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Original Article

Role of Glutathione Reduction in Causation Severe Complications of Coronavirus Disease-2019

Sunober Abdalsamad Mohammed, Asal Aziz Tawfeeq[1], Muhammad Yawoz Noraldin[1]

Department of Microbiology, Azadi Teaching Hospitals, Kirkuk Directorate of Health, [1]Department of Medical Laboratory Techniques, Northern Technical University, College of Health and Medical Techniques, Kirkuk, Iraq

Abstract

Background: Coronavirus is a pandemic disease that emerged in China in 2019 with severe respiratory syndromes in conjunction with organ failure and advanced mortality rate where it has attracted worldwide interest. The pathogenicity of the virus increases by exacerbated oxidative stress that leads to the accumulation of free radicals in the body. Frequently, those free radicals are neutralized by antioxidants, especially glutathione. **Objective:** Thus, this study aimed to evaluate the role of glutathione in the pathogenicity of the virus alongside certain blood parameters. **Materials and Methods:** In this study, 90 participants were examined for glutathione levels using a specific enzyme-linked immunosorbent assay (ELISA) kit in addition to the levels of hemoglobin (Hb). Ferritin and vitamin D3 were also measured as they are linked to the level of glutathione in coronavirus disease-2019 (COVID-19). **Result:** The lowest level of Reduced glutathione in COVID-19 hospitalized patients especially male patients alongside with vitamin D3 and Hb levels. Whereas a significant ($P < 0.05$) increase in ferritin level was recorded. However, a significant ($P < 0.05$) increase in ferritin level was recorded. **Conclusion:** This study showed a significant relationship between glutathione level and disease severity in hospitalized patients with COVID-19, where the depletion of the glutathione level may have a central role in COVID-19 severity and pathophysiology.

Keywords: COVID-19, glutathione, Kirkuk, oxidative stress

Introduction

Coronavirus is a new global epidemic disease that first emerged in Wuhan, China in December 2019 and has since attracted worldwide interest.[1] Coronavirus is an enveloped RNA virus that infects mammals and then is transmitted to humans.[2] This virus causes a fatal cytokine storm and patients die from acute respiratory distress syndrome, whereas in many cases the disease progresses mildly or even asymptomatically.[3] The most important risk factors for severe coronavirus disease-2019 (COVID-19) or death in hospitalized patients are advanced age, sex, hypertension, diabetes, cardiovascular disease, obesity, smoking, and lung disease, especially asthma.[4] The common factor in all COVID-19-associated disorders appears to be disrupted redox (reduction–oxidation system) homeostasis, which is associated with reactive oxygen species (ROS) deposition and is usually neutralized by antioxidants, the most common of which is glutathione (GSH) where it is produced in the liver from three amino acids (glycine, cysteine, and glutamate) and is vital for the immune system.[5,6] Moreover, GSH plays a major role in protecting important cell constituents from the destruction caused by ROS such as free radicals, peroxides, and lipid peroxides.[5,7] Consequently, several researchers suggested that higher GSH levels may improve resistance to viral infections, where it enhances the ability of macrophages to ingest viral particles.[7] However, decreased vitamin D levels, low hemoglobin (Hb), and increased ferritin serum concentrations were all associated with increased severity and mortality of COVID-19 disease.[8-12]

Therefore, this research was suggested to compare GSH levels in patients with COVID-19 and healthy individuals

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Address for correspondence: Sunober Abdalsamad Mohammed, Department of Microbiology, Azadi Teaching Hospitals, Kirkuk Directorate of Health, Kirkuk, Iraq.
E-mail: snasamad94@ntu.edu.iq

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in Kirkuk city of both sexes and different age groups besides evaluation of the role of GSH, vitamin D, ferritin, and Hb in the pathogenicity of COVID-19.

Materials and Methods

Study population

Ninety individuals of different ages and from both sexes were included in this study that was carried out during the period from December 2021 until May 2022 where 5 mL of venous blood samples were collected from the study participants after signing consent to participate in the study and participants were divided into two groups:

Group A: It consisted of 45 patients with COVID-19 diagnosed according to the Iraqi National Guidelines for the diagnosis and treatment of COVID-19 and the interim World Health Organization (WHO) guidelines of polymerase chain reaction (PCR) assay of nasal and pharyngeal swabs in Al-Shifa hospital in Kirkuk city.

Group B: It consisted of 45 healthy individuals without a clear history of previous COVID-19 infection and were all subjected to routine blood testing to monitor their health status.

Glutathione evaluation

Serum-reduced GSH was evaluated according to the manufacturer's instructions of a specific enzyme-linked immunosorbent assay (ELISA) kit (BT-LAB; Bioassay Technology Laboratory, catalog number: E1642 Hu/China/) with a sensitivity of 0.05 ng/mL. The obtained results were analyzed statistically and compared according to standard measurements.

Blood parameters evaluation

All serum samples from Groups A and B were prepared and stored at – 80°C after centrifugation at 1500× *g* for 10 min and the levels of vitamin D3 and ferritin were measured using an automated biochemistry instrument (Cobas e411/Roche Company, Switzerland) according to the manufacturer's instructions, whereas an automated hematology analyzer (Swelab alfa/Boule Medical AB, Sweden) was used to measure Hb levels. Results were evaluated according to the methodology mentioned elsewhere.[8]

Statistical analysis

Results were statistically analyzed using the mini-tab statistical program; the categorical changeable was given as percentages and frequencies. *t* Test was used for comparison between groups. The means were compared according to the Duncan test at the probability levels of 0.05.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patient's verbal and analytical approval before the sample was taken. The study protocol and the subject information and the consent form were reviewed and approved by a local ethics committee according to document number 3657 (including the number and the date in 11/11/2021) to get this approval.

Results

In this study, all of the 90 participants were subjected to reduced GSH level evaluation and the results are shown in Figure 1.

The result of this study showed a statistically significant difference between GSH levels of the Groups A and B. The mean ± standard deviation (SD) for each group was as follows: Group A—patients with COVID-19/GSH = 5.7 ± 2.43 ng/mL, whereas in Group B the mean ± SD was as follows: healthy individuals/GSH = 16.44 ± 3.85 ng/mL with $P < 0.001$.

GSH levels were assessed among the individuals of Groups A and B according to their sex, and the results are shown in Figure 2.

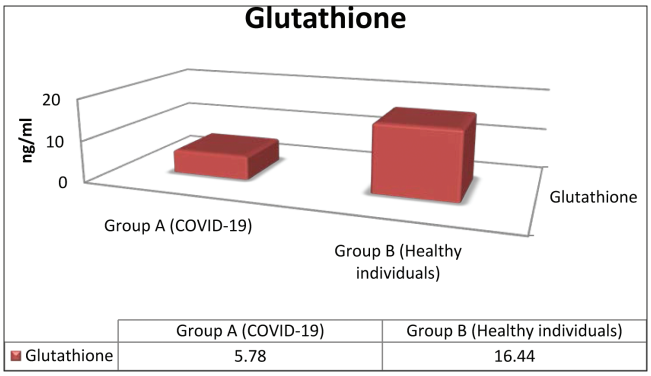


Figure 1: Comparison of reduced glutathione levels between Group A (patients with COVID-19) and Group B (healthy individuals) from both sexes enrolled in this study

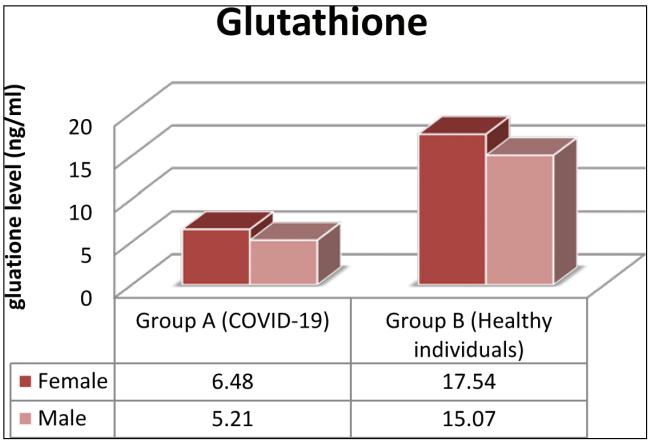
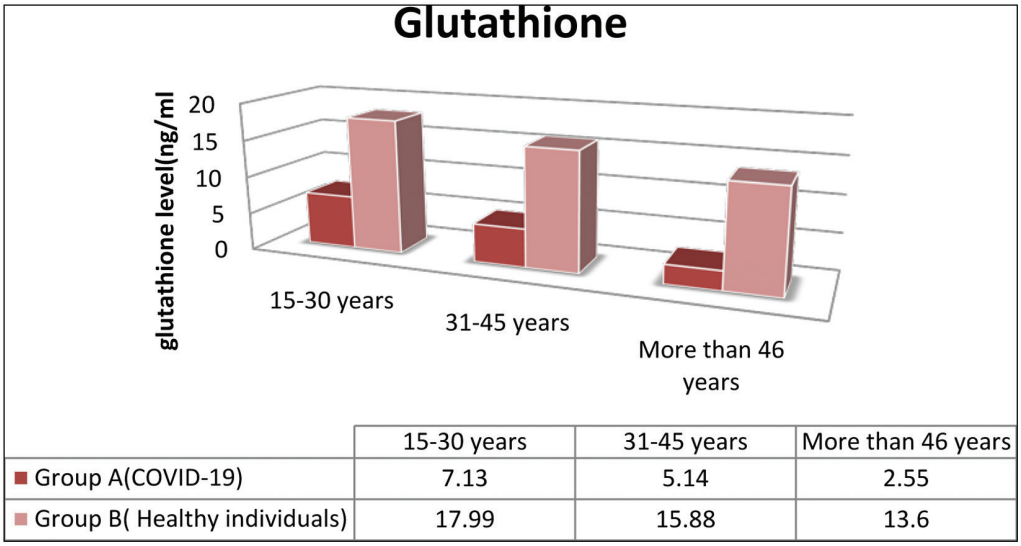


Figure 2: Glutathione levels between Groups A and B based on their sex



	15-30 years	31-45 years	More than 46 years
Group A(COVID-19)	7.13	5.14	2.55
Group B(Healthy individuals)	17.99	15.88	13.6

Figure 3: Glutathione level in different age Groups Among study participants

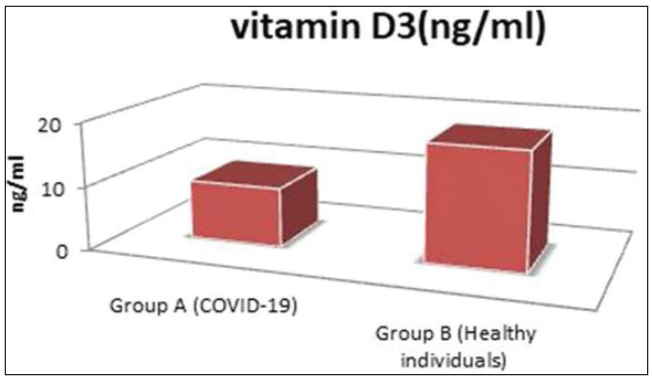


Figure 4: Level of vitamin D3 of the study participants of Groups A and B.

Figure 2 shows a significant difference in GSH levels between sex as a male had lower serum levels of reduced mean GSH (5.21 ± 1.82 ng/mL) than females in Group A (6.48 ± 1.20 ng/mL) compared with Group B (15.07 ± 2.11 male, 17.45 ± 2.95 ng/mL female) with $P < 0.001$. GSH levels were also evaluated among different age groups of the study participants, and the results are shown in Figure 3.

The mean ± SD of reduced GSH level for the age groups 15–30 years, 31–45 years, and over 46 years were 7.13 ± 2.23, 5.14 ± 1.42, and 2.55 ± 0.464 ng/mL, respectively, for Group A, whereas the mean ± SD of reduced GSH level for the age groups 15–30 years, 31–45 years, and over 46 years were 17.99 ± 3.29, 15.88 ± 3.31, and 13.6 ± 2.31 ng/mL, respectively, for Group B.

Vitamin D3 levels were compared between Groups A and B in Figure 4. Group A (COVID-19) had a significant decrease in vitamin D3 levels (9.32 ± 6.34 ng/mL) as compared with Group B (18.28 ± 12.00 ng/mL) with $P < 0.001$.

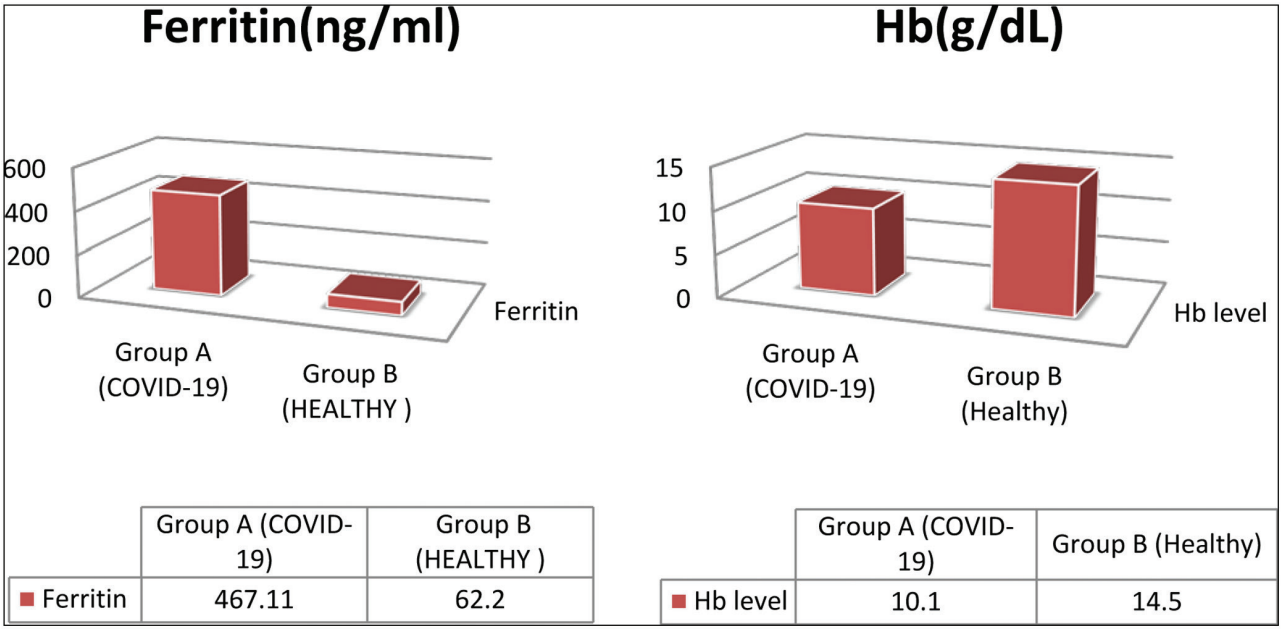
Figure 5 shows a statistically significant increase in ferritin levels in Group A (patients with COVID-19 = 467.11 ng/mL) as compared with Group B (healthy individuals = 62.2 ng/mL). In addition, a significant decrease was noticed in Hb levels between Group A (patients with COVID-19 = 10.1 ± 1.5 g/dL) as compared with Group B (healthy individuals = 14.5 ± 1.5 g/dL).

Discussion

Since the emergence of COVID-19 in 2019, the world has been in a state of contingency to fight the virus, which caused the death of more than 6 million people, and scientists around the world are racing to identify the risk factors associated with the pathogenic mechanism of COVID-19.[1,4]

Accordingly, one of the well-known related factors is the increased cytokine production due to inflammation, resulting in increased oxidative stress in the patient's body.[3,5,6] The most important antioxidant in the body to fight the oxidative stress is reduced GSH, which is considered a health parameter to evaluate the patient's status in many diseases.[7,13]

This study showed that there is a significant decrease in GSH levels in patients with COVID-19 as compared with control groups. The infection with Coronavirus lead to the increase in level of pro-inflammotory cytokines will lead to increase in reactive oxygen species production by the mitochondria which are eliminated by glutathione.[14] Overproduction of ROS could lead to GSH depletion.[15] This result agreed with the results obtained by a study in Moscow in which significantly low levels of total glutathione (tGSH) were recorded among 59 patients with COVID-19 with severe infections and a higher mortality rate.[16]



	Group A (COVID-19)	Group B (HEALTHY)
Ferritin	467.11	62.2

	Group A (COVID-19)	Group B (Healthy)
Hb level	10.1	14.5

Figure 5: Comparison between the levels of Hb and ferritin between Group A (COVID-19) patients and Group B (healthy individuals) enrolled in the study

The results of this study shed light on the infection ratio between males and females during the study period, which showed males were more affected (53.4%) by the virus than females (46.6%). The same results were obtained in a cross-sectional study in Najaf city, which also found that among 1153 patients with COVID-19, two-thirds of them were males.[17]

Several studies have referred to certain risk factors associated with gender behavior, including higher levels of smoking and drinking among men,[18,19] which probably led to the increased level of COVID-19 infections among them.

On the contrary, lower GSH levels were also recorded among males of Group B, which might be related to several factors, including more rapid utilization against increased oxidative stress caused by testosterone and sex-dependent regulation of GSH metabolism.[20,21]

Furthermore, the COVID-19 virus has affected all age groups, with an increased incidence rate in older adults.[22] As shown in Figure 4, patients with COVID-19 of all age groups had significantly lower levels of GSH compared with their levels among individuals of Group B. This result was consistent with the studies conducted for this issue,[14,17,18] where they designated the age-related decrease in GSH levels due to the increased rate of chronic diseases such as hypertension and diabetes.

Therefore, the level of vitamin D3 was estimated and a comparison was carried out between the two study groups as specified in Figure 5. The results showed a lower vitamin D3 level in patients with COVID-19 (Group A) when compared with healthy subjects (Group B). The same results were obtained previously in Kirkuk city by Al-Azzawy *et al.*,[23] where they verified the level of 12.8 ± 3.6 ng/mL in patients with COVID-19 correlated to (29.3 ± 3.1 ng/mL) in the healthy controls.

As a consequence, a relationship was strategized based on this study results between the reduction in the level of GSH and the significant decrease in vitamin D3 levels associated with COVID-19 infection severity. This hypothesis was confirmed by several studies that had reported an important correlation between GSH levels and its positive effect on the active vitamin D ratio in the body where they showed that GSH deficiency is associated with increased oxidative stress where they epigenetically alter vitamin D regulatory genes and, as a result, the suppressed gene expression decreases vitamin D biosynthesis, ultimately leading to a secondary deficiency of vitamin D3.[24-26]

In this study, another relationship was also established between Hb level and ferritin level and the increased severity of COVID-19 infection. In patients with COVID-19, an increased level of ferritin with a lower Hb level is recorded when compared with Group B (healthy subjects). When serum ferritin levels elevated, it causes exacerbated inflammatory conditions and induces oxidative damage and ferroptosis, along with the decrease in GSH levels (which functions as an oxidative stress regulator), creating a series of reactions that resulted in multiple organ failure, pulmonary injury, and reduced lung capacity in the study Group A. Other researchers found the same results when they targeted new diagnostic methods and therapies for iron overload.[27,28,29]

Therefore, GSH reduction does serve as the Trojan Horse of COVID-19 severe complications.[30] As the higher levels of GSH may improve an individual's responsiveness to

viral infections.[30] In particular, GSH is known to protect host immune cells, and it is also responsible for the optimal function of the immune system.[31,32]

Conclusions

This study showed a significant relationship between GSH level and disease severity in hospitalized patients with COVID-19, where the depletion of the GSH level may have a central role in COVID-19 severity and pathophysiology. Also according to the results of this study, GSH is also associated with decreased vitamin D levels. Therefore, increasing the GSH level in tissues may decrease the severity and mortality rates of COVID-19.

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Not applicable.

Conflicts of interest

There are no conflicts of interest.

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