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Association between vitamin D3 and glutathione levels in COVID-19 individuals

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ABSTRACT

Background: COVID-19 is an infectious disease associated with high rates of infection and death, especially in older males with low glutathione (GSH) and vitamin D (vit D) levels. The GSH status is positively associated with bioavailability of vit D. GSH deficiency correlated with increased oxidative stress (OxS) and inflammatory markers, which implicate an increase in the severity of the disease.

Objective: To verify the interaction of vit D and GSH levels among healthy individuals and COVID-19 patients.

Method: The study population involved 166 healthy individuals, who formed the control group, and 171 COVID-19 patients. OxS and antioxidant parameters, and levels of vit D and inflammatory markers were estimated in both groups.

Results: The COVID-19 patients showed significantly higher levels for malondialdehyde (MDA), protein carbonyl group (PC), interleukin-6, tumor necrosis factor alpha, and C-reactive protein and significantly low levels for GSH and vit D compared to the healthy control group. The aged and male COVID-19 groups displayed significantly higher levels of MDA and PC and significantly low levels of GSH compared with the younger and women groups.

Conclusion: The COVID-19 patients displayed higher levels of OxS and inflammatory markers and low levels of antioxidant GSH and vit D, which developed by advancement of age especially within males.

Keywords: *COVID-19; oxidative stress; reduced glutathione (GSH); vitamin D3*

INTRODUCTION

The COVID-19, an infection that is still spreading throughout different countries, has impacted many people. The disease severity in people varied from mild to moderate and to severe critical illness, and without symptoms.¹ The virus enters the human body through the spike protein found on its surface and attach to human cell receptor ACE2. The virus later begins to replicate within the lungs, leading to difficulty in breathing and sometimes death.² The rapid rise in COVID-19 infection resulted in invasion of human immune systems and overcame mechanism of defense and led to “cytokine storm” development.³

The rate of COVID-19 was higher in older-aged people, which suggests the implication of age in making humans more sensitive to environmental stress factors, such as the infection with corona virus SARS-CoV-2.⁴ Different studies have found that the COVID-19 severity was associated with gender. When compared to men, the women were at less risk of contracting the disease, worsening, and death. This is due to the higher levels of glutathione (GSH) in females than in males.⁵

The oxidative stress (OxS) is the generation of excess reactive oxygen species (ROS), correlated with different disorders such as the COVID-19 disease.⁶ Under normal physiological state, OxS is balanced by the antioxidant system. Reduced GSH is considered as a major endogenous antioxidant.⁷⁻⁹ It has different functions such as elimination of free radicals and antiviral property. The level of cellular GSH varies with sex and age.¹⁰

The vit D plays a major role in regulating many cellular pathways and has a critical role in the antioxidant system.¹¹ Low levels of vit D may

result from an increased damage to cells caused by the ROS.¹² Several studies have indicated that vit D deficiency is associated with increased COVID-19 severity, and vit D positively correlates with levels of GSH.^{13,14}

COVID-19 AND GLUTATHIONE

The cellular deficiency of GSH may result from decreased biosynthesis or increased depletion of GSH, which leads to the development of OxS, immunity dysfunction, and viral invasion.¹⁵⁻¹⁷ Data from different sources have confirmed that GSH deficiency can be regarded as the major cause of increased rate of COVID-19 infection in aged population. The old-aged people are more susceptible to damages caused by OxS due to viral infection as a result of a decrease in the GSH levels, a phenomenon that was observed in COVID-19 patients.^{18,19} This results in the exacerbation of inflammation in the lungs, resulting in increased disease severity.²⁰

COVID-19 AND VITAMIN D

Vitamin D is a steroidal hormone,²¹ which plays a significant role in increasing cellular immunity. This is achieved by preventing the cytokine storm by influence of TNF α and interferon- γ ²² and thereby regulating the immune system,²³ which is an important step in inhibiting the replication of respiratory viruses.²⁴ Studies have suggested that the deficiency of vit D can stimulate the renin-angiotensin system, resulting in cardiovascular diseases that impair lung function. People with these comorbidities possess a higher risk of severe COVID-19 outcomes.²⁵

GLUTATHIONE AND VITAMIN D

Many recent observations state a positive relationship between GSH and vit D, with higher levels of GSH related to higher levels of active vit D.²⁶ Low levels of L. cysteine, which is considered as a precursor for reduced GSH, correlated with low vit D levels and vit D binding protein (VDBP).²⁷ Thus, administration of L. cysteine improves reduced GSH, and thereby upregulates the expression of VDBP, 25-hydroxylase, and vit D receptor. Thus, vit D value increases and the level of biomarkers of inflammation decreases.²⁸

GSH deficiency is associated with increase in OxS, which causes alterations in the regulatory genes of vit D, leading to the gene expression suppression and decrease in vit D biosynthesis. The net results of the above process lead to secondary vit D deficiency. Finally, we conclude that GSH is vital for the control of endogenous vit D biosynthesis and may be used as a treatment for vit D deficiency.²⁹

METHOD

Study design and patient collection

A total of 337 individuals, who presented to the Al-Hakeem Hospital, Najaf, Iraq, participated in the study. The study population was categorized into two groups: (1) 166 of them were considered as the healthy control group, (2) and the remaining 171 were marked as the case study group, confirmed with COVID-19 infection.

The case study group (171 patients) was subdivided into two subgroups: (1) the first subgroup (129 patients) was designed considering the age, (2) and the second subgroup (171 patients) was based on the gender of the participants. The aim of the study was to determine the influence of both age and gender on the OxS products MDA and protein carbonyl group (PC), and measure the antioxidant stress, including reduced GSH, vit D, and inflammatory markers such as IL-6, C-reactive protein (CRP), and TNF α for all participants.

Biostatistical analysis

The data were expressed as mean \pm standard deviation. Student's T-test was used to verify the differences among healthy individuals and COVID-19 patients and among the subgroups of COVID-19 case study group, depending on age and sex for all estimated parameters. P-value $<$ 0.05 was considered significant.

RESULT

The analysis of the results revealed significant higher levels for MDA (4.51 ± 1.6 , $P = 0.001$) and PC (1.81 ± 0.92 , $P = 0.003$) and significantly low levels for GSH (2.89 ± 0.42 , $P = 0.001$) in patient group compared with the healthy group (Table 1). The data in Table 2 exhibited significant higher levels for fasting glucose (141.1 ± 11.32 , $P = 0.001$), IL-6 (8.94 ± 1.21 , $P = 0.001$), TNF (5.32 ± 1.03 ,

TABLE 1. Oxidative stress and antioxidant value among healthy individuals and COVID-19 patients.

Parameters	Healthy (Control group) N = 166	COVID-19 (Case group) N = 171	P
BMI (kg/m ²)	23.8 \pm 1.8	24.1 \pm 2.2	0.17
MDA (mmol/L)	1.41 \pm 0.09	4.51 \pm 1.6	0.001
PC (nmol/mg protein)	0.722 \pm 0.3	1.81 \pm 0.92	0.003
GSH (mg/gHb)	4.01 \pm 0.61	2.89 \pm 0.42	0.001

BMI, body mass index; GSH, glutathione; MDA, malondialdehyde; PC, protein carbonyl group.

P = 0.001), and CRP (10.11 ± 2.01 , P = 0.001) and significant low level of D3 (19.71 ± 8.72 , P = 0.001) in the patient group compared to the healthy group. In Table 3, the data demonstrate the influence of age on the disease; it showed significant higher levels for MDA (4.02 ± 1.12 , P = 0.004) and PC (2.05 ± 0.02 , P = 0.028) and low levels for vit D (18.01 ± 6.89 , P = 0.004) and GSH (2.01 ± 0.33 , P = 0.001) in the aging group (67 ± 12 years) compared to the other group having an age of 62 ± 14 years. On the other hand, the gender factor displayed significant higher levels for MDA (4.34 ± 1.81 , P = 0.03) and PC (2.15 ± 0.11 , P = 0.002) and a significant low level for GSH (2.36 ± 0.53 , P = 0.003) in males compared to females. A failure to show significant differences for D3 (21.91 ± 6.96 , P = 0.51) was also observed among two subgroups, as in Table 4.

DISCUSSION

The COVID-19 pandemic is rapidly spreading and new variants are emerging, such as alpha, and sigma, due to the lack of knowledge, proper information, and efficient treatment strategies.³⁰ Therefore, identification of the factors that interact with mechanisms of pathogenicity of the coronavirus is required to lower the hospitalization time and mortality rate. The OxS correlates with disease severity especially when there is a decrement in the levels of antioxidants such as GSH, ascorbic acid, vit D, and others. OxS is associated with different diseases and also certain infections such as COVID-19.³¹

Evidences have revealed that the dearth of GSH is a major cause of disease severity and mortality

TABLE 2. Biochemical and inflammatory markers of healthy individuals and COVID-19 patients.

Parameters	Healthy (Control group) N = 166	COVID-19 (Case group) N = 171	P
Glucose (mg/dL)	96.4 ± 8.31	141.1 ± 11.32	0.001
25(OH) vit D (ng/mL)	33.42 ± 10.13	19.71 ± 8.72	0.001
IL-6 (pg/mL)	3.15 ± 0.11	8.94 ± 1.21	0.001
CRP (mg/L)	2.97 ± 1.1	10.11 ± 2.01	0.001
TNF α (pg/mL)	1.31 ± 0.93	5.32 ± 1.03	0.001

CRP, C-reactive protein; IL-6, interleukin 6; TNF α , tumor necrosis factor alpha.

TABLE 3. Oxidative stress, antioxidant, and vitamin D3 value among COVID-19 patients based on age.

Parameters	COVID-19 patient N = 64	COVID-19 patient N = 65	P
Age years	62 ± 14	67 ± 12	0.03
BMI (kg/m ²)	24.21 ± 1.5	24.62 ± 1.22	0.091
MDA (mmol/L)	3.52 ± 0.81	4.02 ± 1.12	0.004
PC (nmol/mg) protein	1.91 ± 0.51	2.05 ± 0.02	0.028
GSH (mg/gHb)	2.77 ± 0.54	2.01 ± 0.33	0.001
25(OH) vit D (ng/mL)	21.61 ± 7.11	18.01 ± 6.89	0.004

BMI, body mass index; GSH, glutathione; MDA, malondialdehyde; PC, protein carbonyl group; vit D, vitamin D.

TABLE 4. Oxidative stress, antioxidant, and vitamin D3 value among COVID-19 patients based on gender.

Parameters	COVID-19 patients Females = N = 84	COVID-19 patients Males = N = 87	P
Age years	65 ± 10	66 ± 13	0.57
BMI (kg/m ²)	24.32 ± 1.6	23.92 ± 1.17	0.06
MDA (mmol/L)	3.88 ± 0.81	4.34 ± 1.81	0.03
PC (nmol/mg) protein	1.91 ± 0.71	2.15 ± 0.11	0.002
GSH (mg/gHb)	2.61 ± 0.54	2.36 ± 0.53	0.003
25(OH) vit D (ng/mL)	22.68 ± 8.33	21.91 ± 6.96	0.51

BMI, body mass index; GSH, glutathione; MDA, malondialdehyde; PC, protein carbonyl group; vit D, vitamin D.

due to COVID-19.³² The antiviral property of GSH inhibits viral replication, which prevents the liberation of increased massive inflammatory markers into the lungs.¹ GSH also decreases the activity of ACE, reduces ROS synthesis, thereby keeping the cytokine storm under control.³³

Different studies have shown the prevention role of vit D against SARS-CoV2 infection.^{34–36} Meltzer et al. during their study concluded that the rate of COVID-19 infection in humans increased with vit D deficiency.³⁷ Thus, analysis of the role of vit D in keeping the redox status of cells becomes essential, as it demonstrated a significant rate of reduction in the number of infected cells and lowered the levels of proinflammatory markers.^{38–40}

The association between GSH, vit D deficiency, and overproduction of the ROS and pathogenicity of the COVID-19 infection was determined by measuring the intracellular GSH concentrations, OxS parameter, and vit D in individuals affected with the disease.⁴¹

The data in Table 5 exhibit a significant higher level for MDA and PC and a significant low level for GSH in the case study group when compared to the healthy group. The depletion of GSH increases the OxS and carbonylation of proteins resulting in the production of PC,^{42–45} the abnormal production of free radicals results in MDA formation which causes destruction of the lipid cell membranes. It has been revealed that the possible explanation for

TABLE 5. The demographic properties for the study.

Parameters	Controls, N = 166 (Health group)	COVID-19, N = 171 (Case group)
Mean age (year)	62 ± 12	64 ± 14
Gender		
Males	87 (52.41%)	50.88 (87%)
Females	79 (47.59%)	49.12 (84%)
Symptoms		
Mild case	No symptoms	72 (42.11%)
Moderate case		59 (34.5%)
Severe case		40 (23.39%)
Comorbidities		167 (97.66%)
None	No comorbidities	1 (0.59%)
Hypertension		3 (1.75%)
Gall stone		

severity and complication of COVID-19 is GSH deficit.³⁵ Our study findings were compatible with that of the previous studies⁴⁶, such as that of Karkhanei et al.⁴⁷ and Muhammad et al.⁴⁸ These studies also found elevated levels of OxS and reduced antioxidant status of GSH in the patient group.^{49,50}

The data in Table 2 demonstrate significant higher levels for TNF- α , IL-6, and CRP in the patient group compared to the healthy group. In COVID-19 patients, the depletion of GSH increased OxS, resulting in increased levels of TNF- α and IL-6.^{51,52}

Also, the results showed significant higher level for D3 which is associated with low levels of inflammatory markers and OxS in the control group when compared to the case study group. Th vit D plays a significant role in the downregulation of the synthesis of these markers and in reduction of OxS.⁵³⁻⁵⁵ Studies show that GSH stimulates the regulation of vit D gene and elevated concentrations of vit D within cells. On the other hand, vit D influences the biosynthesis of GSH by increasing cellular GSH formation.^{56,14} So, there is positive interaction among D3 and GSH in lowering the severity of COVID-19.⁵⁷

COVID-19 and age

The factors such as age, comorbidities, smoking, and dietary habits are considered as risk agents for COVID-19 infection.⁵⁸ Age is an important factor, and aged subjects are highly susceptible to stressing factors of the environment such as the infectious virus SARS-CoV-2. In addition, aging involves dysfunction of normal immune responses and also induces the dysregulation of inflammation pathways.⁴ The deterioration of redox homeostasis and OxS was revealed to be critical biological processes that could account for enhancing susceptibility to diseases in elderly patients.⁵⁹ The possible cause was the depletion of GSH, which was associated with advancement of age.

Our observations exhibited significant GSH deficiency in the aged group compared to the other groups of patients. Our study results were in agreement with the findings from different studies conducted previously.⁶⁰⁻⁶² The findings suggested that age may be considered as a major factor in the pathogenesis of COVID-19.⁶³

Regarding the level of Vit D in aged patients and its association with disease severity, our results exhibited significant low levels for vit D in the aging group (≥ 67 years) compared with the other group. Many studies have indicated that the aged population is at higher risk toward COVID-19 infection.^{64,65} Our data revealed that the COVID-19 incidence was

significantly greater in older patients especially those with vit D deficiency, and this finding was in agreement with the findings from that of the previous studies.⁶⁶⁻⁶⁸

The less exposure to sunlight, absence of appetite, reduction of vit D absorption, and other factors, common in old-aged patients, contribute to vit D deficiency. Our data indicated significant low levels of vit D and GSH, along with significant higher levels of OxS (MDA and PC) in the aging group. OxS is neutralized with GSH, which is an intracellular antioxidant. An older-aged individual with COVID-19 is at high risk of elevated OxS combined with GSH and vit D deficiencies.^{69,70}

Vitamin D plays a significant role in lowering the OxS by activating the antioxidant cascades and by blocking certain pathways that produce active ROS. Thus, there is a sincere interrelationship between OxS, vit D, and GSH levels especially in the old age.⁷¹

COVID-19 and gender

Gender is also one of the major factors responsible for COVID-19 infection. It was noticed that males are more likely to suffer from COVID-19 infection compared to women.⁷² In addition, our data showed significant lower plasma levels of GSH, and lower, nonsignificant vit D levels in men when compared to women.⁷³ Thus, men are more susceptible to OxS and inflammation,³⁵ observed by the significant elevation in the levels of MDA (4.34 ± 1.81 , $P = 0.03$) and PC (2.15 ± 0.11 , $P = 0.002$) in men compared to that in women, MDA (3.88 ± 0.81 , $P = 0.03$) and PC (1.91 ± 0.71 , $P = 0.002$).

Several studies exhibited lower values for GSH in men due to the rapid utilization of GSH in men compared to women, and the reasons may be due to the implication of male testosterone hormones in exacerbation of free radicals and stimulation of lipid peroxidation.⁷⁴ On the other hand, it was found that the female hormone, estrogen, inhibits the synthesis of ROS, and so women have less probability to depilation of GSH.⁷⁵

From the above data and the evidences collected, we hypothesize that vit D supplementation and GSH or its precursor can be advised in the treatment of COVID-19 severity.⁷⁶

LIMITATION

The major limitations of this study were: (1) small sample size, (2) the data were obtained from only one hospital.

We recommend that future studies consider these limitations, and increase the sample size and measure the levels of other antioxidants as well.

CONCLUSION

The COVID-19 patients display low vit D levels, which is associated with low levels of GSH resulting from increased OxS that implicated lung injury and reflected the severity of disease.

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