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Diagnostic Microbiology and Infectious Disease 105 (2023) 115886

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Clinical, demographic and oxidative profile of patients with COVID-19 and disease severity

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ARTICLE INFO

Article history:
Received 6 September 2022
Revised in revised form 3 December 2022
Accepted 27 December 2022
Available online 30 December 2022

Keywords:
SARS-CoV-2
Oxidative stress
Antioxidant
δ-ALA-D
COVID-19

ABSTRACT

This study aimed to profile the clinical progression, demographics, and oxidative status of COVID-19 patients, correlating with disease severity. The study included 143 participants: 93 patients with COVID-19 (28 outpatients, 65 inpatients), and 50 control participants. Thiobarbituric acid reactive substance (TBARS) was used as an oxidative damage marker. Antioxidant activity was assessed via quantification of Vitamin C, sulfhydryl groups, ferric reduction ability of plasma (FRAP), Uric acid (UA), and evaluation of delta-aminolevulinate dehydratase (δ-ALA-D) enzymatic activity. Geriatric patients, especially men, with comorbidities such as obesity and/or chronic diseases were more likely to develop the most severe form of COVID-19. The activity of the δ-ALA-D was lower in inpatients, and there was no significant difference with the outpatient. Antioxidants decreased in COVID-19 groups, while lipid peroxidation increased. FRAP and Vitamin C decreased with evolution of the disease. Oxidative stress could be used as a predictor of worsening clinical condition.

1. Introduction

Wuhan, capital of the Hubei Province, China, experienced an outbreak of pneumonia cases of unknown etiology in December 2019 [1]. The epidemic later evolved into a global pandemic [1,2]. The etiological agent was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), owing to its phylogenetic similarity to SARS—causative agent of a viral outbreak in 2022; the clinical condition was referred to as coronavirus disease (COVID-19) [3].

SARS-CoV-2 binds to the receptor angiotensin-II converting enzyme (ACE2) within the lung parenchyma; thus, ACE2 is the main mediator of virus entry into human host cells [4–6]. SARS-CoV-2 is transmitted via direct contact with infected patients or respiratory droplets and is detected through real-time polymerase chain reaction (RT-PCR) [1–3,5]. Patients present with a wide range of symptoms, from mild to severe; the most common symptoms are fever, dry cough, fatigue, and shortness of breath. However, symptoms such as sputum production, headache, hemoptysis, diarrhea, and lymphopenia have also been reported [1,5]. The symptoms of COVID-19 are more severe in older age groups with comorbidities; allergies, asthma, and chronic obstructive pulmonary disease (COPD) are also risk factors [1,2].

Oxidative stress occurs due to an imbalance between the production of oxidizing compounds and the antioxidant defense capacity, generating many free radicals [7,8]. This increases reactive oxygen species (ROS), eventually resulting in oxidative damage, cell degeneration, and functional decline, which can further worsen respiratory diseases, including COVID-19 [7,9,10]. Viruses disrupt the homeostasis of infected cells and increase ROS production in phagocytes. Patients with SARS-CoV-2 who present with comorbidities have an increased state of oxidative stress due to chronic diseases and viral diseases, and assessment of oxidative stress is important to determine the severity of COVID-19 [11].

Oxidative stress markers can be used as a tool for decision making, and the correlation between oxidative stress and disease severity should be further studied to identify predictors of disease severity and associated complications. Therefore, this study aimed to profile

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease; ACE2, angiotensin-II converting enzyme; RT-PCR, real-time polymerase chain reaction; COPD, chronic obstructive pulmonary disease; ROS, reactive oxygen species; HUSM, Santa Maria University Hospital; ICU, intensive care unit; CEP, Research Ethics Committee; TBARS, thiobarbituric acid reactive substance; P-SH, Protein thiol groups; NP-SH, Non protein thiol groups; FRAP, ferric reduction ability of plasma; UA, acid uric; δ-ALA-D, delta-aminolevulinate dehydratase enzyme

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https://doi.org/10.1016/j.diagmicrobio.2022.115886
0732-8893/

the clinical condition, demographics, and oxidative status of patients with COVID-19 and correlate them with disease severity.

2. Materials and methods

2.1. Study population

One hundred and forty-three participants with COVID-19 were selected, between August 2020 and March 2021, from the University Hospital of Santa Maria (HUSM), RS, Brazil. The samples were divided into three groups: i) outpatients (n = 28): patients with mild COVID-19, who were in isolation at home; ii) inpatients (n = 65): patients with moderate or severe COVID-19, who were admitted to the HUSM intensive care unit (ICU), following the COVID-19 Treatment Guidelines Panel by the National Institutes of Health [12] and the Guidelines for New Clinical Manifestations of Coronavirus by the World Health Organization [13], and iii) control (n = 50): community volunteers, without COVID-19, collected before the emergence of the pandemic, with sex, age, and comorbidities (such as hypertension and diabetes mellitus) similar to participants with COVID-19. Informed consent was obtained from all patients and participants. This study was approved by the Research Ethics Committee (CEP) of the Federal University of Santa Maria after receiving the certificate of presentation for ethical appreciation (CAAE: 13897319.0.0000.5346). Patients with neoplasms were excluded from the study.

2.2. Sample collection

Venous blood samples were collected in vacuum tubes as follows: 8 mL in a tube with heparin and 2 mL in a tube without any anticoagulant. The whole blood sample in the heparin tube was centrifuged at 3000 rpm for 10 minutes to separate the plasma from the erythrocytes, which were then rinsed three times with a 0.9% NaCl solution. Plasma aliquots were separated and stored at −80°C as soon as they were obtained. This was done for performing the ferric reducing ability of plasma (FRAP) tests at a later stage, while the other tests were performed on the same day. Medical history of patients pertinent to the study was obtained verbally from patients or from electronic medical records. Body mass index (BMI) was calculated by dividing weight by height squared (kg/m^2).

2.3. Oxidative profile

2.3.1. Oxidants

To evaluate lipid peroxidation in erythrocytes and plasma, thiobarbituric acid reactive substance (TBARS) was measured spectrophotometrically at 532 nm using the method described by Lapenna et al. [14] and expressed as nmol MDA/mL of plasma and nmol MDA/mL of erythrocytes.

2.3.2. Antioxidants

Protein thiol groups (P-SH) in plasma and nonprotein thiol groups (NP-SH) in erythrocytes were represented as nmol P-SH/mL of plasma and nmol N-PSH/ mL of erythrocytes and were evaluated according to the Ellman and Boyne [15] method modified by Jacques-Silva et al. [16]. Estimation of Vitamin C levels in plasma was performed as described by Galley et al. [17] with some modifications by Jacques-Silva et al. [16], and the results are expressed in μg vitamin C/ mL of plasma. The ferric reducing ability of plasma (FRAP), technique described by Benzie and Strain (1996) [18] was applied on a Mindray BS 380 automated analyzer, Shenzhen, China, and the results were expressed in μmol/L. The uric acid (UA), measured in serum were assessed by standard method with a commercial kit (Labtest).

2.3.3. Enzymatic activity

The Berlin and Schaller [19] technique was used to determine the activity of δ-ALA-D. The results are expressed as U/l (*U* = nanomole of PBG/hour/milligram of Hb).

2.4. Statistical analysis

The software Graph Pad Prism 8.0.2 software (San Diego, California) was used to analyze the data. The normality of the distribution was tested with the Shapiro-Wilk test. For the analysis of nonparametric data, the Kruskal-Wallis test was used and the results were expressed as median (interquartile range) and for the parametric data, the One-Way analysis of variance (ANOVA) followed by the Tukey post hoc test to compare groups and data were expressed as mean standard deviation (SD). Categorical variables analyzed by the Pearson chi-square test. For correlation, Spearman's correlation coefficient was used for nonparametric data. Differences in the probability of rejecting the null hypothesis of 5% ($P < 0.05$) were considered statistically significant.

3. Results

Ninety-three COVID-19 patients confirmed using the RT-PCR test were included in the study; 28 patients had mild symptoms and were assigned to the outpatient group, 65 patients had moderate/severe symptoms, and were assigned to the inpatient group, and 50 volunteers were assigned to the control group. Table 1 shows the clinical and demographic details of the study participants.

The mean age of the outpatients was lower than that of inpatients and the control group. The inpatient group had the highest weight and BMI, corresponding to grade I obesity. There was no significant difference between the heights of the three analyzed groups. Inpatients had a higher mortality rate, predominantly among men than other groups. The outpatients and the control group experienced zero fatalities. Hypertension (39/65) and diabetes mellitus (21/65) were the most common comorbidities among inpatients, and arterial hypertension was the only observed comorbidity (2/28) among outpatients. In the control group, hypertension (18/50) and diabetes mellitus (7/50) were the observed comorbidities.

Fig. 1 shows the oxidative stress markers and the activity of the δ-ALA-D enzyme and their relationships with the three groups. TBARS in erythrocytes, an oxidative damage marker, were significantly higher in the outpatient and inpatient groups than in the control group and did not differ between the outpatient and inpatient groups. Regarding antioxidant defenses, NP-SH, Vitamin C, FRAP and Uric acid were significantly lower in the infected patients than in the control group, and Vitamin C and FRAP are significantly reduced with disease worsening in the inpatient group compared with the outpatient group. The activity of the δ-ALA-D enzyme was lower in patients with moderate/severe COVID-19 (3.00 ± 0.93), but not in the outpatients (3.13 ± 0.70), when compared to the control group (3.77 ± 1.14), and there was no significant difference between the outpatient and inpatient groups. No significant differences were found in plasma TBARS and P-SH between the groups.

Table 2 shows a negative Spearman's correlation between Vitamin C and FRAP with COVID-19 and a positive correlation between FRAP and Vitamin C, indicating that antioxidant defenses decrease as disease severity increases.

4. Discussion

The carried out study detected, in an unprecedented way, a decrease in the activity of the δ-ALA-D enzyme in patients with moderate/severe COVID-19 when compared to the control group. Antioxidant defenses (NP-SH, Vitamin C, FRAP e Uric acid) also showed a decrease in out and in patients groups, while lipid peroxidation was

Table 1
Clinical and demographic characteristics of participants.

Characteristics		Control group (n = 50)	Hospitalization No (n = 28)	Yes (n = 65)	*P* value
Age (y)		51.6 (21–81)	38.7 (19–62)[a]	59.7 (14–91)[a,b]	<0.001
Weight (Kg)		74.8 (58–110)	72.0 (45–105)	87.7 (52–157)[a,b]	0.001
Height (m)		1.7 (1.55–1.90)	1.7 (1.53–1.89)	1.7 (1.48–1.85)	0.625
BMI (Kg/m^2)		26.2 (20.98–37.20)	25.6 (19.22–36.10)	30.9 (20.06–48.50)[a,b]	0.001
Sex (n, %)					
Male		30 (60.00)	11 (39.29)	40 (61.54)	
					0.071
Female		20 (40.00)	17 (60.71)	25 (38.46)	
Death n (%)	No	-	28 (100)[a]	32 (49.23)[a,b]	0.001
	Yes,Male	-	-	20 (30.77)[a,b]	
	Yes, Female	-	-	13 (20.0)[a,b]	
Comorbidity, n (%)					
Systemic hypertension	No	32 (64.00)	26 (92.90)[a]	26 (40.00)[a,b]	<0.001
	Yes	18 (36.00)	2 (7.10)[a]	39 (60.00)[a,b]	
Congestive heart failure	No	-	28 (100)	52 (80.00)[a,b]	0.011
	Yes	-	-	13 (20.00)[a,b]	
Diabetes mellitus	No	43 (85.00)	28 (100)	44 (67.70)[a,b]	0.001
	Yes	7 (15.00)	-	21 (32.30)[a,b]	
Obesity	No	-	28 (100)	53 (81.50)[a,b]	0.015
	Yes	-	-	12 (18.50)[a,b]	
COPD	No	-	28 (100)	54 (83.10)[a,b]	0.02
	Yes	-	-	11 (16.90)[a,b]	

Values represented as median (interquartile range) or n (%). Results analyzed by Kruskal-Wallis or Pearson's chi-square test. Statistical significance was set at $P < 0.05$. BMI, body mass index; COPD, chronic obstructive pulmonary disease.
[a] $P < 0.05$, compared to control group.
[b] $P < 0.05$, comparing the out and inpatient groups.

shown to be increased, representing a data of greater oxidative stress in patients with COVID-19. FRAP and Vitamin C decreased with evolution of the disease, thus, oxidative stress in clinical practice is an indicator of deterioration of clinical condition.

COVID-19 is a new disease associated with high morbidity and mortality rates [20]. COVID-19 patients often present with mild symptoms such as cough, sore throat, and fever; however, patients also present with severe forms of the disease known as severe acute respiratory syndrome (SARS), which is associated with a high mortality rate [21]. The geriatric population, pregnant women, indigenous populations, and patients with comorbidities are further predisposed to the infection due to lowered immune defenses and are thus considered a high-risk group [22,23]. In this context, our study showed (Table 1) that older adults with high BMI, especially obese patients and those with chronic diseases such as hypertension, diabetes mellitus, heart failure, and chronic lung disease, develop more severe forms of the disease and often require admission to the ICU.

Our study also demonstrated that patients in the inpatient group had a high mortality rate of approximately 50%, and the majority were men. Bayrak et al. [20] also found high mortality rates of patients in the ICU (62.8%) and the predominance of deaths among men.

The oxidative stress relationship, which is the loss of balance between pro-oxidants and antioxidants, has been linked to several diseases such as cancer, diabetes, hypertension, and viral infections, including respiratory diseases [24–27]. Some studies have already demonstrated the association between oxidative stress and SARS-CoV-2 infection and its complications [21,23].

TBARS was used to evaluate the peroxidation of lipids present in cell membranes of erythrocytes. This technique is based on the reaction between malondialdehyde (MDA) with thiobarbituric acid and is considered a good marker of oxidative stress in clinical conditions [28,29]. Our results showed that TBARS levels in erythrocytes were higher in COVID-19 patients than in the control group, showing the existence of an oxidative imbalance in these patients. There was no significant difference in erythrocyte TBARS levels between outpatients and inpatients. In Muhammad et al.'s 2020 study, higher levels of MDA were found in COVID-19 patients than in the control group, indicating overproduction of free radicals.

The continuous generation of free radicals leads to the development of antioxidant defense mechanisms to limit the intracellular levels of such reactive species and control the occurrence of resulting damage [7]. The thiol groups (-SH or sulfhydryl) plays an important role because of its potential for chelation, reduction, and protection against damage caused by oxidative stress [30]. In our study, there was a decrease of (NP-SH) in patients with COVID-19. Vitamin C donates electrons to ROS, acting as a reducing agent, thus destroying them before they react with lipoproteins and membranes and, in turn, preventing lipid peroxidation and reducing oxidative stress [31]. The FRAP technique tests the antioxidant strength of a sample in reducing ferric to ferrous ions [32]. The main advantage of the test is that it represent the antioxidant capacity of all compounds present in a biological sample, whether endogenous or exogenous, and not an isolated compound [32]. Our study showed a decrease in the antioxidant defenses of COVID-19 patients compared with the control group, with the lowest levels of Vitamin C and FRAP, associated with the severity of the disease, showing statistical significance between the inpatient and outpatient group, and both with the control group. These data are confirmed by the correlation study presented in Table 2, where a negative correlation is observed between Vitamin C and COVID-19 as well as between FRAP and COVID-19, proving that as the severity of the disease increases, there is a decrease in antioxidant defenses. A positive correlation between FRAP and Vitamin C was observed, both markers decrease as disease severity increases. In a pilot study conducted by Arvinte et al. [33], among critically ill COVID-19 patients hospitalized in Colorado, older age and low Vitamin C levels appeared to be co-dependent risk factors for COVID-19-associated mortality.

Uric acid (AU) is a hydrophilic antioxidant, being generated during the metabolism of purine nucleotides, being frequently associated with renal dysfunction, however, it is also considered a marker of tissue damage and oxidative stress [34,32]. Its antioxidant effect is due

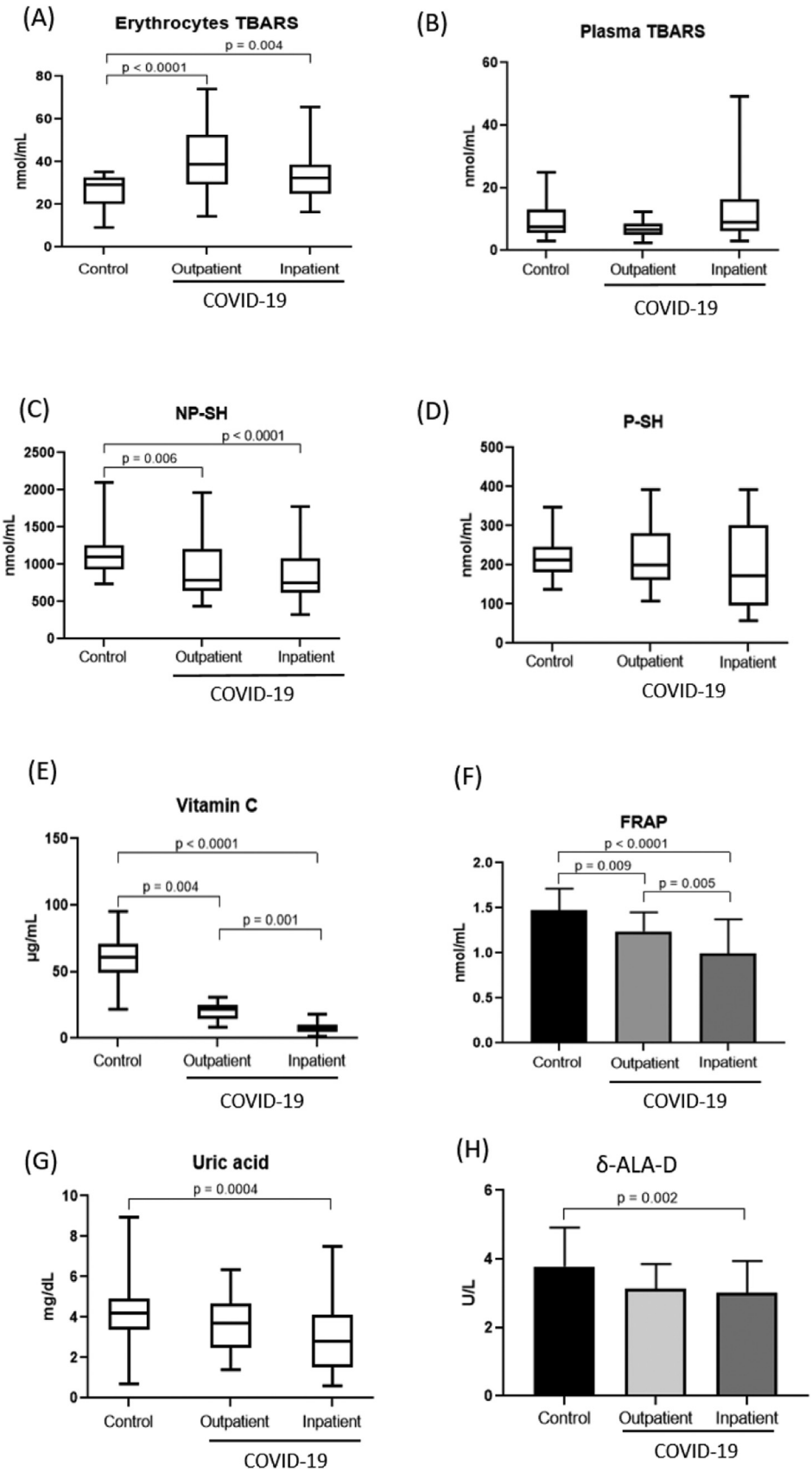

Fig. 1. Oxidative stress markers in the groups: Erythrocyte TBARS (A), Plasma TBARS (B), NP-SH (C), P-SH (D), Vitamin C (E), FRAP (F), Uric acid (G) and Enzyme activity of δ-ALA-D (H) and their relationships with the three groups. Nonparametric results were determined by Kruskal-Wallis and represented as median (interquartile range). Parametric results were determined by ANOVA followed by Tukey's test and represented as mean standard deviation (SD). Values with $P < 0.05$ were considered statistically significant. P-SH: protein thiol groups; NP-SH: non-protein thiol groups; FRAP: ferric reduction ability of plasma; TBARS: thiobarbituric acid reactive substance and δ -ALA-D: delta-aminolevulinate dehydratase.

Table 2
Correlations of oxidative stress parameters and disease severity in COVID-19 patients.

Correlations	Spearman r	*P* value
TBARS plasma X COVID-19	249	0.020
Vitamin C X COVID-19	-648	0.00
FRAP X COVID-19	-370	0.001
FRAP X Vitamin C	315	0.005

Correlation of data performed using Spearman correlation coefficient (n = 93). Values of $P < 0.05$ were considered statistically significant.
FRAP = ferric reduction ability of plasma; TBARS = thiobarbituric acid reactive substance.

to the fact that AU is able to inhibit the action of free radicals on organic molecules, creating stable complexes with iron ions, inhibiting the Fenton and Haber-Weiss reaction, thus preventing the formation of the hydroxyl radical. This is considered the most important antioxidant effect of AU, however, it is also related to the improvement of endothelial function, by preventing the loss of NO in endothelial cells exposed to peroxynitrite, a potentially harmful oxidant [35–37]. In the study by Lucca et al. [32]. AU was related to tissue damage and oxidative stress in women with preeclampsia. Our study shows a higher AU value in the control group, and a reduction in antioxidant defenses was observed as the severity of the disease progresses, with the lowest AU value in patients admitted to the COVID-19 ICU.

Regarding the study of the activity of δ-ALA-D, which is an enzyme that contains sulfhydryl groups in its structure, it is sensitive to pro-oxidant elements, causing changes in the synthesis of the heme group of hemoglobin, thus being inhibited in situations of oxidative stress; therefore, the enzyme can be suggested as an indirect marker of oxidative stress [38,39]. To our knowledge, the activity of the δ-ALA-D enzyme has not been studied in COVID-19 patients. In our study, the activity of the δ-ALA-D enzyme was lower in patients with moderate/severe COVID-19, but not in outpatients, when compared with the control group and there was no significant difference between the outpatient and inpatient groups. In the control group the enzyme was more active, and the oxidative stress in this group was lower. A decrease in enzyme activity was observed as the disease severity advanced, with the lowest enzyme value found in patients who were hospitalized in the ICU with moderate/severe COVID-19, proving the presence of more free radicals in this group, which were able to decrease the enzyme activity.

Our study had some limitations, such as the low number of participants, mainly in the nonhospitalized group and the variation in the diet of participants.

5. Conclusion

Geriatric male patients with comorbidities were more likely to develop severe COVID-19, requiring hospital intervention. The mortality rates among this group were also relatively higher. Furthermore, the activity of the δ-ALA-D enzyme was lower in patients with moderate/severe COVID-19, than in the control group. Antioxidant defenses also showed a decrease in out and inpatients groups, while lipid peroxidation was shown to be increased. FRAP and Vitamin C decrease with worsening of the disease, thus, oxidative stress in clinical practice is an indicator of deterioration of clinical condition.

Author Contributions

Protocol and Project Development: Andressa de Azambuja Pias Weber and Thissiane de Lima Gonçalves. Data collection or Management: Andressa de Azambuja Pias Weber, Jovana Simonetti Bulegon, Manoela Dias de Souza, Silmara Ana Vendrame and Larissa Venturini. Data Analysis: Andressa de Azambuja Pias Weber, Jovana Simonetti Bulegon, Wendel Mombaque dos Santos and Thissiane de Lima Gonçalves. Manuscript Editing and Writing: Andressa de Azambuja Pias Weber, Jovana Simonetti Bulegon, Manoela Dias de Souza, Silmara Ana Vendrame, Larissa Venturini, Wendel Mombaque dos Santos and Thissiane de Lima Gonçalves.

Acknowledgments

The authors thank all of the volunteers who participated in this study. Additionally, we thank the University Hospital of Santa Maria (HUSM), Rio Grande do Sul, Brazil, for support in this study.

Funding

This work was supported by the Coordination of Improvement of Higher Level Personnel (CAPES) and the Graduate Support Program (PROAP).

Disclosures

The authors reported no potential conflict of interest.

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