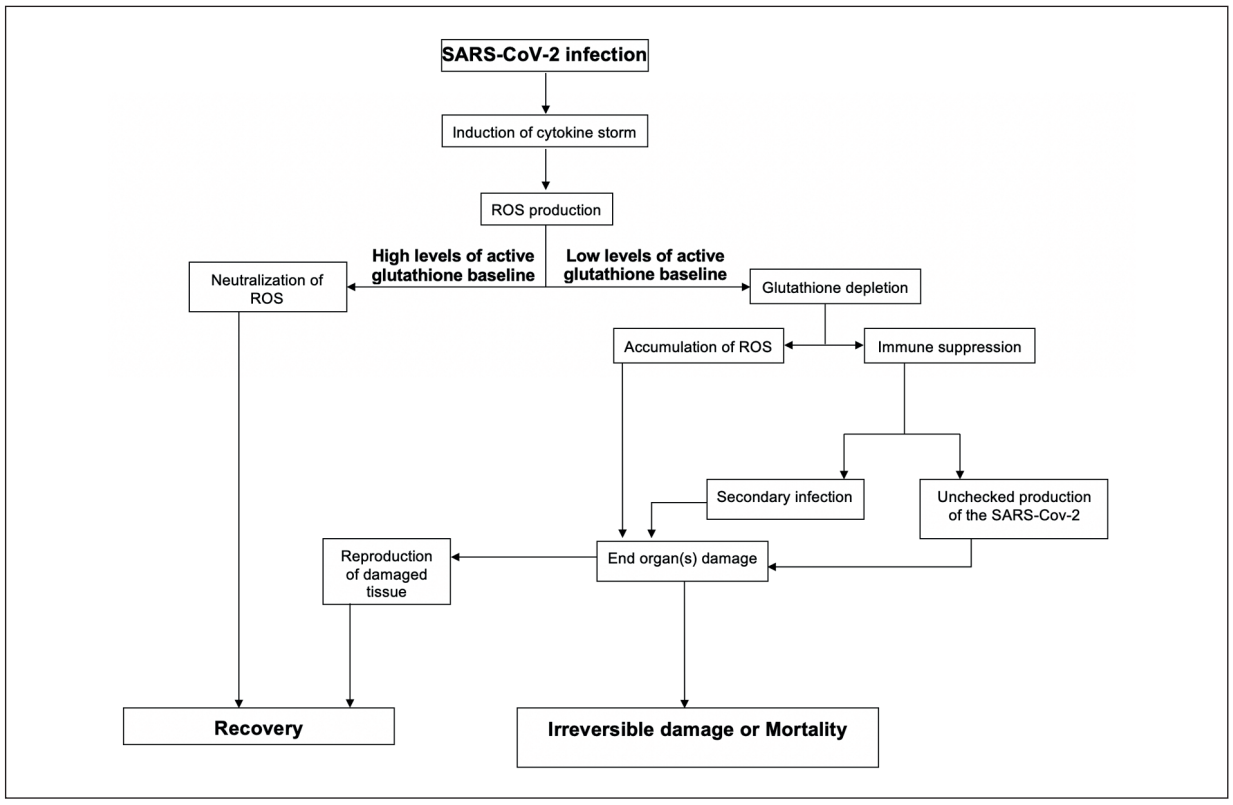

Figure 1. The hypothetical role of GSH in COVID-19 mortality. ROS: reactive oxygen species. Active glutathione: the reduced form. Baseline: the level before infection.

severity of COVID-19[58,63]. A massive elevation of these pro-inflammatory cytokines will lead to a large increase in reactive oxygen species production by the mitochondria[64]. These reactive oxygen species are toxic to intracellular organelles and cell function, which can induce acute lung injury/acute respiratory distress syndrome and lymphocyte dysfunction/lymphopenia[64-68].

The scavengers of these reactive oxygen species are the intracellular anti-oxidation systems from which the GSH and its regulatory enzymes are considered the most important[69,70]. Overproduction of reactive oxygen species could lead to GSH depletion[69].

GSH depletion leads to a loss of its protective functions in terms of the end organs and endothelium. GSH depletion can lead to the impairment of the function of lymphocytes, macrophages, and neutrophils, rendering an immunocompromised state, which may explain the high incidence of secondary infections in COVID-19 patients[2]. Moreover, GSH depletion triggers the apoptotic cascade in lymphocytes, leading to lymphopenia, affecting mainly T lymphocytes[71,72]. Low lymphocytic counts (absolute or relative) at the time of presentation are associated with a high mortality rate in COVID-19 patients[2,73]. The assumed effect of GSH level depletion on COVID-19 will be discussed in the following sections.

Glutathione and the Immune System

GSH is extremely important for the proper function of the immune system in general and lymphocytes in particular[74,75] as low GSH levels inhibit T lymphocytes proliferation and subsequently disturbs the immune response[76,77]. This could be reversed by administering N-acetylcysteine[75,78], which elevates GSH levels in the tissues by providing the amino acid cysteine[31,32]. A low GSH level inhibits interleukin-2 production, which induces lymphocyte proliferation[79].

The role of GSH on the immune system in COVID-19 patients could be established from its role in other viral infections associated with low lymphocytic count. For example, in HIV infection, low levels of GSH were associated with low levels of CD4 T lymphocytes and lower survival rates[80,81]. The opposite was also true; high GSH levels (by means of supplementation) improved CD4 T lymphocyte function in HIV patients[82]. Consequently, administration of GSH in COVID-19 patients may share similar positive effects as in HIV patients.

Even in non-viral conditions, inhaled N-acetylcysteine improved the lymphocytic count in the lungs of patients with cystic fibrosis[83]. Enhancing the function and proliferation of immune cells was also noticed after GSH administration to healthy adults[72]. In addition, GSH supplementation increased the cytotoxicity of natural killer cells and the proliferation of lymphocytes[72].

A more profound effect of low GSH levels on the immune system would be the induction of lymphocytes' apoptotic cascade. In particular, GSH depletion is necessary for apoptosis to be triggered in the lymphocytes regardless of reactive oxygen species[71]. Interestingly, it was noticed that to induce apoptosis of leukemic Jurkat T lymphocytes (immortal cells), GSH has to be pumped out of the cells[84].

This effect on apoptosis combined with proliferation inhibition could explain why patients with COVID-19 experience a low T lymphocytic count and the subsequent failure of the immune system. This was associated with a high mortality rate in COVID-19 patients[2,73]. This resultant failure could lead to uncontrolled replication of the virus, secondary infections and continuous shedding of the virus in patients who die from COVID-19 regardless of the time elapsed from the start of the infection[3,2].

Of lesser importance, the effect of GSH in the immune system is also observed on macrophages and neutrophils. GSH is essential for the efficiency of phagocytosis in neutrophils[85] and was found to improve macrophage function in HIV patients[86]. These findings further emphasize the importance of GSH in the immune system.

As shown above, this resultant failure of the immune system combined with the loss of GSH's protective effect as an antioxidant may explain the progression of the disease into an acute respiratory distress syndrome/acute lung injury.

Glutathione as an Antioxidant

As explained earlier, COVID-19 results in a cytokine storm and a subsequent reactive oxygen species. GSH is the most important endogenous antioxidant and is fundamental in detoxification of these reactive oxygen species[51], which are the culprits to be blamed for lung damage resulting from the inflammatory conditions[87]. Reactive oxygen species and their byproducts (e.g., reactive nitrogen species) are produced in the mitochondria in response to variable cytokines, such as interleukin 1B and TNF-α[87-89]. Those reactive species will produce hydrogen peroxide in the

mitochondria, which will diffuse to the cytosol resulting in intracellular and/or extracellular damage[90].

This hydrogen peroxide is regulated by multiple cell systems, like catalase, thioredoxin peroxidase, and, most importantly, GSH peroxidase[51,91]. GSH peroxidase utilizes reduced GSH in detoxification of the reactive oxygen species and their byproducts (free radicals, nitrogen reactive species, and hydrogen peroxide) in a process that may lead to GSH depletion in the cell[51]. Such depletion can result in severe and irreversible damage to the cell, which can be fatal as in acetaminophen toxicity[51]. The inability to detoxify reactive oxygen species and their byproducts will result in inflammation, increased vascular permeability, and end-organ damage[51]. On the contrary, the administration of GSH and/or its precursors was beneficial in managing some inflammatory conditions[92-94].

It is worth mentioning that the mechanism of action of GSH is to prevent tissue damage induced by oxidative stress and not to treat an already established damage in the end-organ. This would explain the lack of clinical improvement in patients with an established systemic inflammatory response syndrome or acute respiratory distress syndrome, as shown in some articles after the administration of N-acetylcysteine[95]. In addition, some studies showed that taking N-acetylcysteine did not decrease the mortality in patients with established acute respiratory distress syndrome; however, it did shorten the patients' stay in the intensive care unit (ICU)[96-99]. Nonetheless, an N-acetylcysteine nebulizer was shown to be effective in preventing ventilator-induced pneumonia, shortening the lengths of ICU stay and hospital stay[100]. Its use was associated with a significant increase in the number of patients attaining complete recovery[100].

In addition, prophylactic administration of N-acetylcysteine enhanced recovery in patients with lung injury[93] and prevented acute kidney injury post-contrast[101]. Further, nebulized N-acetylcysteine with unfractionated heparin improved survival rates and decreased the development of acute respiratory distress syndrome after smoke inhalation injury[102]. Moreover, administration of N-acetylcysteine improved the antioxidant status in male patients with chronic obstructive pulmonary disease[103], improved the inflammatory markers in patients with community-acquired pneumonia[104] and improved FEV1 in cystic fibrosis[105].

As shown above, the protective effect of GSH against oxidative stress experienced during acute or chronic inflammatory conditions is well documented. GSH depletion makes the end-organ prone to damage by reactive oxygen species. However, the administration of GSH may have a protective effect against end-organ damage, especially acute respiratory distress syndrome/acute lung injury.

Antiviral Effect of GSH

GSH enhances the ability of macrophages against different viruses, probably by functioning as an intracellular signal[106]. It has shown antiviral properties against influenza viruses *in vitro* and *in vivo*[107,108]. In particular, it decreased the lung inflammation induced by the H1N1 influenza virus[109]. In addition, it enhanced the effect of oseltamivir and prevented the development of fatal influenza[110]. N-acetylcysteine has shown inhibitory effects against H5N1 influenza virus[111].

Additionally, GSH has shown antiviral effects against dengue virus and herpes simplex virus type 1[112-114]. N-acetylcysteine suppressed HIV expression[115], decreased the infectivity of rotavirus[116,117], and decreased the damage experienced in piglets after being infected by the porcine epidemic diarrhea virus (which is a delta coronavirus)[118].

Interestingly, Xu et al[119] investigated viral shedding during the convalescent phase and showed that the protein structure of SARS-CoV-1 has been destroyed by N-acetylcysteine administration.

This confirms that GSH/N-acetylcysteine has antiviral activity toward a wide range of viruses. Nothing was common among these viruses, which showed sensitivity to GSH. They range from DNA viruses to retroviruses and from respiratory viruses to enteral viruses. This stresses the potential antiviral activity of GSH/N-acetylcysteine.

Recently, a case report of two patients was published after the completion of this article. Two patients with shortness of breath due to COVID-19 pneumonia were treated with GSH and showed a dramatic and rapid response within hours[120].

Conclusions

GSH is essential for proper functioning of the immune system, especially T lymphocytes and macrophages. Intracellular GSH is responsible for proper T lymphocyte function, proliferation, and prevention of apoptosis. In addition, proper

GSH levels should be maintained in patients with severe inflammatory response syndrome to counteract oxidative stress and end-organ damage. Finally, GSH has an inhibitory effect on many viral strains. These data may explain why people with depleted GSH levels are prone to mortality from COVID-19. This is particularly true in patients with risk factors for COVID-19 mortality.

GSH may be at the core of COVID-19 pathophysiology.

This preclinical study may form a basis for more in-depth studies concerning the efficacy of GSH and its precursors in managing COVID-19 as an adjunct treatment with the most acceptable protocols.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Authors' Contribution

All authors contributed to the preparation of this manuscript, which has been available to all authors to this point.

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