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Involvement of oxidative stress in post-acute sequelae of COVID-19: clinical implications

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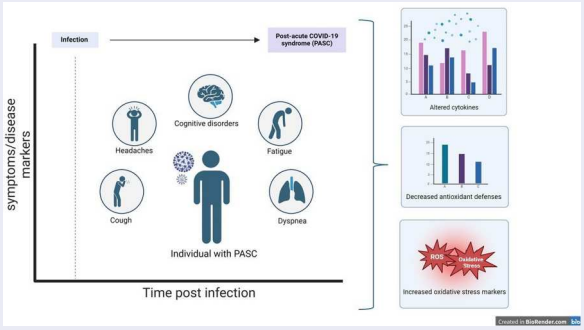
ABSTRACT

Oxidative stress (OS) plays a key role in the pathophysiology of COVID-19 and may be associated with sequelae after severe SARS-CoV-2 infection. This study evaluated OS and inflammation biomarkers in blood from individuals with post-acute sequelae of COVID-19 (PASC). 64 male and female participants were distributed into three groups: healthy individuals (n = 20), acute COVID-19 patients (symptoms for <3 weeks, n = 15), and PASC patients (symptoms for >12 weeks, n = 29). Analyses included inflammatory cytokines, myeloperoxidase (MPO) activity, and OS markers, such as superoxide dismutase (SOD), catalase (CAT), glutathione *S*-transferase (GST), gamma-glutamyl transferase (GGT), reduced glutathione (GSH), uric acid (UA), thiobarbituric acid reactive substances (TBARS), and protein carbonyls (PC). Individuals with PASC showed increased IL-6 and IL-8. Both COVID-19 groups exhibited decreased SOD and CAT. GST decreased only in the acute group. Elevated GGT and GSH were found in the PASC group. High UA levels were observed in PASC individuals. There were no changes in TBARS values in the PASC group. However, PC concentrations were elevated only in this group. Correlations were identified between inflammatory markers and OS parameters. These findings suggest that individuals with PASC pronounced OS, which potentially exacerbates disease complications. Monitoring OS biomarkers could aid in patient prognosis and management.



KEYWORDS

SARS-CoV-2; long-COVID; chronic COVID syndrome; reactive oxygen species; cytokines; inflammation; oxidative stress; antioxidants

1. Introduction

On December 31, 2019, the World Health Organization (WHO) was notified of multiple cases of pneumonia in the city of Wuhan, China, caused by a new strain of coronavirus called SARS-CoV-2 [1]. Coronavirus disease 2019 (COVID-19) presents with a variable clinical spectrum, potentially causing mild, moderate, or severe symptoms [2].

Acute SARS-CoV-2 infection triggers a complex response in the organism, inducing oxidative stress (OS) through an imbalance in the redox state and promoting systemic inflammation and deregulation of the renin–angiotensin–aldosterone system, ultimately exacerbating inflammatory responses and oxidative damage [3,4]. Recent studies demonstrated that patients with more severe forms of the disease exhibit intense inflammatory responses, evidenced by elevated levels of pro-inflammatory cytokines, increased OS, and reduced antioxidant defenses [3,5]. Decreased thiol/disulfide ratio of plasma proteins occurs mainly in inflammatory diseases, which appears to be also associated with severe complications of COVID-19 [6].

Individuals who survive the disease, even those with moderate cases, may develop post-acute sequelae of COVID-19 (PASC), also known as chronic COVID syndrome or long COVID [7]. This condition is characterized by a variety of symptoms that persist for weeks or months [8]. Fatigue represents the most prevalent symptom, commonly accompanied by headaches, dyspnea, cognitive dysfunction,

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impaired capacity to perform daily activities and work, loss of taste and smell, fever, and cough [8].

It has been reported that some viral infections for example, infection with the hepatitis C virus (HCV) and the human immunodeficiency virus (HIV), can result in persistent symptoms, such as fatigue, joint and muscle pain, and liver complications [9–11] Likewise, persistent OS may play a crucial role in maintaining prolonged PASC symptoms. Chronic inflammation associated with prolonged OS can perpetuate cellular damage [12] and contribute to persistent fatigue, respiratory difficulties, and other clinical manifestations observed in patients with this syndrome [8]. Furthermore, evidence indicates that OS can negatively affect the vascular system, contributing to endothelial dysfunction and potentially aggravating the cardiovascular complications associated with PASC [13].

Research on the association of OS and PASC is scarce. Understanding the role of the oxidative state in COVID-19 is essential to develop effective and safe management strategies for both the acute phase of infection and the long-term challenges that many individuals face. This study aimed to characterize the antioxidant status, OS, and inflammation biomarkers in individuals with PASC.

2. Materials and methods

2.1. Study design

In a recent investigation conducted by our laboratory, inflammatory cytokines and OS biomarkers were evaluated in blood samples from patients with acute SARS-CoV-2 [3]. The findings were correlated with disease severity using a protocol and experimental design similar to that of the current study. Here, inflammatory cytokines and OS biomarkers were evaluated in the blood of PASC patients.

Patients were recruited from the Specialized Center (*Centro Especializado em Reabilitação da Associação de Pais e Amigos dos Excepcionais* – CER/APAE), Campo Grande, MS, Brazil. Sociodemographic, clinical, and laboratory data were collected. Individuals with a laboratory diagnosis of SARS-CoV-2 infection were included in the study and categorized by symptom duration. A group of healthy volunteers with normal inflammatory markers was also included. The study protocol was approved by the Human Research Ethics Committee at the Federal University of Mato Grosso do Sul (UFMS), Brazil, according to national and international guidelines on research involving human subjects (National Health Council Resolution No. 1996) (CAAE protocol No. 37596720.7.0000.0021).

The sample comprised 64 male and female subjects. Participants were distributed into three groups: Group 1, healthy subjects ($n = 20$); Group 2, patients with acute COVID-19 (symptoms for <3 weeks, $n = 15$); and Group 3, patients with PASC (symptoms for >12 weeks, $n = 29$). Inclusion criteria were as follows: individuals aged between 18 and 40 years with a positive laboratory test (rapid test or molecular diagnosis) for COVID-19 as well as individuals with persistent post-COVID-19 symptoms. Exclusion criteria were as follows: patients with other systemic pathological conditions, such as infections, cancers, and autoimmune disorders; patients with clinical or biological findings suggestive of chronic kidney disease; patients who were participating in other studies; and patients taking supplements or medications with anti-inflammatory (e.g. corticosteroids) or antioxidant (e.g. vitamins E and C, acetylcysteine) action that may interfere with the results of the current study (Figure 1).

2.2. Sample preparation and equipment

Blood was collected from the antecubital vein into chilled tubes with EDTA as an anticoagulant and without EDTA for serum collection. Plasma and erythrocytes were separated by refrigerated centrifugation of whole blood at $2500 \times g$ for 10 min. Hemolysates for analysis of CAT (catalase), SOD (superoxide dismutase), and GST (glutathione S-transferase) were prepared by washing erythrocytes twice with saline solution, followed by centrifugation ($5000 \times g$ for 3 min), and subsequent freezing and thawing procedures. After a final centrifugation ($5000 \times g$ for 5 min), hemolysate supernatants were stored at −80 °C, except for plasma samples used for analysis of MPO (myeloperoxidase), GGT (gamma-glutamyl transferase), TBARS (thiobarbituric acid reactive substances), and PC (protein carbonyls). For these analyses, plasma samples were stored in liquid nitrogen (−170 °C) until use. Whole blood aliquots were precipitated in 12% trichloroacetic acid (TCA) (1:4 v/v) and immediately stored in liquid nitrogen for analysis of GSH (reduced glutathione) content. Serum samples were used for cytokine and uric acid quantification. All stages of blood sample collection and processing were performed quickly to avoid possible degradation or oxidation of analytes present in the sample. All groups were collected during the same period and in pairs to standardize the time between collection, processing and analysis between groups. All analyses were conducted in duplicate or triplicate (TBARS assays). Spectrophotometric and flow cytometric analyses were performed using a microplate reader (Thermo Fisher Scientific Oy®) and flow cytometer (CytoFLEX Flow Cytometer – Beckman Coulter©), respectively.

2.3. Cytometric bead array (CBA)

Serum concentrations of cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12, and TNF), were measured using the BD™ Cytometric Bead Array (CBA) Human Inflammatory Cytokines multiplex kit (BD Biosciences®, USA), following the manufacturer's protocol. Serum samples were gently thawed on ice and homogenized. The pool containing the six cytokine-binding beads was prepared and distributed into microtubes to then add the standard curve (0–5,000 pg dL^{-1}) or plasma samples. Detection reagent containing the fluorophore phycoerythrin (PE) was added to the mixture, and the tubes were incubated with protection from light for 3 h at room temperature. After the incubation, the samples were washed with collection, centrifuged at 200 *g* for 5 min. The supernatant was discarded and the pellet was resuspended with 300 µL of collection to then be read on the flow cytometer. The data obtained from the standard curve were used to define the cytokine concentrations of the samples. Data analysis was performed using FlowJo software version 10.8 (BD Biosciences®, USA), and cytokine concentrations were expressed in pg dL^{-1}.

2.4. Myeloperoxidase assay

Myeloperoxidase (MPO) activity was assessed spectrophotometrically [14]. Samples were thawed at room temperature,

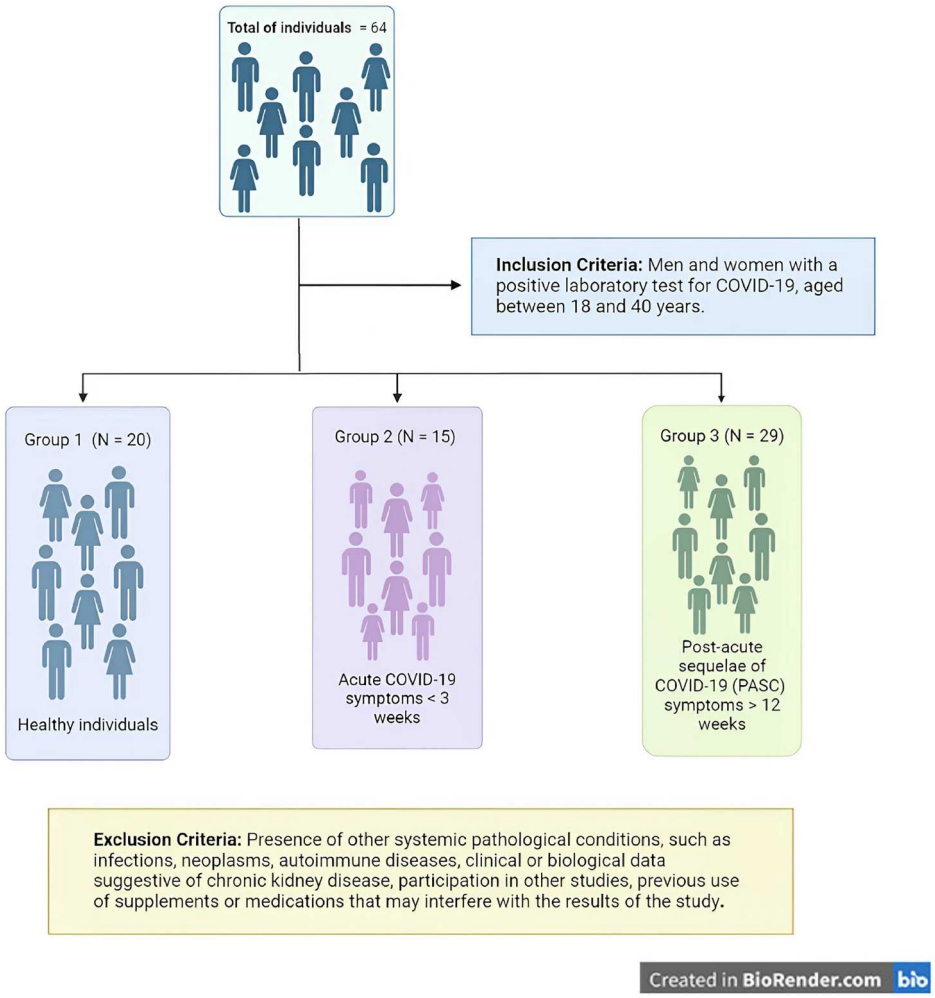

Figure 1. Study design and eligibility criteria. Image created using Biorender® (https://biorender.com/).

and 20 µL was transferred to the wells of a microplate. The biochemical reaction was initiated by adding 150 mL of a solution containing 165 mL of o-dianisidine-2-HCl, 50 mL of 30% H_2O_2, distilled H_2O, and 50 mM NaH_2PO_4. After 15 min of incubation at room temperature, the enzymatic reaction was halted with the addition of 15 mL of 1% sodium azide. Optical density was measured at 450 nm using a microplate reader. The results were compared against a standard curve of MPO activity (0.7–140 mU mL^{-1}). The equation [$y = (X - 0{,}03014)/0{,}0009545$] obtained from the standard curve was used to calculate MPO activities. MPO values are expressed in mU mL^{-1}.

2.5. Antioxidant enzyme assays

Catalase (CAT) activity was assessed by monitoring its reduction in a freshly prepared solution of 10 mM hydrogen peroxide (H_2O_2) at 240 nm [15]. Superoxide dismutase (SOD) activity was measured at 480 nm based on the epinephrine autoxidation method [16], with some modifications [17]. Glutathione *S*-transferase (GST) activity was determined at 340 nm using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate and 0.15 M GSH [18]. Gamma-glutamyl transferase (GGT) activity was measured spectrophotometrically at 405 nm using a diagnostic kit (Analisa®). SOD, CAT, and GST activities are expressed per mL whole blood as USOD mL^{-1}, mmol min^{-1} mL^{-1}, and µmol min^{-1} mL^{-1}, respectively. GGT values are expressed as UL^{-1} plasma.

2.6. GSH assay

Reduced glutathione (GSH) levels were assessed at 412 nm [19] using DTNB reagent (2.5 mM 2-dithionitrobenzoic acid). GSH concentrations were calculated using a molar extinction coefficient (ε) of 14.1 $mmol^{-1}$ cm^{-1} and as expressed as µmol mL^{-1} whole blood.

2.7. Determination of serum uric acid

Serum total uric acid concentration was assessed spectrophotometrically using a commercial kit (Analisa®). The method is based on the oxidation of total uric acid by uricase, leading to the formation of a red chromophore that can be measured at 505 nm. Results are reported in mg dL^{-1}.

2.8. Lipid peroxidation assay (TBARS levels)

Plasma lipid peroxidation was determined at 535 nm, based on the reaction of oxidation products, particularly malondialdehyde (MDA) with thiobarbituric acid (TBA) forming TBA Reacting Substances (TBARS) [20]. Lipoperoxidation levels are expressed in nmol mL^{-1}

2.9. Protein carbonyls assay

Protein carbonyls (PC) content was determined using DPNH reagent (10 mM 2,4-dinitrophenyl-hydrazine in 2.0 N HCl),

and the absorbance was measured at 360 nm [21]. The results were normalized by the total protein content in each sample. The total protein concentration measurement was determined using an albumin standard curve (0-1.0 mg mL^{-1}) [22]. PC content is expressed in mmol mg^{-1} protein.

2.10. Statistical analysis

The normality distribution of data sets was tested using Kolmogorov–Smirnov test. For non-normally distributed data, comparisons between multiple groups were conducted using the Wilcox test and Kruskal–Wallis test, followed by the Dumus post-test. For data that showed normal distribution, the comparison was performed using analysis of variance (ANOVA), followed by the Tukey–Kramer test at a level of significance of at least $p < 0.05$. Correlations (r) were assessed using Pearson's correlation analysis. All analyses were performed using GraphPad Prism software version 10.1.2.

3. Results

There were significant differences in inflammatory cytokines among acute COVID-19 and PASC individuals (Figure 2(A)). Serum IL-1β and IL-6 values were elevated in PASC patients (144,3% and 184.6%, respectively) compared with healthy individuals. Individuals with acute (Group 2) SARS-CoV-2 infection showed decreases of 95.0% serum IL-8 concentration compared with healthy individuals (Group 1).

As for IL-10, the PASC group exhibited an increase of 150.0% compared with the control. No significant differences were observed in serum IL-12 and TNF levels compared with the control group. MPO values were elevated in individuals with acute COVID-19 (13.4%) and PASC (11.1%) (Figure 2(B)).

SOD activity decreased in individuals with acute COVID-19 and PASC (52.3% and 33.8%, respectively) compared with healthy subjects (Table 1). CAT activity showed a similar decrease in individuals with acute COVID-19 (64.0%) and PASC (58.4%) compared with healthy controls. GST activity decreased by 41.0% in the acute COVID-19 group compared with the healthy group, which did not differ from the PASC group. Unlike other enzymes, GGT was increased in individuals with PASC (186.0%) and decreased in individuals with acute COVID-19 (62.7%) compared with healthy individuals. Similarly, GSH contents were considerably lower in individuals with acute COVID-19 (96.0%) compared to the control. The UA levels of PASC patients were considerably lower (13.0%) than those of healthy individuals.

TBARS concentrations were 45.0% higher in Group 2 and 37.0% higher in Group 3 than in Group 1 (Figure 3(A)). However, PC levels were considerably elevated only in Group 3 (20.0%) in comparison with Group 1 (Figure 3(B)).

Several strong and very strong correlations were detected between OS markers and pro-inflammatory cytokine levels. Very strong positive correlations ($r = +0.971/+0.997$) (Figure 4(A)) and very strong negative correlations ($r = -0.961/-0.999$) (Figure 4(B)) were observed between the levels of the pro-inflammatory cytokines IL-1β, IL-6, IL-10, IL-12, and GGT. Furthermore, strong positive correlations ($r = 0.700$-0.900) were observed between PC and MPO levels (Figure 3(A)). Interestingly, these cytokines showed very strong positive correlations ($r = +0.971/+0.997$) with uric acid levels. IL-8 showed very strong negative correlations ($r = -0.961/-0.999$) with TBARS. IL-6 exhibited a moderate correlation ($r = 0.500/0.700$) with PC values.

4. Discussion

Recent studies have highlighted the prevalence of persistent symptoms in individuals who contracted mild forms of COVID-19 [23,24]. A review conducted by [8] showed that 10% to 35% of COVID-19 patients continued to face symptoms, such as fatigue and shortness of breath, for more than four weeks after the initial infection [8]. Additionally, related studies have demonstrated that individuals suffering from persistent post-COVID-19 fatigue exhibit increased formation of reactive oxygen species (ROS), indicating a state of OS [25].

A previous study by our research group found that elevated production of cytokines, was correlated with COVID-19 severity and progression [3]. However, in the present study, these cytokines exhibited diverse patterns in individuals with long COVID. Overall, serum levels of the inflammatory cytokines were altered in individuals with PASC compared to a group of healthy individuals (Group 1) and patients with acute COVID-19 (Group 2) (Figure 2(A)).

Pro-inflammatory cytokines IL-1β and IL-6 showed increased serum expression in the long COVID group than in control and acute COVID-19 groups. A recent systematic review highlighted that elevated levels of IL-6 after SARS-CoV-2 infection are associated with prolonged persistence of symptoms, suggesting its potential use as a prognostic biomarker for the disease [26]. Furthermore, [27] demonstrated that increased IL-1β and IL-6 levels in bronchoalveolar lavage fluid are associated with chronic lung disease in preterm infants. These findings indicate that these pro-inflammatory cytokines play a crucial role in inflammatory response and immune regulation, and their elevated levels may reflect the presence of chronic inflammation in affected individuals.

On the other hand, IL-10 is a cytokine that exerts pleiotropic effects in regulating the immune system and inflammatory response [28]. Recent studies showed that individuals with PASC who presented persistent neurological symptoms, such as memory/concentration, vision, and balance issues, expressed increased serum levels of IL-10 and IL-6 [29,30]. Similarly, in this study, the PASC group exhibited elevated IL-10 levels compared to the control group, corroborating previous findings and suggesting an association between elevated IL-10 and the severity and duration of symptoms (Figure 2(A)).

Interleukins are essential proteins for communication between different cells of the immune system, coordinating the inflammatory response, especially during SARS-CoV-2 infection [31]. Among them, IL-8 stands out for its ability to attract neutrophils to infection sites [32]. Once recruited, activated neutrophils release various enzymes, including MPO [33]. This enzyme is responsible for forming structures that help capture and eliminate invading microorganisms, such as SARS-CoV-2 [34]. However, during exacerbated inflammation, this enzyme produces hypochlorous acid (HClO), responsible for the irreversible modification of proteins and lipids. HClO also raises low-density lipoprotein levels, contributing to OS and chronic inflammation [35,12].

Increased MPO oxidative activity has been associated with severe progression of various conditions, such as acute

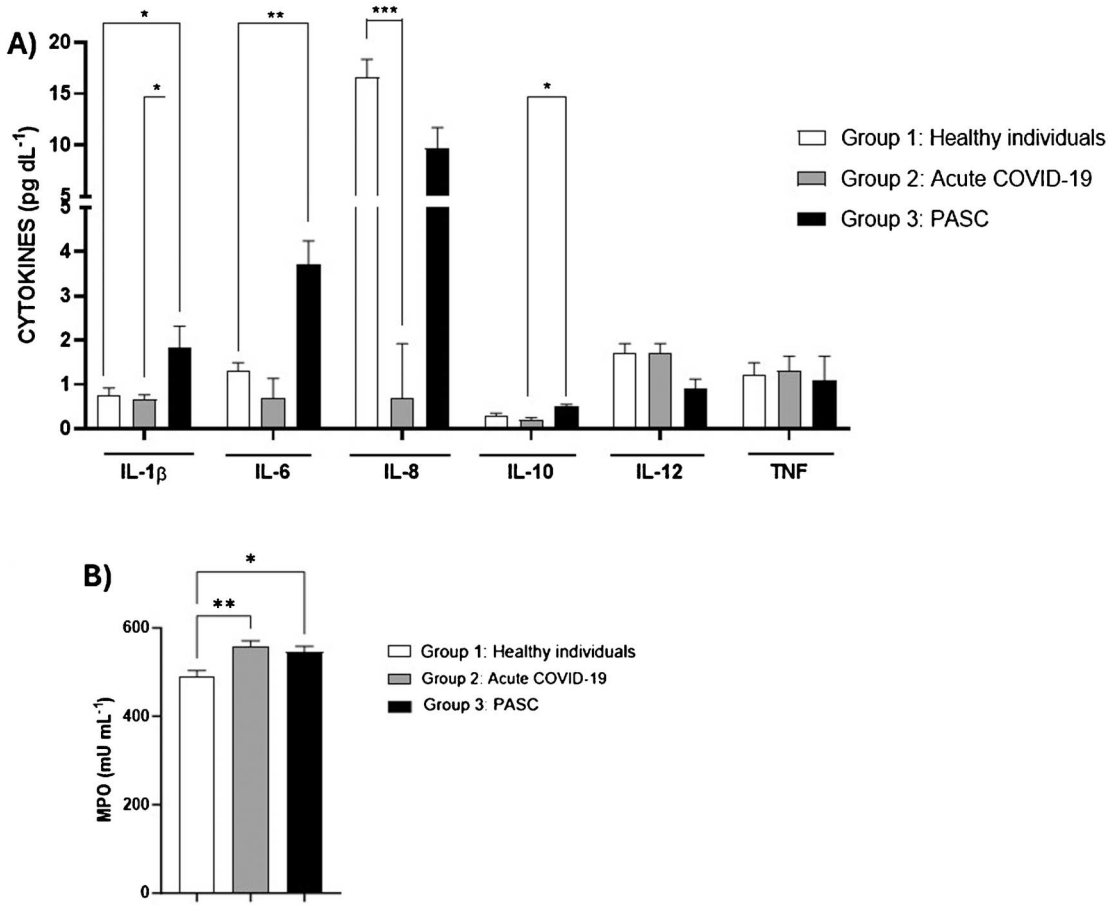

Figure 2. Inflammatory markers. (A) Cytokine (IL-1β, IL-6, IL-8, IL-10, IL12, and TNF) concentration and (B) myeloperoxidase (MPO) activity in serum samples of healthy individuals, patients with acute COVID-19, and patients with post-acute sequelae of COVID-19. (*), (**), and (***) represent significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. IL-1β and MPO were analyzed by ANOVA test; Other results were analyzed by Kruskal–Wallis test.

coronary syndrome [36], chronic obstructive pulmonary disease [37], hepatitis C [38] and COVID-19 [3,34]. Recently, [39] evaluated the levels of MPO, MDA, PC, glutathione peroxidase (GPx), and nitric oxide (NO) in individuals with PASC presenting neuropsychiatric symptoms in comparison to a control group [39]. The authors observed that deficiencies in antioxidant defenses, may play a crucial role in maintaining long COVID symptoms. They also identified associations between elevated MPO levels, OS markers, and persistent symptoms similar to those found during the acute infection phase [39].

Table 1. Antioxidant defenses.

Antioxidant defense	Group 1	Group 2	Group 3
SOD (USOD mL^{-1})	132.21 ± 6.66	58.70 ± 3.59 ***	81.56 ± 5.54***
CAT (mmol min^{-1} mL^{-1})	237.06 ± 45.09	85.24 ± 20.76*	98.51 ± 18.31*
GST (μmol min^{-1} mL^{-1})	9.05 ± 0.77	5.31 ± 0.81**/##	9.04 ± 0.67
GGT (U L^{-1})	23.99 ± 2.10	8.93 ± 2.39 ***	68.53 ± 9.37***
GSH (μmol mL^{-1})	0.08 ± 0.006	0.002 ± 0.006***/##	0.06 ± 0.009
Uric acid (mg dL^{-1})	1.32 ± 0.40	1.27 ± 0.43#	1.50 ± 0.04**

Enzymatic activity of superoxide dismutase (SOD), catalase (CAT), glutathione *S*-transferase (GST) in hemolysates, and gamma-glutamyl transferase (GGT) in serum, as well as contents of reduced glutathione (GSH) and uric acid in blood acid extract and plasma, respectively. Group 1, healthy subjects (control); Group 2, individuals with acute COVID-19; Group 3, individuals with post-acute sequelae of COVID-19 (PASC).

Note: (*), (**), and (***) indicate significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, compared to Group 1 (control). (#), (##) and (###) indicate significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively, compared to Group 3. SOD, CAT and GGT were analyzed by Kruskal–Wallis test; Other results were analyzed by ANOVA Test.

The results obtained in this study corroborate the literature, as significant differences were observed between individuals with PASC (Group 3), healthy subjects (Group 1), and individuals with acute COVID-19 (Group 2) (Figure 2(B)). However, there were no significant differences between individuals of Groups 2 and 3 (Figure 2(B)). These findings suggest an association between MPO activity, exacerbated inflammation, and persistent symptoms.

Several studies demonstrated an increase in inflammatory enzymes and a decrease in antioxidant defenses during the peak of COVID-19 [40–44]. Such antioxidant depletion is associated with OS and endothelial dysfunction, contributing to the severity of SARS-CoV-2 infection [45]. Additionally, a decrease in antioxidant defenses has been related to chronic infections, leading to a persistent state of OS [46–48].

In the present study, individuals from Group 2 and Group 3 showed alterations in enzymatic and non-enzymatic antioxidant defenses (Table 1). SOD and CAT levels were decreased in both groups compared with healthy individuals. Similar decreases in the activity of these enzymes have been reported for conditions of chronic inflammation, such as HCV and HIV infections, whereby continuous ROS production exceeds the endogenous antioxidant capacity [47,48]. Additionally, the prolonged immune response in COVID-19 may increase the demand for antioxidants, resulting in their depletion [49]. Interestingly, very strong positive correlations ($r = 0.900/1.000$) were found between SOD and CAT and interleukins, such as IL-8 (Figure 4(A)). These findings suggest that a deficiency in these enzymes is linked to persistent

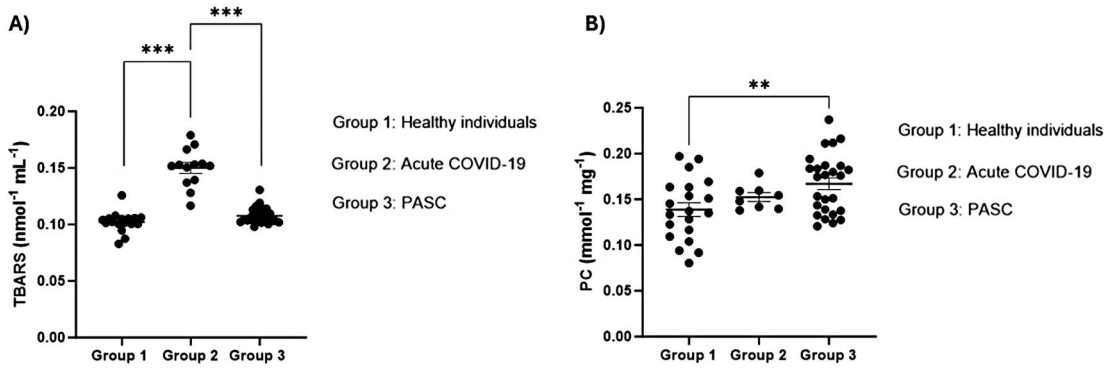

Figure 3. Markers of oxidative damage. (A) Plasma contents of thiobarbituric acid reactive substances (TBARS) and (B) protein carbonyls (PC). (**) and (***) indicate significant differences at $p < 0.01$ and $p < 0.001$, respectively. Results were analyzed by ANOVA test.

immunoinflammatory activation and OS, characterizing a possible underlying mechanism in the pathophysiology of long COVID.

Of note, significant differences in GST, GGT, and GSH levels were observed across the different phases of COVID-19. Previous studies investigated changes in the levels of these enzymes in various pathological conditions, including viral infections, such as HCV and HBV [50,51], and acute respiratory syndromes, such as acute respiratory distress syndrome [52]. A significant decrease in GST activity was observed in the acute phase of COVID-19. This finding suggests an overload of the body's antioxidant systems, owing to the excessive production of ROS during viral replication. However, in the post-acute syndrome, GST levels returned to values similar to those of healthy individuals.

Another result that corroborates the data from this study, and consistent with the GSH assay results, is the enzymatic activity of GGT (Table 1), which showed an increase in individuals with PASC compared to the control group and acute COVID-19. Classically, GGT is considered a marker of liver function and is located on the outer surface of the plasma membrane, where it facilitates the synthesis of the tripeptide GSH. GGT initiates the degradation of extracellular GSH by hydrolysis of the γ-glutamyl-cysteine bond of the tripeptide where it facilitates the restoration of intracellular GSH levels [11,53].

GSH content showed a significant decrease in the acute infection phase compared to the post-acute phase and healthy individuals (Table 1). Furthermore, this enzyme had a very strong positive correlation ($r = 0.900/1.000$) with GST levels (Figure 4(A)). During the acute phase of COVID-19, the body experiences exacerbated OS, resulting in a significant decrease in GST and GSH levels [54] as observed in Group 2. However, as the infection progresses to the post-acute syndrome, the body restores GSH concentrations; with this, OS becomes milder but remains persistent. This pattern was also observed for uric acid values, which were restored in the post-COVID-19 phase. GSH and uric acid play important roles as ROS scavengers; for instance, uric acid is responsible for approximately 60% of plasma ROS elimination [55].

The restoration of GSH and uric acid levels after the acute phase suggests the body's attempt to rebalance the redox state, indicating that these antioxidants continue to play a crucial role in neutralizing the remaining ROS. However, uric acid elevation may also reflect the presence of tissue ischemia and OS, as observed in pulmonary hypertension [56]. The persistence of OS, despite the partial recovery of the antioxidant systems, is evidenced by other markers of oxidative damage.

Recent studies from an Italian group suggested that some viral infections can alter thiol redox balance in lung tissue and lining fluids, which may influence the risk of infection as well as the host's ability to respond to the infectious agent and avoid serious complications [57]. Furthermore, *in vitro* work using SARS-CoV2-infected Vero E6 cells showed that the virus depleted intracellular thiol content, particularly GSH levels, while increased levels of oxidized glutathione (GSSG) accompanied by protein glutathionylation were also detected [58].

Although TBARS levels were higher in Group 2 patients, the increase in Group 3 was not significant in comparison to healthy individuals (Figure 3(A)). On the other hand, PC levels were elevated only in Group 3 compared to healthy individuals (Figure 3(B)). These findings suggest significant oxidative damage to proteins in Group 3 patients, even after recovery from the acute infection, contributing to

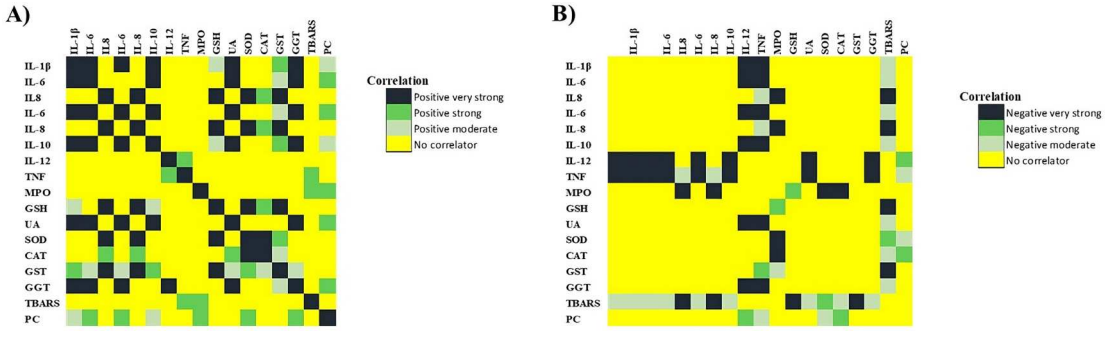

Figure 4. Pearson's correlation matrix. (A) Correlations positive and (B) Correlations negative, both classified as moderate. ($r = 0.500–0.700$), strong ($r = 0.700–0.900$), or very strong ($r = 0.900–1000$).

cellular dysfunction and persistent symptoms, as commonly observed in individuals with PASC. A mouse study conducted by [59] demonstrated that, despite significant lung inflammation during influenza virus infection, TBARS levels were not significant. However, high PC levels indicated considerable oxidative damage to proteins, suggesting that chronic viral infections may lead to increased protein oxidation without a corresponding increase in lipid peroxidation [59].

Continuous OS and chronic inflammation may be associated with the persistence of elevated PC levels in various chronic conditions, including chronic obstructive pulmonary disease and viral infections such as HCV and HIV infection [47,48,60]. In the case of COVID-19, the maintenance of high PC levels in the PASC group suggests a failure of the body's ability to restore redox balance and repair protein damage, resulting in prolonged cellular dysfunction. Additionally, PC was strongly and positively correlated with uric acid, MPO, GGT, and IL-6 ($r = 0.700/0.900$) (Figure 4(A)), as well as strongly negatively correlated with IL-12 ($r = -0.700/-0.900$) (Figure 4(B)).

Proteins are primary targets of HOCl produced by MPO resulting in the formation of several oxidative by products [61]. The chlorination of tyrosine and the formation of chloramines and carbonyls are some of the main modifications induced [61]. A study by Yan and collaborators [62] demonstrated that the carbonyl content in proteins increases with oxidation by hypochlorite, but this effect can be inhibited in a concentration-dependent manner in the presence of HOCl scavengers. Thus, compounds such as lipoic acid, cysteine, and GSH, in addition to antioxidants such as uric acid and ascorbic acid, can play an essential role in preventing the formation of carbonyls in proteins [62].

In summary, various studies have demonstrated the complexity of long COVID, characterized by persistent symptoms affecting a significant portion of individuals after acute COVID-19 infection [63,64]. These symptoms are accompanied by an increase in ROS formation, indicating a persistent state of OS [65]. Analysis of the levels of the inflammatory cytokines revealed significant alterations, indicating a prolonged inflammatory response that may be associated with symptom severity and duration. Additionally, increased MPO activity and elevated PC levels in long COVID patients suggest continuous oxidative damage to cellular proteins, potentially contributing to persistent cellular dysfunction. The decrease in antioxidant defenses during the acute phase and their restoration in the post-acute phase probably indicate a compensatory immune response to the redox state, although OS persists. These findings reinforce the need for a deeper understanding of the underlying pathophysiological mechanisms of long COVID to develop effective management and treatment strategies.

One of the main limitations of this study is the number of participants, which made it impossible to stratify individuals into different groups based on severity and stage of infection. The number limited of subjects in each group reduces capacity to detect differences in some parameters among groups and limits the generalizability of the findings. Furthermore, this limitation makes it difficult the categorization of patients with PASC according to the types of symptoms they exhibit, which may hinder a more detailed understanding of the heterogeneity of this study. Studies associating different symptoms with OS are scarce in PASC. However, this study can be a starting point for studies categorizing each symptom. While the results provide valuable insights, further studies with larger cohorts are necessary to validate these observations, allowing for a more comprehensive classification of PASC symptoms.

Another limitation of the present study is the lack of macrophage phenotyping to assess the shift from a pro-inflammatory (M1) to an anti-inflammatory (M2) phenotype, which is closely associated with increased IL-10 levels. The complexity of accurately characterizing macrophage phenotypes in clinical studies, given their plasticity and the dynamic nature of their activation states, also represents a challenge. Future studies addressing macrophage phenotyping in these patients could help clarify the role of IL-10 in modulating macrophage function during disease progression and recovery.

Potential therapeutic strategies are being investigated to mitigate the effects of long COVID. One such approach might be the use of *N*-acetylcysteine (NAC), which has shown potential in reducing OS and inflammation under various conditions [58,66–68]. Other antioxidant and anti-inflammatory approaches are also being explored, underscoring the importance of targeted therapies for managing persistent symptoms which could improve the quality of life of patients [69].

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability

The data that support the findings of this study are available on request from the corresponding author, RTP or EBP. The data are not publicly available due to privacy.

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