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Original Article

Oxidative stress in diverse clinical conditions of SARS-CoV-2 Cuban hospitalized patients

[Estrés oxidativo en diferentes condiciones clínicas de pacientes cubanos hospitalizados con SARS-CoV-2]

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Abstract

Context: COVID-19 related to SARS-CoV-2 infection generates inflammation with increased reactive oxygen species production. Drug treatment and others factors could influence systemic oxidative stress during pathogenic insult.

Aims: To determine the redox status in COVID-19 patients with different clinical conditions and explore the relationship between redox and hematological hemochemical variables.

Methods: In this comparative longitudinal study, blood samples were drawn from 160 individuals divided into four groups: COVID-19 asymptomatic, COVID-19 symptomatic (low and moderate symptoms), COVID-19 convalescent, and presumable healthy subjects. Demographic, redox, hematological, and hemochemical indices were assessed. Statistical analyses compared the median values of each variable and explored individual, simultaneous indices, and multivariate alteration.

Results: Relative to the healthy group, acute COVID-19, and convalescent groups had significant differences in global damage indices and antioxidant status ($p<0.05$). The convalescent group showed significantly higher damage (malondialdehyde, advanced oxidation protein products, nitric oxide) and lower antioxidant enzymatic activities and glutathione concentration compared to other groups ($p<0.05$). Global modification of redox indices showed that more than 80% of studied individuals in acute conditions had simultaneous detrimental differences compared to a healthy status. The discriminant analysis permitted obtaining two canonical functions ($p< 0.05$) that reflect 98% of redox variables with 95% of variances with successful case classifications.

Conclusions: These results corroborate that oxidative stress occurred in different COVID-19 and post-acute conditions with different molecular alterations of redox indices. Redox diagnosis should be considered in early diagnosis and treatment of infection, which would be worthwhile to conduct a more comprehensive study and management of disease evolution.

Keywords: antioxidant status; COVID-19; oxidative stress; oxidative damage; SARS-CoV-2.

Resumen

Contexto: El COVID-19 relacionado con la infección por SARS-CoV-2 genera inflamación con aumento de la producción de especies reactivas del oxígeno. El tratamiento farmacológico y otros factores podrían influir en el estrés oxidativo sistémico durante el insulto patogénico.

Objetivos: Determinar el estado redox en pacientes con COVID-19 con diferentes condiciones clínicas y explorar la relación entre las variables redox y hemoquímicas.

Métodos: En este estudio longitudinal comparativo, se extrajeron muestras de sangre de 160 individuos divididos en cuatro grupos: COVID-19 asintomáticos, COVID-19 sintomáticos (síntomas bajos y moderados), COVID-19 convalecientes y sujetos presuntamente sanos. Se evaluaron los índices demográficos, redox, hematológicos y hemoquímicos. Los análisis estadísticos compararon los valores medios de cada variable y exploraron las alteraciones en los índices individuales, simultáneos y multivariadas.

Resultados: En relación con el grupo sano, los grupos COVID-19 agudo y convaleciente presentaron diferencias significativas en los índices de daño global y en el estado antioxidante ($p<0,05$). El grupo convaleciente mostró un daño significativamente mayor (malondialdehído, productos proteicos de oxidación avanzada, óxido nítrico) y menores actividades enzimáticas antioxidantes y concentración de glutatión en comparación con los otros grupos ($p<0,05$). La modificación global de los índices redox mostró que más del 80% de los individuos estudiados tenían diferencias perjudiciales simultáneas en comparación con el estado saludable. El análisis discriminante permitió obtener dos funciones canónicas ($p< 0,05$) que reflejan el 98% de las variables redox con el 95% de las varianzas con clasificaciones de casos acertadas.

Conclusiones: Estos resultados corroboran que el estrés oxidativo se presentó en diferentes COVID-19 y condiciones post-agudas con diferentes alteraciones moleculares de los índices redox. El diagnóstico redox debe ser considerado en el diagnóstico y tratamiento precoz de la infección, lo que valdría la pena para realizar un estudio y manejo más exhaustivo de la evolución de la enfermedad.

Palabras Clave: daño oxidativo; COVID-19; estado antioxidante; estrés oxidativo; SARS-CoV-2.

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INTRODUCTION

The SARS-CoV-2 infection, an outbreak discovered in Wuhan, China, resulted in a highly contagious disease that spread throughout 230 countries causing global havoc, more than 300 million infections, and five million deaths globally in third/fourth waves associated with different viral variants that continue to prompt fear and misery (Chen et al., 2020a; WHO, 2020; Zhou et al., 2020).

Patients with COVID-19 (infected by SARS-CoV-2) presented symptoms such as myalgia, fever, diarrhea, fatigue, and dry cough, among others. Recently published findings evidenced that SARS-CoV-2 induced immune and inflammatory responses, which trigger both a "cytokine storm", and apoptosis of different cells through epithelial and endothelial. Afterward, abnormal T and cell macrophage responses concomitantly with vascular leakage conduce and encourage oxidative distress (ODS) status (Conti et al., 2020; Delgado-Roche and Mesta et al., 2020; Dosch et al., 2009; Ye et al., 2020).

Diverse host cellular elements (oxidant and antioxidant metabolites) have been recognized as bioactive in viral infection and are also involved in the different life cycle steps required for viral product generation. Those metabolites could interfere with cellular homeostasis, activating a stressed tone in the host cell. Overall, cellular metabolism progressively yields reactive oxygen species (ROS), as a byproduct of the physiological pathways, by compartmental enzymes located at the endoplasmic reticulum (ER), the peroxisome, or the mitochondria, and concurrently the oxidized molecules are removed to keep the equilibrium (Delgado-Roche and Mesta, 2020; Fang, 2011; Pickering et al., 2013; Sies et al., 2017). Furthermore, immunological cells generate ROS as an effector mechanism of immune response to pathogens, given their antimicrobial properties (Cecchini and Cecchini, 2020; Phaniendra et al., 2015). The interaction of the biological elements at play during stress escalation is partly understood at individual levels of biological organization, but how these horizontal and vertical responses are coordinated in time and space remains to be defined. This system uses cascading chemical reactions of short-lived small redox-active molecules at the biochemical level for sensing and signaling purposes, defined as the ''Reactive Species Interactome'' (RSI) (Pickering et al., 2013; Sies et al., 2017).

ODS is defined as an imbalance between the generation of oxidants (ROS) and the active antioxidant systems of cells, besides the capacity of damage repair pathways to contribute to an increase of oxidants that influence redox circuits. ODS may disturb a cell's normal oxidation-reduction state, impacting the adaptation-resolving oxidant tone (Palipoch and Koomhin, 2015; Pickering et al., 2013). This altered state can contribute to tissue injury or produce toxic species to all cell components affecting signaling and cellular function (Palipoch and Koomhin, 2015; Phaniendra et al., 2015).

Recent literature shows that ODS is associated with aging and several human diseases or conditions, for instance, arteriosclerosis, diabetes, eye diseases, inflammation, hypertension, cancer, skin diseases, autoimmune disorders, and infectious diseases, among others (Ceriello and Testa, 2009; Khomich et al., 2018; Mao et al., 2019; Pisoschi and Pop, 2015; Xuan et al., 2019). Lately, the ODS hypothesis has strongly motivated researchers to elucidate the role of antioxidant systems in eliminating ROS, i.e., the biochemical role of antioxidant enzymes and non-enzymatic antioxidants, and how they protect the essential biomolecules (Muralidharan and Mandrekar, 2013; Patlevič et al., 2016). Evidence has been reported about the positive effect of redox modulation related to the adaptation of cells to ODS and the capacity to improve the cellular antioxidant system response (Ahmed et al., 2017; Ifrim et al., 2014; Lee, 2018) through Nrf2 activation. Hence, the preservation of favorable intracellular and extracellular redox surroundings is critical for the host to sustain the energetic process, signaling, and comeback against infections (Liguori et al., 2018). Compared to previous studies, redox biomarkers are needed to determine metabolic status and the effect of infections on the oxidant/antioxidant systems and their relation to the clinical outcome (Amatore et al., 2015; Marrocco et al., 2017; Sebastiano et al., 2016).

COVID-19 is described as a sickness that mainly disturbs the lungs, but since the ODS generated is involved in inflammatory pathophysiology and is influenced by drug toxicity, it can also damage many other organs. These damages may increase the risk of long-term health problems possibly related to disease sequelae (Azkur et al., 2020; Gemelli Against COVID-19 Post-Acute Care Study Group, 2020; Tay et al., 2020).

The latest findings advise that 87% of COVID-19 patients, despite being negative for the viral nucleic acid assessment, still had a high level of inflammation and sustained symptoms for at least two months after infection onset and the resolution of viral compromise (Dasgupta et al., 2020; Greenhalgh et al., 2020; Kemp et al., 2020). A substantial number of patients who have been previously confirmed as positive for SARS-CoV-2 continued sick for three weeks or months after the resulting negative test. After recovery, the most

frequent persistent symptoms are joint pain, dyspnea, fatigue, and chest pain (Dasgupta et al., 2020). These events are caused in part by persistent viremia in fluids and organs other than the nasopharyngeal because of low or poor antibody response, reinfection or relapse, inflammatory and other immune responses, deconditioning, and psychological factors such as post-traumatic stress, which may all lead to and could also be interrelated to oxidative tone (Bhaskar et al., 2020; Mittal et al., 2014).

Numerous scientists have described that in a high ODS, meanly generated by sustained immune system activation, the evolution of COVID-19 infection can be encouraged in the host (Cecchini and Cecchini, 2020; Kosanovic et a., 2021; Polonikov, 2020). The redox breach could influence the modulation and cause the activation of transcriptional mediators, leading to a specific cellular process, including apoptosis and tissue impairment, which in turn may conduce to disease-related dysfunction or not (Fakhri et al., 2020; Kosanovic et a., 2021; Liguori et al., 2018).

The fact that various clinical conditions and a high mortality rate occur in patients with COVID-19 emphasizes the importance of identifying the alteration of redox biomarkers associated with diverse conditions. These situations suggest a potential amplifying mechanism involved in CoV-induced ODS. That is why the ODS characterization permitted the identification of several key targeting steps in the pathophysiology process that could bring about different outcomes (Polonikov, 2020).

MATERIAL AND METHODS

Ethical considerations

The research was performed in agreement with the Good Clinical Practice Guidelines of the International Conference on Harmonization and the principles of the Declaration of Helsinki. The exploratory research procedures were agreed upon by the Scientific and Ethical Committees of the three hospitals (by fast tract) on February 17, 2021. The results presented in this document considered the initial values of three types of patients: those included in the two clinical trials and asymptomatic patients from an IPK study. All of them were compared with a group of supposedly healthy individuals who were recruited at the IPK to carry out the exploratory correlation study. The results of the trial interventions are not reported here. The three approval documents related to the studies were issued: from Salvador Allende Hospital (CEIHSA-014-21), where mild-to-moderate symptomatic patients were admitted; from IPK, where asymptomatic patients were hospitalized (CEIPK 024-21); and from Ernesto Guevara UCI Hospital (CEIHUCI-2-21), which hospitalized convalescent patients.

The hospitals' source documents were archived in the medical recording system. Clinical results and laboratory analyses were also accessible locally. The positive subjects and convalescent status consisted of individuals whose SARS-CoV-2 infections were previously confirmed by real-time polymerase chain reaction (RT-PCR). According to the in-house agreement, post-acute COVID-19 convalescent conditions were established for those individuals with persisting symptoms after a month from a negative RT-PCR test. All recruited patients during the acute COVID-19 phase received a combination of Kaletra (Lopinavir/Ritonavir), chloroquine, and interferon α2b according to the Cuban protocol approved on April 2020 by a Designed Commission by the Ministry of Public Health. Non-probabilistic convenience selection was applied to identify the appropriate patients based on the inclusion criteria, namely history of fever, any respiratory symptoms such as cough or rhinorrhea, and male or female aged 32-85 years at the time of inclusion who did not take part in other clinical studies within the last three months. One hundred and twenty patients were enrolled and hospitalized: 40 were asymptomatic, 40 individuals presented with low or moderate symptomatic infection, and 40 had a negative RT-PCR test after 4 weeks. An exhaustive oral and written explanation of the protocol, researchers' names and institutions, potential benefits, and probable adverse effects of the interventions were described to the participants, and additional information was provided upon request. Informed participants signed a written informed consent.

Forty supposedly healthy individuals (SHI) were enrolled and evaluated as a reference group.

At baseline assessment, all patients completed anthropometric (weight, height), demographic, and age data prior to study initiations. A complete physical exam was accomplished, and data of preexistent disease (chronic heart disease, asthma, stroke, hypertension, diabetes, and arthritis, among others) and toxic habits (smoking, alcoholism) were computed. The clinician ordered specific laboratory tests related to the study design. All participants took hospitalized-adjusted diets and had limited physical activity during the study.

Biochemistry measurements of multiorgan biomarkers

One blood extraction has been considered to develop the study. After 48 hours of confirmed infection and overnight fasting (10-12 hours), venous blood sample (20.0 mL) was collected at 8:30–9:30 a.m. No

medication was used at the moment of blood extraction. After initial evaluation and physical exams following Cuban protocols, a combining therapy, which included Kaletra, chloroquine, and interferon α2b, was recommended to each positive patient. Otherwise, other therapies such as oxygenation, analgesic, and anticoagulant were encouraged throughout the disease and depending on the patients' clinical outcomes. Some hematological and chemical indices were assayed. The remaining blood was put into a dry tube for serum extraction. Sera were transferred into clean micro-tubes in aliquots for conservation after determining the proposed schedule of indices measurement.

According to international recommendations (Polonikov, 2020), redox, hematological, and chemical indices were determined using analytical methods for human diagnosis.

For assays of SOD and CAT, hemoglobin was extracted from hemolysate. For the others indices, 3 mL of serum were used. Serum samples were frozen at -70°C and conserved out of light exposure until analyses were measured out.

All redox parameters were assessed by spectrophotometric methods using Zuzi Spectro-photometer from Japan.

Glutathione determination

Serum GSH concentrations were determined using a kinetics assay with a glutathione reductase reaction (Schieber and Chandel, 2014). Autoxidation of GSH to GSSG was avoided by the addition of N-ethylmaleimide to the samples. GSH from Sigma, St. Louis, M.O., USA, was employed to produce standard curves.

Malondialdehyde determination

Malondialdehyde (MDA) concentrations were assessed with the LPO-586 kit from Calbiochem (La Jolla, C.A., USA). In this test, stable chromophore production occurred after 40 min of incubation at 45°C and was determined at a wavelength of 586 nm by ZuZi Spectrophotometer (Japan). To prevent lipid oxidation during the analysis, BHT [0.01% (v/v) of a 2% stock solution in ethanol] and EDTA (1 mM final concentration) were added to the sample before assay development. Newly prepared solutions of bis [dimethyl acetal] (Sigma, St. Louis, M.O., USA) assayed under similar conditions were considered as reference standards. MDA concentration in serum samples was obtained using the standard curve, and values were expressed as nmol/g Hb (Erdelmeier et al., 1998).

Superoxide dismutase (SOD) activity quantification

SOD activity was evaluated by a modified pyrogallol autoxidation procedure (Marklund and Marklund, 1974).

Catalase (CAT) activity quantification

CAT activity was measured according to the method of Clairborne. A molar extinction coefficient of 43.6 M^{-1} cm^{-1} and the rate of the first 30 s were utilized to calculate the CAT activity. CAT activity was expressed as U/mg Hb (Clairborne, 1986).

Advanced oxidation protein products (AOPP) determination

Serum AOPP was determined agreeing to the methods of Witko-Sarsat et al. (1998). The values were expressed as chloramine T equivalents and corrected by serum albumin concentrations.

Nitric oxide (NO)

Nitrite/nitrate levels as a measure of NO were determined by the Griess reaction after first converting nitrates to nitrites using nitrate reductase (Boehringer-Mannheim Italy SpA, Milan, Italy) (Granger et al., 1996).

Biochemical indices

Blood indices such as erythrocyte sedimentation rate (ESR), hemoglobin, and hematocrit were measured by the hematological counter MICROS 60. Others as alanine aminotransferase activities, cholesterol, creatinine, aspartate aminotransferase, and triglycerides, were performed by standard procedures in COBAS analyzer, all in a specialized laboratory at IPK hospital.

Statistical analysis

Kolmogorov- Smirnov test was applied to evaluate the normality of data, and variance homogeneity was checked by Levene s test. Means and standard deviations were calculated using descriptive statistics of continuous variables, whereas categorical variables were expressed as proportions. Comparisons between the groups with respect to the healthy group were assessed using the t-Student test for non-pared samples followed by a post hoc Newman Keuls method. A two-sided p-value less than 0.05 was considered statistically significant. All analyses were achieved using SPSS software (Version 22, SPSS Inc., Chicago, IL, USA).

Table 1. Age, gender, ethnicity, body mass index and other characteristics of studied groups in 2021.

Variables		Supposedly health	Asymptomatic	Moderate	Convalescents
N		40	40	40	40
Age		40.25 ± 5.06	43.70 ± 13.01	45.20 ± 9.46	55.60 ± 11.93
BMI (kg/m^2)		24.07 ± 3.34	25.48 ± 5.29	27.51 ± 6.42	26.33 ± 4.58
Color of skin	White	31	28	30	14
	Black	3	7	8	16
	Mixture	6	5	2	10
Gender	Male	33	24	25	36
	Female	7	16	15	4
Preexistent diseases	Hypertension	-	5	4	3
	Asthma	-	3	6	4
	Diabetes	-	4	3	4
Toxic habits	Smoking	-	5	8	7
	Alcoholism	-	6	3	4

Data are expressed as mean ± standard deviation. No significant differences were detected in the comparison between variables for the different groups ($p<0.05$). BMI: body mass index; Source: Clinical History archived in the Medical record department in each hospital; Period of hospitalization from April to September 2021.

It was examined if the values and the mean of the biochemical and hematological indices were in the reference interval reported in the literature.

Each redox, biochemical and hematological index was considered to determine how many patients had at least one deviation standard difference with respect to the healthy group values.

Simultaneous differences in some indices were also analyzed for each group. A combinatorial variable evaluates the difference in the antioxidant system and damage of biomolecules (simultaneous change in AOPP, CAT, SOD, GSH, NO, and MDA) with respect to the healthy group. For each patient, the global alteration was considered. The frequency of global alteration was described for each group. Null hypotheses test and a magnitude-based inference were developed.

An exploratory multivariate discriminate study was accomplished relating redox index values for each group. Results were presented in tables and graphically using SPSS.

RESULTS

The hospitalized patients were admitted when SARS-CoV-2 RT-PCR tests were positive (April – September 2021) due to the active screening system of transmission protocol established in Cuba since March 2020. The baseline characteristics of the 120 hospitalized subjects and the 40 healthy individuals are shown in Table 1. No statistically significant differences were detected between the groups according to demographics, gender, BMI, and the number of patients ($p>0.05$). As a real-world study, 30% of all patients had chronic conditions (asthma, diabetes, hypertension), and 30% had previous toxic habits (smoking, alcoholism). Statistical differences were not observed between groups related to chronic conditions or previous toxic habits. A major percentage of patients were older than 30 years, had white skin color, and were on long-term treatment due to a history of previous diseases. Almost all patients were on normoweigth status, and nutritional support was guaranteed and monitored by hospital specialists.

The mean value of all biochemical and redox indices evaluated in the reference and the three studied groups are provided in Table 2.

Most of the redox indices values showed a significant difference concerning the values of the healthy group. MDA (a marker of lipid peroxidation) and AOPP serum concentrations were significantly higher ($p<0.05$) in the convalescent group with respect to the healthy and the other two groups. On the other hand, the GSH levels of the convalescent group were significantly lower ($p<0.05$) with respect to the other groups. The activity of the erythrocyte antioxidant enzyme CAT was significantly higher in the studied groups with respect to the healthy ($p<0.05$). SOD showed a higher value in the convalescent group, significantly different from all other group values. Related to NO, low values were observed in the three studied groups with respect to the referential value ($p<0.05$). In the case of NO, no significant differences were detected among groups ($p>0.05$) (Table 2). No

significant differences were observed when comparing redox indices values in patients with and without health conditions (asthma, hypertension, diabetes) or previous toxic habits.

Significant differences ($p<0.05$) were observed in the mean values of hemoglobin, ALAT, UA, and TP of each COVID-19 group with respect to the healthy group. The mean values of the moderate group were the highest (Table 2).

Regarding ASAT and creatinine mean values, no significant differences in SARS CoV-2 groups were observed compared with the healthy group ($p>0.05$).

No significant differences ($p>0.05$) were found when comparing multiorgan index values in patients with and without preexistent conditions (asthma, hypertension, diabetes) or previous toxic habits. According to this finding, each group was considered representative of the studied conditions. The mean values of both parameters remaining on the interval were considered physiological-reference. Correlation analysis among redox and biochemical variables mean values revealed a significant relationship between MDA and ALAT ($r = 0.52$, $p = 0.02$), and AOPP and UA ($r = 0.39$, $p = 0.041$), suggesting subclinical liver or kidney deficiency, which in the first days of the disease probably contributed to an increase of oxidative stress. Individual analyses identified detrimental redox indices values in more than 80% of patients in each acute group related to MDA, GSH, and CAT alterations. Detrimental AOPP values were found in moderate and convalescent groups and combined with MDA, GSH, and CAT values, 62% of the patients presented alterations in the convalescent group (Fig. 1).

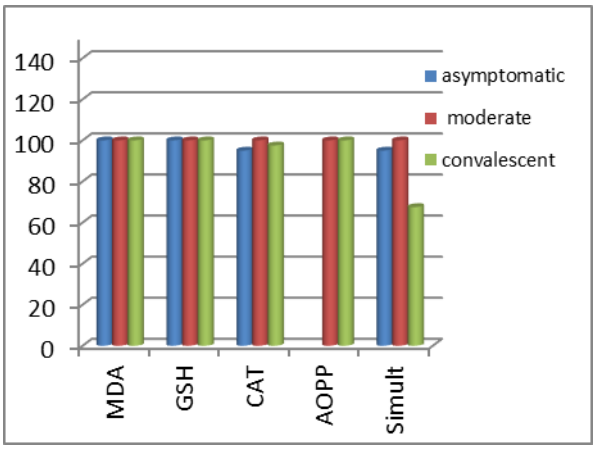

Figure 1. Number of patients that present simultaneously oxidative damage indices (AOPP, MDA), antioxidant indices (CAT, GSH), or global (Simult) markers with respect to the healthy group.

CAT: catalase; MDA: malondialdehyde; GSH: glutathione; AOPP: advanced oxidation protein product.

Simultaneous analyses permit the identification of the percent of patients who presented modification in a set of redox variables with respect to outcome (Fig. 1). The moderate group presented the highest values.

Multivariate discriminate exploration, including redox indices in each group, is shown in Fig. 2.

Discriminant analysis revealed that 95.7% of the variation between groups accounted for the two first discriminant functions ($p = 0.000$).

The biochemical redox indices were correlated in these two functions with loadings of 0.982 and 0.814, respectively.

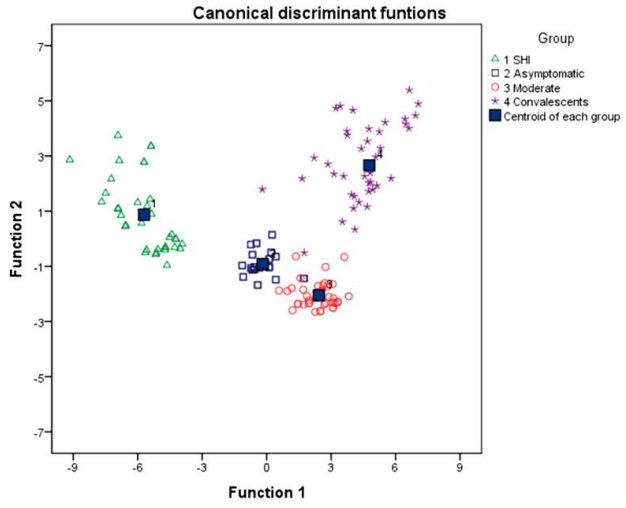

Figure 2. Canonical discriminate analysis representation related to redox indices in studied groups.

1 SHI: supposedly healthy individuals

2 asymptomatic

3 moderate

4 convalescents

5 centroid of each evaluated group

Function 1 = 0.336 MDA – 0.722 GSH + 0.605 CAT + 0.488 AOPP

Lambda de Wilks = 0.002 ($p<0.05$)

Function 2 = 0.601 MDA + 0.539 GSH + 0.341 CAT – 0.262 AOPP

Lambda de Wilks = 0.125 ($p<0.05$)

Table 2. Hematologic, hemochemical, and redox indices data in the different studied groups.

Parameter	Supposedly healthy volunteers	Asymptomatic	Moderate	Convalescents
Hematologic and hemochemical indices				
Hemoglobin (g/L) (RI: 11.0-16.0)	13.45 ± 0.64	10.35 ± 1.4[a]	12.89 ± 1.14[b]	10.6 ± 1.02[a]
Alanine amino transferase (U/L) (RI: M < 33 and F < 25)	31.48 ± 1.18	36.27 ± 3.86[a]	32.19 ± 2.40[b]	37.28 ± 2.53[a]
Aspartate amino transferase (U/L) (RI: < 42 U/L)	39.53 ± 2.15	43.45 ± 2.07	40.52 ± 1.71	42.34 ± 2.60
Creatinine (mmol/L) (RI: < 5.18)	3.86 ± 1.34	4.12 ± 1.23	3.31 ± 1.01	4.25 ± 1.46
Uric acid (µmol/L) (RI: M = 202-416 y F = 142-339)	346.5 ± 37.63	461.2 ± 22.37[a]	403.2 ± 30.2[b]	440.6 ± 37.63[a]
Total protein (g/L) (RI: 60-83)	75.7 ± 3.22	40.5 ± 1.60[a]	69.3 ± 2.37	39.4 ± 3.16[a]
Redox indices				
MDA (nmol/g Hb)	2.31 ± 0.33	3.01 ± 0.14[a]	3.13 ± 0.24[a]	5.78 ± 1.49[abc]
GSH (µM/g Hb)	1215 ± 207.4	604.4 ± 64.19[a]	405.4 ± 105.3[ab]	446.0 ± 240.8[ab]
CAT (U/mg Hb min)	144.5 ± 22.29	220.8 ± 31.07[a]	248.1 ± 34.92[a]	358.8 ± 76.82[abc]
AOPP (µM Cloramine T)	13.70 ± 2.51	16.38 ± 0.99	21.21 ± 1.68[ab]	21.71 ± 2.89[ab]
SOD (U/mg Hb min)	2.82 ± 0.69	2.99 ± 0.18	2.76 ± 0.5	4.38 ± 1.58[abc]
NO^{-3}/NO^{-2} (µM)	67.82 ± 22.44	34.54 ± 7.26[a]	33.13 ± 9.70[a]	32.29 ± 10.82[a]

Data are expressed as mean ± standard deviation. The different letter represents significant differences ($p<0.05$): [a] significant differences respect the healthy group; [b] significant differences respect the asymptomatic group; [c] significant differences with respect to the moderate group. SD: standard deviation; RI: reference interval; M: males; F: female; CAT: catalase; SOD: superoxide dismutase; MDA: malondialdehyde; GSH: glutathione; AOPP: advanced oxidation protein product, NO^{-3}/NO^{-2}: estimation of nitric oxide- nitrate-nitrite ratio.

DISCUSSION

Previous clinical findings report that elevated ROS levels closely correlated with inflammation (Mittal et al., 2014; Palipoch and Koomhin, 2015; Patlevič et al., 2016), oxidative injuries (Amatore et al., 2015; Cecchini and Cecchini, 2020; Imai et al., 2008), and replication and infection of various viruses, affecting immune reactions (Khomich et al., 2018; Majewska et al., 2004; Sebastiano et al., 2016). Accordingly, a growing number of leukocytes are recruited, contributing to ROS and cytokine release, which results in hyperinflammation and cytokine storm syndrome producing a vicious cycle in different diseases (Fakhri et al., 2020; Khomich et al., 2018; Phaniendra et al., 2015).

ROS are a variety of partially reduced metabolites formed by oxygen, sulfur, nitrogen, and other atoms, with strong oxidizing competence. They are mainly generated due to cellular metabolism: the electron transport chains in mitochondria, endoplasmic reticules flow, and cytochrome P450 functions (Deavall et al., 2012; Fakhri et al., 2020; Phaniendra et al., 2015). The other major source is oxidases (e.g., NADPH oxidase), which are ubiquitously present in various cells, particularly phagocytes and endothelial cells. But also their endogenous bioavailability depends on exogenous sources such as smoking, alcohol, environmental contaminants, food, and drugs, among others (Fang, 2011; Phaniendra et al., 2015).

Circulating levels of dietary antioxidants depend on culture, habits, food disponibility, and others but also have been shown to be influenced by individual genetic variation (Di Meo et al.,2016).

ROS could be excessively formed during a pathological situation, and insufficiently presented antioxidant enzymes may contribute to H_2O_2 accumulation locally or systematically. Peroxides could produce the

oxidization of proteins on their sulfur residues (cysteine and methionine) and reaction with transition metals (e.g., iron). This reaction produces mainly chloramines that could be evaluated as AOPP (Fakhri et al., 2020; Palipoch and Koomhin, 2015; Pisoschi and Pop, 2015). Those elements were demonstrated in previous research developed in COVID-19 patients (Chen et al., 2020b; Delgado-Roche and Mesta, 2020; Pickering et al., 2013; Polonikov, 2020). Also, lipids, carbohydrates, and nucleic acids could be oxidized, and these products might be identified by damage-associated molecular patterns (DAMPs) triggering activation of NF-κB, which conduce to inflammatory gene activation. Thus contribute to the persistent inflammatory cycle and tissue damage. Oxidized biomolecules generating downstream ROS are also highly active (Mittal et al., 2014; Schaefer, 2014).

Some evidence has been provided concerning COVID-19 patients and improved oxidative stress related to innate immune response stimulation, inflammation, and medication detoxification. The evidence of the oxidation chemical reaction with the specific ability to generate both biomolecule functional alteration and redox-regulated cell death signal was demonstrated in different tissues (Azkur et al., 2020; Cecchini and Cecchini, 2020; Polonikov, 2020). The results obtained in the present research confirm the plasmatic oxidative altered status in diverse COVID-19 patients´ outcomes concerning their physiological conditions. It seems rational to consider that oxidative machinery activation related to innate immunity functions has been exacerbating host antiviral response. In that sense, oxidative stress is characterized by imbalance favoring oxidant conditions.

Altered biochemical indicators such as Hb, erythrocyte sedimentation rate, alanine aminotransferase, uric acid, and total protein previously reported by other authors have also been observed in the present study. Those findings could be related to increased oxidization tone demonstrated in blood, which could produce environmental biomolecule lesions due to the cytotoxic nature of ROS (Chen et al., 2020b; Kosanovic et al., 2021; Marrocco et al., 2017). All evidence contributes to deciphering the ongoing mechanism of SARS-CoV 2 infection, but on this occasion, a percentage of COVID-19 patients who presented comorbidities or toxic effects were recruited. It is possible that the patients in the present study showed altered values because of preexistent diseases such as hypertension, cardiovascular disease, asthma, and diabetes (Palipoch and Koomhin, 2015) or because of smoking or alcohol consumption. Nevertheless, no discrimination with respect to oxidative stress-based pathologies or toxic habits was identified in COVID-19 Cuban patients because no differences were detected with respect to patients with or without those features in each group. Probably, it could be associated with the small size of samples (30% - 13 patients with respect to 40% -17 patients). No previous studies analyzing those aspects in clinical samples were found.

One emphasis had to be placed on GSH concentration, which is a tripeptide with a secondary antioxidant capacity. This molecule is a co-substrate in multiple biochemical reactions (Ballatori et al., 2009). In different pathological conditions and also in COVID-19 infection, they were found depleted. The GSH reductions in systemic circulation will contribute to the oxidation of biomolecules and organ injury (Kalem et al., 2021). Previous results observed redox alteration and increased inflammation in the context of disease and convalescent conditions (Azkur et al., 2020; Polonikov, 2020).

MDA, a recognized biomarker of lipid oxidative injury, was higher in different Cuban COVID-19 conditions with respect to the healthy group, but it was not associated with other redox biomarkers, comorbidities, or toxic habits in the evaluated groups. These oxidized lipids could be accumulated, producing cytotoxic events in the liver and contributing to liberating enzymes (ALAT) in the blood (Pincemail et al., 2021; Schaefer, 2014). Since MDA and ALAT were altered, this could be associated with macromolecular modification of MDA in organ biomolecules such as the liver.

AOPP values were also altered in COVID-19 patients. This index refers to dityrosine, pentosidine, and carbonyl-containing protein products related to hypochlorous acid reaction (HClO). During an innate antimicrobial immune response, this acid is the main product of activated myeloperoxidase enzyme from neutrophils. Multiple mechanisms of biological damage could be developed by HClO as hemoglobin iron oxidation, reduction of O_2 saturation, hemoglobin-heme alteration, and iron liberation. The free iron could produce a Fenton reaction to generate OH• and other ROS mediating biomolecule injury that impacts renal function. The latter was possibly related to a significant correlation between AOPP and UA indices (Goud et al., 2021; Mittal et al., 2014; Muhammad et al., 2021; Palipoch and Koomhin, 2018).

Previous reports showed that COVID-19 might disturb different parts of the human organism, like the lung, renal, and cardiac tissues (Bhaskar et al., 2020; Kosanovic et al., 2021). In the present study, low GSH values were observed in different COVID-19 conditions with respect to the reference group. Other damage mechanisms could also be involved, such as those related to NO bioavailability, which may impact contract and relaxation events on blood vessels that

could mediate coagulopathies and other features recognized in COVID-19 patients. All these possibilities are related to increased ROS generation (Di Meo et al., 2016; Liguori et al., 2018).

The increased availability of ROS contributing to redox alterations in these patients could be related not only to the results of host-virus interaction, but also to nutritional differences and individual genetic variations in the sources of oxidant generation and antioxidant protection, not explored in the study.

Simultaneous modification analyses of redox status in each COVID-19 group allowed us to identify that almost all patients had variations in CAT, MDA, GSH, and AOPP compared to the healthy group. Furthermore, other indicators such as Hb, TP, UA, creatinine, ALAT, and ESR were altered in patients presenting nonspecific signs, as previously reported (Channappanavar and Perlman, 2017; Chen et al., 2020b). All evaluated metabolites by redox and biochemical indices reach the totality of organs by systemic circulation.

Observations suggest a link between altered redox balance and SARS-CoV-2 physiopathology. The COVID-19 pathological characteristics are of the highest importance to better comprehend the extent and nature of redox damage associated with this infection in order to adopt known or experimental rationale-based clinical therapeutic strategies (Bhaskar et al., 2020; Kobayashi et al., 2016; Liu et al., 2020; Vlahos et al., 2011).

Besides, modulation of a cytokine pattern when mononuclear/macrophage cells in peripheral blood are activated by ODS has been suggested. It contributes to developing responses to remove and deal with certain challenges, and it may conduce to extensive tissue injury by enhancing NF-κB gene expression and enhancing the cell to improve inflammation signal response (Azkur et al., 2020; Ifrim et al., 2014; Mittal et al., 2014).

Discriminant analysis considering redox indicators allows conforming one or more linear arrangements of predictors, producing a new latent variable for each function. These functions, named discriminant functions, could represent accumulated variance contemning data.

These studies conduce to an integral observation and synthetic representation of several indicators and summarize possible complex correlations. The interaction of redox metabolites and species with biomolecules occurs very quickly in cellular and fluid microenvironment and correspondingly with antioxidants, so the correlation between diverse redox indices could be multifactorial and nonlinear (Gadotti et al., 2021; Muralidharan and Mandrekar, 2013; Sies and Jones, 2020). These relations have been visualized in a synthetic manner using the two obtained functions.

Considering that the causes of polypathologies are complex and multifaceted, the recognition of the molecular and cellular concert involved is essential. An underlying relationship concerning some components, such as modified macromolecule and chemistry-hematological status, has arisen, but the process through which these molecular and biochemical pathways ensue remains to be established (Cecchini and Cecchini, 2020; Delgado-Roche and Mesta, 2020; Goud et al., 2021).

Several previous studies have also shown that antiretroviral treatment, including lopinavir-ritonavir, has an additional impact on pre-existing ODS related to the condition as observed in HIV infection treatment (Deavall et al., 2012; Dysangco et al., 2017). Some researchers have suggested the consumption of antioxidants to reduce the ODS consequence, also by infection or treatment due to it could impact the development of other related diseases that interventions were assessed (Chiscano-Camón et al., 2020; Gemelli Against COVID-19 Post-Acute Care Study Group, 2020; Pisoschi and Pop, 2015; Vlahos et al., 2011).

Several findings pointed to an imbalanced redox system as the causal element influencing morbidity and mortality in patients with COVID-19 and post-COVID outcomes (Azkur et al., 2020; Channappanavar and Perlman, 2017; Dasgupta et al., 2020; Polonikov, 2020). Future research should assess interventions to reduce oxidative stress in patients with COVID-19 and may use redox biomarkers as endpoints (Lee, 2018; Marrocco et al., 2017).

The present results support the conception that some redox indices evaluations will therefore become potentially useful to characterize COVID-19 metabolic conditions and interventions (Di Meo et al., 2016; Fakhri et al., 2020; Kalem et al., 2021).

The present investigation has some limitations. First, the groups' amount of individuals is small. Second, the confidence interval (95%) for the adjusted estimates was wide and did not exclude a 20–30% decline in the coefficient on days to show clinical improvement. Third, no individual genetic or nutritional variations were explored. Fourth the possibility of residual unmeasured confounding cannot be excluded, as it happens with most observational studies.

The effect of comorbidities can not be avoided by recruiting subjects with diseases associated with marked oxidative stress but regulated. Otherwise, data evidence the involved role of oxidative imbal-

ance in SARS-CoV-2 infection. Thus support a possible beneficial effect of bioxidative therapies.

The strengths of this research consist of its practical, real-world COVID-19 population, objective primary clinical outcome use, and risk adjustment applying approaches of regression modeling studies.

CONCLUSION

Redox biomarkers showed some alterations in the acute course of the COVID-19 disease. Special consideration should be noted since the pattern of changes differs in clinical outcomes. Our results suggested that systemic oxidative stress could be involved in patients' active clinical status and long-term consequences sequelae of COVID-19. The concentration of this cumulative damage may directly impact cell functioning. Their etiology, including the roles of non-virus and viral-related effects and treatment-associated factors, requires ongoing investigation. These conclusions are also methodologically important for the follow-up and management of infected individuals.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Gil-del Valle L	Gravier-Hernández R	Delgado-Guerra MM	Sánchez-Márquez JA	López-Fernández OE	Acosta-Suárez MA	Rosell-Guerra T	Suárez-Iznaga R	Martínez-Casanueva R	Zamora-Rodriguez Z	Fernández-García LA	Bermudez-Alfonso Y	Hernández-Gonzalez-Abreu MC	Garrido G
Concepts or ideas	x		x	x	x		x	x				x		
Design	x		x		x		x	x			x	x		
Definition of intellectual content	x		x		x									
Literature search	x	x							x	x				x
Clinical studies			x	x	x	x					x		x	
Experimental studies	x		x	x	x				x	x	x		x	
Data acquisition	x						x	x	x	x				
Data analysis	x		x		x							x	x	
Statistical analysis	x	x												
Manuscript preparation	x		x		x									x
Manuscript editing					x		x	x						x
Manuscript review	x	x	x	x	x	x	x	x	x	x	x	x	x	x

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