Original Article

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Oxidative Stress and Biochemical Parameters among Recovered COVID-19 Patients: A Case-control Study

Biochemistry Section

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ABSTRACT

Introduction: The Coronavirus Disease-2019 (COVID-19) pandemic has spread rapidly, infecting more than 194 million and killing more than 4 million people worldwide. Algeria has not escaped this scourge; according to World Health Organisation (WHO), 162,155 confirmed cases and 4,063 deaths have been recorded from 3rd January 2020 to 26th July 2021. Recent studies have indicated the critical role of an altered immune system, and oxidative stress in the pathological process contributing to several complications during COVID-19 disease.

Aim: To determine blood markers, oxidant/antioxidant status and biochemical parameters in patients recovered from COVID-19 and compare with those who have never contracted COVID-19; considered as controls.

Materials and Methods: The present case-control study was conducted in Tiaret, Algeria, between May 2021 and June 2021. Thirty healthy volunteers who had never contracted COVID-19 and 16 volunteers who recovered from COVID-19 in the last six months were included in the study. Blood samples were taken after 8 to 12 hours of fasting, the blood markers and biochemical parameters were evaluated. The participant with chronic diseases (diabetes, hypertension, cardiovascular diseases, kidney disease) was excluded. Student's t-test was performed for statistical comparison between the two groups. Statistical analysis was performed using Excel Microsoft 2010 software.

Results: The control group consisted of 46.7% males (n=14) and 53.3% females (n=16). While, the case group consisted of 62.5% males (n=10) and 37.5% females (n=6). The plasma levels of Low Density Lipoprotein-Cholesterol (LDL-C), p-value=0.004** and creatinine increased significantly in the cases compared to the controls. While, total cholesterol, p-value=0.04* and Glutamate Pyruvate Transaminase (GPT), p-value=0.03* increased significantly in the case group on comparision to the control group. On the other hand, erythrocyte Malondialdehyde (MDA) levels, p-value=0.009** increased very significantly in the case group compared to controls. The erythrocyte activity catalase decreased significantly in the case group compared to the controls. But erythrocyte Reduced glutathione (GSH) decreased very significantly in group cases compared to controls.

Conclusion: The findings in the present study confirmed the persistence of metabolic alterations and oxidative stress in COVID-19 patients after recovery. Antioxidant supplementation is recommended to improve redox status and reduce oxidative stress after recovery.

Keywords: Catalase activity, Coronavirus disease-2019, Malondialdehyde, Metabolism, Reduced glutathione

INTRODUCTION

The Coronavirus Disease-2019 (COVID-19) pandemic is due to Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), which was first discovered in December 2019 in Wuhan, China [1]. Until July 2021, COVID-19 has infected more than 194 million and killed more than four million people worldwide. In Algeria, according to WHO, there have been 162,155 confirmed cases of COVID-19 with 4,063 deaths from 3rd January 2020 to 26th July 2021 [2]. Earlier, WHO has reported that during June 2021, there was a 12% drop in cases worldwide, but an increase of more than 40% in Africa. Also, mortality increased in Africa (more than 20%) and South-east Asia (more than 12%) but decreased in Europe (less than 17%) and North and South America (less than 7%) [3].

With the new variants, especially delta, the situation has become much more alarming in Algeria and all world countries. The COVID-19 infection could be symptomatic or asymptomatic. The most common symptoms in those affected are fever, dry cough, and fatigue. While the less common symptoms are body aches, sore throat, diarrhea, conjunctivitis, headache, loss of smell or taste, rash, or discoloration of the fingers or toes. The most severe symptoms are difficulty in breathing or shortness of breath, tightness or pain in the chest and loss of speech or motor skills [4].

The pathogenesis of COVID-19 is still incompletely understood. Nevertheless, recent studies have indicated the key role of altered immune system, oxidative stress and excessive inflammation in the pathological process, which contributes to several complications during COVID-19 [5,6].

Oxidative stress corresponds to an imbalance between the pro-oxidant and antioxidant systems in favor of the pro-oxidant. According to a previous study, it is involved in the onset of several diseases such as arteriosclerosis, cancer, cardiovascular diseases, inflammatory diseases and the aging process [7]. Recent studies show that oxidative stress plays a vital role in the pathogenesis of COVID-19, especially in the respiratory stage, which leads to the death of the infected patient [8,9]. In addition, advanced age, black and South Asian ethnicity, male sex, low socio-economic status, hyperglycaemia, and obesity represent the main risk factors associated with COVID-19 infection [10]. These risk factors are associated with a state of significant oxidative stress [11-16].

The accumulation of free radicals coupled with the weakened antioxidant system leads to oxidative stress, further aggravating respiratory illnesses, COVID-19 included. A strong relationship between coronavirus infection and mitochondrial dysfunction, inducing excessive Reactive Oxygen Species (ROS) release, has been reported [17]. In addition, the virus induced modulation of the host cellular antioxidant defense system determines the severity of viral diseases [18].

The persistence of a state of oxidative stress after recovery from COVID-19 is well documented in a very recent study [19]. Reducing oxidative stress is a priority in the follow-up of patients recovered

from COVID-19. Authors are currently underway on the benefit of intravenous vitamin C supplementation to reduce the state of oxidative stress in these people. Targeting oxidative stress in COVID-19 recovery may provide a promising approach that leads to a successful healing. It is then essential to determine to what extent oxidative stress is present during recovery [19].

There is a paucity of data regarding the state of oxidative stress in COVID-19 patients and those who have recovered within the last six months. Because in the six months after infection, there is always symptoms and complications risk.

The present study aimed to identify blood markers (plasma and erythrocyte) of the oxidant/antioxidant status (Vitamin C, MDA, catalase activity, Reduced Glutathione (GSH) and biochemical parameters (total cholesterol, triglycerides, urea, creatinine, uric acid, Glutamic Oxaloacetic Transaminase (GOT), Glutamate Pyruvate Transaminase (GPT), total plasma proteins in people recovered from COVID-19 compared to people who have never contracted COVID-19, considered as controls.

MATERIALS AND METHODS

This case-control study was carried out in Boubeker khaled polyclinic centre, Tiaret, Algeria, between May 2021 and June 2021. Investigations of patients, as well as blood sampling conditions, were subjected to a strict code of ethics. The protocol was approved by the Tlemcen Hospital Committee for Research on Human Subjects (D01N01UN1301202019). All participants were informed about the objective of this study and were asked to give their written consent before starting the work.

Inclusion criteria: The 16 volunteers, who recovered from COVID-19 in the last six months and 30 volunteers age, sex matched with healthy volunteers who had never contracted COVID-19 were included.

Exclusion criteria: Patients with chronic diseases (diabetes, hypertension, kidney disease, thyroid disease, cardiovascular diseases) and pregnant female were excluded from this study for both control and case groups.

Sample size calculation: To obtain a power of 80% sample size calculator (Statistical solutions, Sigma) was used.

Study Procedure

The 5 mL of blood was taken in Ethylenediamine Tetraacetic Acid (EDTA) tubes after 8 to 12 hours of fasting and transported in an ice chest to the laboratory. Blood tubes were centrifuged at 3000 rpm for 15 minutes. Plasma was stored at -20°C for biochemical parameters (total cholesterol, triglycerides, urea, serum creatinine, uric acid, Glutamic Oxaloacetic Transaminase (GOT), Glutamate Pyruvate Transaminase (GPT), total plasma proteins) and oxidant/antioxidant status markers determinations (Vitamin C and MDA). The remaining erythrocytes were recovered, washed and haemolysis by adding cold distilled water (1/4) and then incubated for 15 minutes in the refrigerator. Cell debris was removed by centrifugation at 4000 rpm for 15 minutes. Then, the lysate was recovered for erythrocyte oxidant/antioxidant status markers assay (MDA, GSH and catalase activity).

The erythrocyte lysate preparation and vitamin C dosage were carried out on the same day, the sample was taken.

Measurement of biochemical parameters: The determination of biochemical parameters (total cholesterol, triglycerides, LDL-C, HDL-C, urea, creatinine, uric acid, GOT, GPT, total plasma proteins) were carried out by enzymatic spectroscopic methods using Biomaghreb kits (Tunis, Tunisia) [Table/Fig-1].

Measurement of oxidant/antioxidant status markers: Plasma vitamin C was measured according to the method of Jagota SK and Dani HM [20], using the folin's reagent and a calibration range of ascorbic acid. The absorbance was measured at 769 nm.

Then, plasma and erythrocyte MDA; the most marker used to determine the lipid peroxidation process, was assayed by the method of Draper HH and Hadley M [21]. After hot acid treatment, the aldehydes react with Thiobarbituric Acid (TBA) to form a chromogenic condensation product. The absorbance of this chromogen was measured at 532 nm. The MDA concentrations were expressed as micromoles of MDA, using the molar extinction coefficient (1.56×105 $M^{-1}cm^{-1}$). While, Catalase Activity (CAT, EC 1.11.1.6) was determined by the Claiborne method [22], which is based on the decomposition rate of hydrogen peroxide at 240 nm by the catalase enzyme. Erythrocyte GSH levels were assayed using to the colorimetric method using Ellman's reagent (DTNB). The reaction involves cutting the 5,5-dithiodis-2-nitrobenzoic acid (DTNB) molecule with GSH, which releases Thionitrobenzoic acid (TNB). At pH equal to 8-9, alkaline TNB exhibits an absorbance at 412 nm with an extinction coefficient equal to 13.6 $mM^{-1}cm^{-1}$ [23].

Parameters	Values	References
Cholesterol (g/L)	1.4-2.2	Biomaghreb kit, Tunisia
Triglycerides (g/L)	Women: 0.40-1.40 Men: 0.60-1.65	Biomaghreb kit, Tunisia
HDL-C (mg/dL)	High risk: Men <35 Women <45	Biomaghreb kit, Tunisia
LDL-C (mg/mL)	High risk >160	Biomaghreb kit, Tunisia
Urea (g/L)	Serum or plasma 0.15-0.40	Biomaghreb kit, Tunisia
Creatinine (mg/dL)	Serum or plasma: 70-105	Biomaghreb kit, Tunisia
GOT at 25°C (µL/L)	Women up to 16 Men up to 19	Biomaghreb kit, Tunisia
GPT at 25°C (µL/L)	Women up to 16 Men up to 22	Biomaghreb kit, Tunisia
Total plasma proteins (g/L)	67-87	Biomaghreb kit, Tunisia

[Table/Fig-1]: Measurement of biochemical parameters.

STATISTICAL ANALYSIS

The results are presented as mean±standard deviations. Statistical analysis was performed using excel Microsoft 2010 software. After variance analysis, the significant differences between the two groups were determined by student's t-test. Differences were significant for p-value <0.05.

RESULTS

Characteristics of the study population: Analysis of age and Body Mass Index (BMI) (Kg/m^2) were not significantly different between the two groups (p-value=0.6 for age and 0.4 for BMI). The control group consisted of 46.7% males (n=14) and 53.3% females (n=16). While, the case group consisted of 62.5% males (n=10) and 37.5% females (n=6) [Table/Fig-2].

Variables	Controls (n, %)	Cases (n, %)
Gender		
Men	14 (46.7%)	10 (62.5%)
Women	16 (53.3%)	6 (37.5%)
Age (years) means±standard deviation	43±3	38±4
BMI (Kg/m^2) means±standard deviation	23.52±3.34	24.60±3.26
Marital status		
Married	17 (56.7%)	11 (68.7%)
Single	13 (43.3%)	5 (31.3%)
Education		
Educated	29 (96.7%)	15 (93.7%)
Illiterate	1 (3.3%)	1 (6.3%)
Profession		
Employed	22 (73.3%)	14 (87.5%)
Unemployed	8 (26.7%)	2 (12.5%)

[Table/Fig-2]: Comparison of demographic characteristics between cases and controls. Student's "t" test after analysis of variance. Qualitative variables presented as percentages

The present study showed that 68.75% (n=11) of recovered persons were married, and 93.75% (n=15) were educated. On the other hand, 96.66% (n=29) of the control group were educated [Table/Fig-2]. Demographic characteristics such as family members, work environment and type of profession are a primary factors in the possibility of COVID-19 infection.

Biochemical parameters in cases and controls: Plasma levels of triglycerides, HDL-C, uric acid, urea, GOT, and total plasma proteins were identical between the two groups. On the other hand, plasma levels, LDL-C and creatinine increased very significantly in the cases compared to the controls (p=0.004** for LDL-C and 0.005** for creatinine). Total cholesterol and GPT risen considerally in the case group compared to the control group (p=0.04* for total cholesterol and 0.03* for GPT) [Table/Fig-3].

Parameters	Controls	Cases	p-values
Total cholesterol (g/L)	1.29±0.01	1.40±0.04	0.04*
Triglycerides (g/L)	1.25±0.05	0.85±0.05	0.51
HDL-C (g/L)	0.32±0.04	0.33±0.01	0.75
LDL-C (g/L)	0.65±0.06	0.85±0.02	0.004**
Uric acid (mg/L)	42.57±1.18	40.38±1.23	0.67
Urea (g/L)	0.34±0.01	0.28±0.01	0.16
Creatinine (mg/L)	8.03±0.11	9.63±0.19	0.005**
GOT (µL/L)	32.31±1.15	33.72±1.01	0.67
GPT (µL/L)	13.80±1.87	18.50±1.30	0.03*
Plasma total proteins (g/L)	71.58±3.64	74.69±3.47	0.10

[Table/Fig-3]: Biochemical parameters in cases and controls.
Each value represents the mean±standard deviation. HDL: high density lipoproteins; LDL: Low density lipoproteins; GOT: Glutamic oxaloacetic transaminase; GPT: Glutamate-pyruvate transaminase. The comparison of the means is carried out by Student's "t" test after analysis of variance
People recovered of COVID-19 (cases) compared to controls: *p<0.05; ** p <0.01

Oxidant/antioxidant status markers in cases and controls: erythrocyte MDA levels increased very significantly in the case group compared to controls (p=0.009**) [Table/Fig-4]. In addition, erythrocyte catalase activity decreased significantly in the cases compared to the controls (p=0.0002***) [Table/Fig-5]. While erythrocyte GSH decreased very significantly in patients compared to controls (p=0.007**) [Table/Fig-5].

Markers	Controls	Cases	p-value
Plasmatic MDA (µmol/L)	1.10±0.04	0.87±0.01	0.30
Erythrocyte MDA (µmol/L)	2.43±0.04	4.50±0.06	0.009**

[Table/Fig-4]: Oxidant status (plasmatic and erythrocyte MDA) in controls and cases.
Each value represents the mean±standard deviation. MDA: Malondialdehyde. The comparison of the means is carried out by Student's "t" test after analysis of variance
People recovered of COVID-19 (cases) compared to controls: **p<0.01

Markers	Controls	Cases	p-value
Vitamin C (µmol/L)	49.82±0.90	57.56±1.30	0.47
Erythrocyte catalase activity (µL/L)	96.80±3	27.70±1.8	0.0002***
Erythrocyte GSH (mmol/L)	2.07±0.12	1.34±0.11	0.007**

[Table/Fig-5]: Antioxidant status in controls and cases.
Each value represents the mean±standard deviation. GSH: reduced glutathione.
The comparison of the means is carried out by Student's "t" test after analysis of variance
People recovered of COVID-19 (cases) compared to controls: **p<0.01, ***p<0.001

DISCUSSION

The findings in the present study showed a significant alteration in the level of biochemical parameters and oxidant/antioxidant markers. The COVID-19 has surprised every one because of its unprecedented high spread around the world. It drew the attention of researchers to seek explanations on the pathophysiology of this infection and the risk factors that can play a role in its evolution and complications. Previous studies described several metabolic alterations in patients during the infection [24]. It has been demonstrated that COVID-patients suffer from alterations in kidney function, resulting in elevated proteinuria, haematuria, and even acute kidney injury among severe cases of the infection [25-28], or slight elevation in creatinine, possibly due to renal tropism of the virus and multiple organ failure [29]. These patients presented too altered lipids levels.

It has been found that triglycerides, diglycerides and fatty acids were increased in infected people [30]. While LDL-cholesterol, HDL-C and total cholesterol levels decreased significantly in patients compared to controls. Also, it has been reported that reduced serum high density lipoproteins concentration is associated with the severity of COVID-19 [31]. On the other hand, it has been shown that reduced LDL-cholesterol levels were associated with a high rate of 30 day mortality among COVID-19 patients [32]. Inversely, HDL-C is recognised to play a scavenger for viruses, an immune modulator and a mediator of viral entry [33]. Regarding liver function, it has been shown that COVID-19 infection induced liver damage with elevated liver enzymes attributed to the immune mediated damage and direct cytotoxicity [34,35].

In the present study, the authors found that plasma levels of LDL-C and creatinine increased very significantly in the cases compared to the controls. While total cholesterol and GPT risen considerably in the case group compared to the control group. The elevation of cholesterol could be the result of the liver's inability to eliminate all the cholesterol, and this has been proven by the increase in the liver enzyme GPT. Authors believed that the increase in creatinine was due to decreased in renal filtration [34].

Besides, it is well known that SARS-CoV-2 induces an increase in ROS formation in response to high levels of infected cells, and acts on antioxidant mechanisms creating an imbalance status between oxidants/antioxidants resulting in cell damage [36]. The COVID-19 infection has distinguished itself by a multiorgan system inflammation and oxidative stress. However, the recovery and rehabilitation process in infectious disease survivors are not clearly understood. In addition, pre-existing metabolic abnormalities, such as diabetes, obesity, cardiovascular diseases and hypertension, are important risk factors for severe and critical cases of this infection [37]. Diabetes, obesity and hypertension represent the significant comorbidities in people with COVID-19 and are responsible for the progression of COVID-19 disease to very critical stages [38].

Few data have been done on the period after recovery [19]. The long term metabolic consequences for survivors of COVID-19 have been observed in patients with these diseases cited above [39]. However, no information has been given for patients without metabolic diseases who recovered from COVID-19. Therefore shows the originality of the present study on cured patients compared to controls who have never been infected with the virus.

According to the results from the present study, there was no difference in BMI and age between the two groups. The present study highlighted the persistence of metabolic abnormalities after recovery from COVID-19 infection despite the absence of any metabolic diseases. It is believed that the metabolic disturbances observed in the present study are due to the viral involvement during the infection period.

Authors have proposed the association between oxidative stress and the pathogenesis of COVID-19. The COVID-19 patients exhibited a high serum level of oxidative stress and inflammatory markers and a low serum level of antioxidants, compared to the healthy control group, especially in patients admitted to intensive care [6]. Invitro and invivo study has shown that certain viruses can modify the redox balance of the cell [40]. The onset of oxidative stress by viral infection is necessary for the activation of innate immunity through the production of cytokines [41]. In addition, the oxidative stress induced by several viruses involved in the facilitation of viral replication inside the cell [40].

The MDA is an important indicator of lipid peroxidation, especially for its stability as a final product of this chain process. Mehri F et al.,

2021 [39] found serum MDA levels nearly three times higher in the group of COVID-19 patients compared to controls. In the present study, the authors found that erythrocyte MDA levels increased significantly in the case group compared to controls.

Currently, the management of COVID-19 mainly involves nutritional support, including taking micronutrients, mainly zinc and vitamin C. The vitamin C is known for its antiviral effect against several respiratory viruses and other viruses. This effect is likely the result of an enhanced immunological response against viral infections rather than a direct effect against viral replication. More, vitamin C has potent antioxidant and anti-inflammatory effects, which reduce the risk of tissue damage from oxidative stress and suppress the excessive inflammatory response mediated by cytokines. Vitamin C also increases interferon production and stimulates lymphocyte proliferation, enhancing the host's antiviral immune response [42]. Low levels of vitamin C was found in patients with respiratory distress syndrome; this could be due to increased metabolic consumption following an increased inflammatory response, glomerular hyperfiltration, dialysis, decreased blood pressure and gastrointestinal absorption or decreased recycling of dehydroascorbate to ascorbic acid [43]. The results from the present study indicated that plasma vitamin C levels were similar between the two groups

According to research, the deficiency of antioxidant enzymes and the synergy between the SARS-CoV-2 virus and a bacterial pathogen, *Streptococcus pneumoniae* is the most likely cause of death associated with COVID-19. A decrease in catalase activity induces H_2O_2 accumulation in the body. On the other hand, the additional production of H_2O_2 by the virus increases the concentration of H_2O_2 to a critical level in the respiratory tract. The high level of H_2O_2 is responsible for lung damage [44]. The authors of the present study indicated that erythrocyte catalase activity decreased significantly in the cases compared to the controls.

The GSH represents the most important endogenous antioxidant non enzymatic of the whole organism. The GSH depletion is associated with COVID-19 mortality. At the same time, the high levels of GSH are associated with a decrease in the severity and mortality rate due to the infection [43]. In addition, it has been demonstrated that thiol can block the angiotensin converting enzyme 2, so preventing the penetration of SARS-CoV-2 into cells [44]. In the present study, erythrocyte GSH levels decreased significantly in the cases compared to the controls.

The results of the present study showed that redox status was still altered in COVID-19 patients after recovery. In these patients, erythrocyte MDA levels were high, while catalase activity and GSH levels were low compared to controls. These findings pointed out the persistent oxidative stress after recovery. This oxidant/antioxidant imbalance could be due to dysfunctional endothelial cells and inflammation state observed during COVID-19 which persisted after recovery. Authors also supposed that increased circulated lipid levels noted in the present study such as LDL-C and total cholesterol and decreased HDL-C concentration could be responsible for this oxidative stress state.

Limitation(s)

The principal limitation of the present study was the small sample size. Second, the study was carried out during confinement representing an obstacle. In addition, other oxidative stress markers and lifestyle parameters are necessary to understand the period after recovery. Thus, further studies with a huge sample size are essential, considering other potential parameters like lifestyle factors, type of treatment during the infection and the duration of the disease.

CONCLUSION(S)

In conclusion, the present study results confirmed metabolic alterations and oxidative stress persistence in COVID-19 patients after recovery. These alterations should not be neglected and should be supported by adequate treatment. Antioxidant supplementation is also recommended to improve redox status and reduce oxidative stress after recovery.

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