

Investigation of Antioxidant Enzymes (GST, GSH, R-GSSG) in Erythrocytes of COVID-19 Patients: A One-Month Observation

Elżbieta Cecerska-Heryć¹, Natalia Serwin¹, Ewa Pius-Sadowska², Klaudia Głowacka¹, Aleksandra Polikowska¹, Małgorzata Goszka¹, Radosław Birger¹, Marta Budkowska³, Bogusław Machaliński², Barbara Dołęgowska¹

¹Department of Laboratory Medicine, Pomeranian Medical University of Szczecin, Szczecin, Poland; ²Department of General Pathology, Pomeranian Medical University of Szczecin, Szczecin, Poland; ³Department of Analytical Medicine Pomeranian Medical University of Szczecin, Szczecin, Poland

Correspondence: Elżbieta Cecerska-Heryć, Email elzbieta.cecerska.heryc@pum.edu.pl

Introduction: Oxidative stress (OS) occurs when there is an imbalance between reactive oxygen species (ROS) production and the body's ability to neutralize them. This can lead to cellular damage and is associated with aging and various diseases. Glutathione (GSH) acts as a vital non-enzymatic antioxidant, while enzymes such as glutathione S-transferase (GST) and glutathione reductase (GR) help maintain the body's redox balance. OS from viruses, including coronavirus, can disrupt inflammation regulation and cause prolonged tissue damage.

Aim: This study examines GSH levels and the activities of GST and R-GSSG in the erythrocytes of COVID-19 patients compared to those of healthy controls.

Materials and Methods: The study involved 85 COVID-19 patients (42 females and 43 males), with blood samples collected at the time of disease detection and at 7, 14, and 28 days later. The control group included 85 healthy individuals. Antioxidant levels (GSH, GST, R-GSSG) were measured using spectrophotometric methods.

Results: COVID-19 patients exhibited a significant decrease in GSH concentration ($p < 0.001$) and an increase in R-GSSG ($p < 0.001$) and GST ($p = 0.046$) activity. Lower GSH levels correlated with a higher risk of death ($p = 0.008$). Multivariate analysis revealed relationships between GSH and factors such as time since detection, survival rate, hospital stay, and disease severity.

Conclusion: COVID-19 disrupts antioxidant enzyme balance, potentially worsening disease severity and increasing mortality risk, especially in older patients.

Keywords: antioxidant enzymes, GST, GSH, R-GSSG oxidative stress, COVID-19

Introduction

Aerobic organisms, including humans, rely on oxygen for their survival and metabolic processes. However, the metabolic pathways that utilize oxygen also generate reactive oxygen species (ROS), which can be detrimental to cellular health. Under normal physiological conditions, ROS play critical roles, including aiding in the elimination of pathogens, modulating cellular signaling, and regulating vital processes such as inflammation and cell proliferation. Despite their necessary functions, an overproduction of ROS can lead to oxidative stress, an imbalance between ROS and antioxidant defenses. This oxidative stress is known to exacerbate with aging and is associated with a myriad of chronic diseases, including diabetes, cardiovascular disorders, and skeletal muscle dysfunction.¹

Amid the COVID-19 pandemic, the importance of antioxidant enzymes, particularly within erythrocytes (red blood cells), has garnered significant attention. Numerous studies have documented that individuals suffering from COVID-19 often present with heightened levels of oxidative stress, alongside decreased activity of vital antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR). The diminished functionality of these

enzymes in erythrocytes has been associated with increased disease severity and a higher mortality rate among patients.^{2–4}

Glutathione (GSH), the primary non-enzymatic antioxidant present in the human body, plays a crucial role in neutralizing reactive oxygen species (ROS) and facilitating the regeneration of other antioxidant enzymes. Research has shown that patients experiencing severe manifestations of COVID-19 have markedly lower levels of glutathione (GSH). This depletion may contribute to the onset of cytokine storms, a hyper-inflammatory response that can result in significant tissue damage and systemic complications.^{5,6}

Furthermore, genetic polymorphisms in the genes that encode for enzymes involved in glutathione metabolism, such as glutathione S-transferase (GST), may influence an individual's susceptibility to severe COVID-19 and impact the variability of their antioxidant responses. Such genetic factors can potentially lead to differences in how individuals respond to oxidative stress during the illness.

The objective of this study is to perform a thorough analysis of glutathione (GSH) levels and the enzymatic activities of two specific antioxidant enzymes—glutathione S-transferase (GST) and glutathione reductase (GR)—in erythrocyte samples obtained from patients diagnosed with COVID-19 in comparison to healthy control subjects. This longitudinal analysis will span a one-month observation period, allowing us to assess changes in antioxidant capacity as the disease progresses. By examining the interplay between oxidative stress and the antioxidant responses of erythrocytes in COVID-19 patients, we aim to derive valuable insights that could inform the development of potential therapeutic strategies and ultimately enhance patient survival outcomes.

Materials and Methods

Characteristics of the Study Group

The study group (SG) comprised 85 COVID-19 patients (43 males and 42 females) aged 46 to 72 years. Blood samples were collected at the time of disease detection (first draw) and then again at 7 days (second draw), 14 days (third draw), and 28 days (fourth draw) after disease detection. Real-time PCR confirmed SARS-CoV-2 virus infection by testing for viral material in nasopharyngeal swabs. Patients were treated in the Observational and Infectious Diseases, Tropical Diseases, and Acquired Immunodeficiencies Unit of SPWSZ Hospital in Szczecin, Poland, and the Temporary COVID Unit at SPSK2 in Szczecin.

The control group (CG) comprised 85 healthy volunteers (43 males and 42 females) aged 31 to 51 years. Their absence of the virus was confirmed on the day of sample collection through negative ELISA test results for IgM and IgG antibodies specific to SARS-CoV-2. Antibody testing was performed using the ELISA method (Human SARS-CoV-2 Spike [trimer] Ig Total ELISA Kit by INVITROGEN, Thermo Fisher Scientific, USA). Each participant completed a detailed general health questionnaire.

Physicians assessed the severity of COVID-19 in the study group at the Infectious Diseases Hospital on Arkońska Street. The classification was based on the Modified Early Warning Score (MEWS), recommended by the Polish Society of Epidemiology and Infectious Diseases. MEWS relies on the following parameters: systolic blood pressure, heart rate, respiratory rate, body temperature, and neurological symptoms. Additionally, all patients underwent a thorough physical examination to determine symptoms such as cough, fatigue, dyspnea, fever, body and headaches, diarrhea, chest pain, and disorders of smell and taste, as well as the duration and severity of these symptoms. Based on this assessment, the study group was categorized into mild, moderate, and severe cases. Patients with mild symptoms were classified as having a mild course, while those with moderate but non-life-threatening symptoms were classified as having an average course. Patients displaying severe life-threatening signs were grouped in the severe category.

Exclusion criteria for the study included: 1. advanced hepatic or renal dysfunction that could affect the synthesis, distribution, and elimination of antibodies; 2. presence of HIV and congenital or acquired humoral and cellular immune deficiencies; and 3. lack of written consent to participate in the study. The severity of symptoms was defined retrospectively, with patients in severe conditions requiring intensive care. At the same time, moderate cases involved individuals with saturation levels below 95% who did not need admission to the intensive care unit. Mild cases included

asymptomatic or mildly symptomatic patients who did not require hospitalization for COVID-19, as detailed in an earlier publication.

All subjects provided informed consent before participating in the study. The study was conducted by the Declaration of Helsinki and approved by the Bioethics Committee at Pomeranian Medical University (No. KB-0012/83/2020). The study group (SG) comprised 85 COVID-19 patients (43 males and 42 females) aged between 46 and 72 years. Blood samples were collected at the time of initial disease detection (first draw) and subsequently at 7 days (second draw), 14 days (third draw), and 28 days (fourth draw) post-detection. Real-time PCR was employed to confirm SARS-CoV-2 virus infection by detecting the presence of viral material in nasopharyngeal swabs. The patients received treatment in the Observational and Infectious Diseases, Tropical Diseases, and Acquired Immunodeficiencies Unit of the SPWSZ Hospital in Szczecin, Poland, as well as in the Temporary COVID Unit at SPSK2 in Szczecin.

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Exclusion criteria for the study included the following: 1. advanced hepatic or renal dysfunction that could interfere with the synthesis, distribution, and elimination of antibodies; 2. the presence of HIV or congenital or acquired humoral and cellular immune deficiencies; and 3. lack of written consent from subjects to participate in the study. The severity of symptoms was classified retrospectively, with individuals in severe conditions requiring intensive care. At the same time, moderate cases involved patients with oxygen saturation levels below 95% who did not necessitate admission to the intensive care unit. Mild cases included asymptomatic or mildly symptomatic patients who did not require hospitalization for COVID-19, as detailed in prior publications.

All participants provided informed consent before participating in the study. The investigation was conducted according to the Declaration of Helsinki and received approval from the Ethics Committee of the Bioethics Committee at Pomeranian Medical University (No. KB-0012/83/2020). [Table 1](#) presents the characteristics of the study and control group.

Table 1 Characteristics of the Study Group (SG) and Control Group (CG)

Parameter	Study Group (SG)	Control Group (CG)	p-value
Sex [M – male, F – female]	M-43 / F-42	M-43/ F-42	NS
Age [years]	59±13	44±13	NS
Duration of hospitalization [days]	15±9	-	-
COVID-19 course (mild, moderate, severe – I draw)	Mild – 52 Moderate – 7 Severe – 26	-	-

(Continued)

Table 1 (Continued).

Parameter	Study Group (SG)	Control Group (CG)	p-value
COVID-19 course (mild, moderate, severe – II draw)	Mild – 52 Moderate – 7 Severe – 26	-	-
COVID-19 course (mild, moderate, severe – III draw)	Mild – 52 Moderate – 5 Severe – 17	-	-
COVID-19 course (mild, moderate, severe – IV draw)	Mild – 52 Moderate – 4 Severe – 12	-	-
Death [yes; no] – I draw	Y – 14 No – 71	-	-
Death [yes; no] – II draw	Y – 14 No – 71	-	-
Death [yes; no] – III draw	Y – 6 No – 63	-	-
Death [yes; no] – IV draw	Y – 0 No – 57	-	-

Notes: Mean $\pm$ standard deviation; NS- non-statistical results between SG and CG (Fisher test, chi-square).

Material

The study material was blood collected from an ulnar vein on K₂EDTA. Then, blood was centrifuged (10 minutes, 2600 rpm, 20°C), and the resulting plasma was transferred to tubes, which were frozen at –80°C until biochemical parameters (glucose, cholesterol, triglycerides, HDL, creatinine, uric acid, total protein, and albumin) were analyzed. The results of the biochemical analysis are presented in Table 2.

The blood was collected on K₂EDTA. Red blood cells were rinsed three times with a 0.9% NaCl solution. After rinsing, third centrifugation, and removal of NaCl, erythrocytes were transferred to new, properly labeled tubes and frozen at –80°C until analysis.

Table 2 Biochemical Tests of the Study and Control Groups

Parameter	Study Group (I Draw) n=85	Study Group (II Draw) n=85	Study Group (III draw) n=74	Study Group (VI Draw) n=68	Control Group n=85	P*	P**
Albumin [g/dL]	4,9 $\pm$ 1,3 4,8	4,6 $\pm$ 1,07 4,5	5,0 $\pm$ 1,0 4,6	5,2 $\pm$ 1,4 4,6	4,50 $\pm$ 0,8 4,3	NS	0,031
Total protein [g/dL]	5,3 $\pm$ 1,7 5,5	4,9 $\pm$ 2,0 4,8	5,5 $\pm$ 1,6 5,0	6,4 $\pm$ 1,6 6,2	2,6 $\pm$ 1,2 2,2	0,003	<0,001
HDL [mg/dL]	55,7 $\pm$ 30,6 59,7	59,1 $\pm$ 33,4 63,2	60,6 $\pm$ 31,0 60,3	65,0 $\pm$ 31,8 71,5	23,9 $\pm$ 20,6 17,3	NS	<0,001
Cholesterol [mg/dL]	129,6 $\pm$ 49,5 133,5	138,1 $\pm$ 43,4 142,4	134,0 $\pm$ 39,9 150,1	144,3 $\pm$ 39,5 152,7	131,7 $\pm$ 26,8 128,7	0,042	NS
Triglycerides [mg/dL]	200,01 $\pm$ 6,1 188,4	199,5 $\pm$ 45,8 188,0	202,0 $\pm$ 41,3 184,4	206,2 $\pm$ 75,1 185,8	184,81 $\pm$ 26,2 171,7	NS	NS

(Continued)

Table 2 (Continued).

Parameter	Study Group (I Draw) n=85	Study Group (II Draw) n=85	Study Group (III draw) n=74	Study Group (VI Draw) n=68	Control Group n=85	P*	P**
Creatinine [mg/dL]	0,57±0,5 0,4	0,6±0,7 0,4	0,8±0,8 0,5	0,5±0,3 0,4	0,85±0,3 0,8	NS	NS
Glucose [mg/dL]	126,7±48,6 113,4	138,1±70,6 116,4	109,4±21,1 102,9	104,8±22,9 100,3	99,8±33,4 94,3	0,011	<0,001
Uric acid [mg/dL]	5,6±2,52 4,6	5,3±2,1 4,9	5,0±0,9 4,8	5,4±1,2 5,2	6,6±2,7 6,0	NS	<0,001

Notes: Mean ± standard deviation, median. p*- p value between study groups (I–IV draw); p** - p value between SG and CG (Kruskal–Wallis ANOVA).

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; NS, non statistical results.

Methods

Before determining the levels of selected antioxidants, appropriate dilutions of erythrocyte samples (hemolysates) were prepared. For the first dilution (hemolysate I), 150 µL of the erythrocyte sample was mixed with 450 µL of distilled water. The hemoglobin level of each erythrocyte sample was measured after that, and based on the formula, the volume of water to be added to 200 µL of hemolysate I was calculated to obtain the subsequent dilution (hemolysate II). Hemolysate II was used to determine the levels of glutathione (GSH) and glutathione S-transferase (GST). For R-GSSG, 62.5 µL of the erythrocyte sample was diluted in 1250 µL of distilled water immediately before the analysis began. Antioxidant enzyme activity (GST, R-GSSG) and reduced glutathione concentrations were measured spectrophotometrically by Lambda P450 spectrophotometer (Perkin Elmer, USA).⁷

Statistical Analysis

The results of the studies were analyzed using Statistica PL 13 Trial (StatSoft). The Shapiro–Wilk test was used to check the normality of the variables' distributions, revealing some non-normal distributions. Each analyzed parameter was characterized by arithmetic mean, median, standard deviation, sample size, and lower and upper quartile (except for biochemical parameters). Kruskal–Wallis rank ANOVA was used for non-parametric, unrelated variables to assess differences between study parameters. The *U*-test was used for post-hoc analysis of study parameters, while Fisher's exact test and Chi-square tests were used to analyze qualitative data. Spearman's rank-sum coefficient measured parameter correlations. A multivariate regression model was used to evaluate the associations between parameters, with GSH concentration, GST, and R-GSSG activities as dependent variables, and various independent variables. Significance was set at $p < 0.05$.

Results

Antioxidant Levels in Erythrocyte Samples Between COVID-19 Patients Considering the Time of Blood Collection and Control Subjects

The study found a significant correlation between the concentrations and activities of GSH, GST, and R-GSSG in both COVID-19 patients, categorized by time since virus detection, and a control group, with significance levels of $p < 0.001$, $p = 0.046$, and $p < 0.001$, respectively. GSH levels were significantly higher in the control group and appeared to be at their lowest 28 days after virus detection among patients (Figure 1). Conversely, R-GSSG activity was substantially elevated in the patient group compared to the control group, with the most pronounced activity observed at the initial detection of the virus and 7 days thereafter (Figure 2). GST activity peaked at the onset of COVID-19, contrasting with its lowest levels in the control group (Figure 3). The comprehensive results, including mean values, standard deviations, medians, and quartile ranges for the antioxidant enzymes studied, are detailed in Table 3.

Furthermore, the study indicated a statistically significant difference in GSH concentrations in the study group at different sampling times - at the time of disease onset and 7, 14, and 28 days after disease detection, with a p-value of

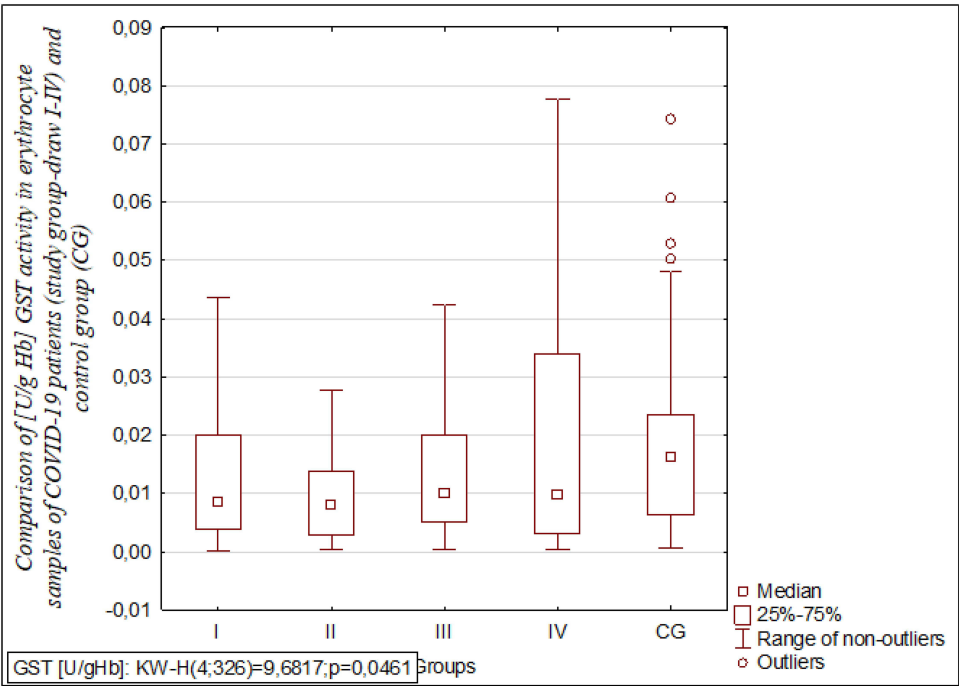


Figure 1 Comparison of the GSH concentration [μmol/g Hb] in erythrocyte samples of COVID-19 patients (study group-draw I–IV) and control group (CG); $p<0,001$).

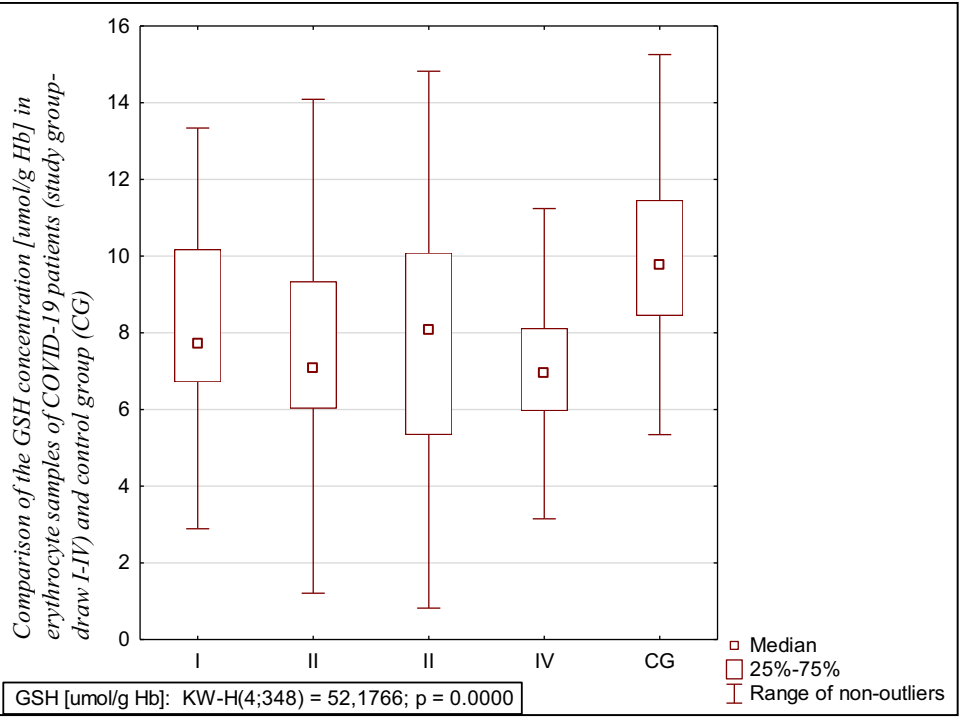


Figure 2 Comparison of [U/g Hb] GST activity in erythrocyte samples of COVID-19 patients (study group-draw I–IV) and control group (CG); $p=0,046$).

0.0355 (Figure 4). The lowest GSH concentration was observed 28 days following disease detection, while the highest concentration occurred 14 days after viral detection.

A post-hoc analysis was conducted to compare individual enzyme results between the study and control groups, as well as across different collection points within the study group (I–IV). Table 4 presents statistically significant findings.

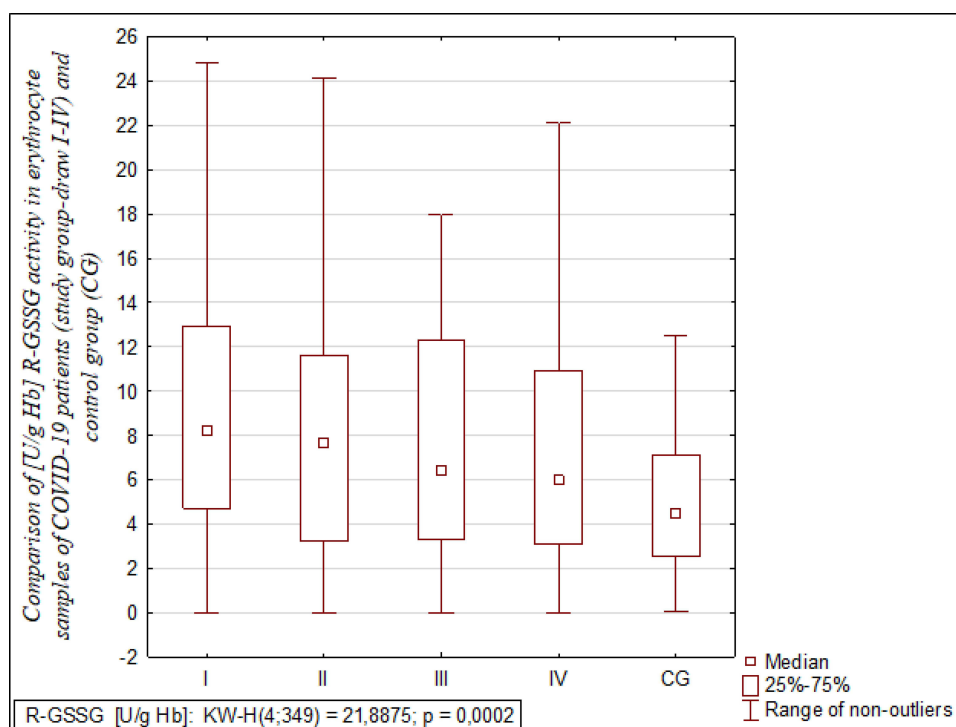


Figure 3 Comparison of [U/g Hb] R-GSSG activity in erythrocyte samples of COVID-19 patients (study group-draw I-IV) and control group (CG); $p=0,002$.

Antioxidant Levels in Erythrocyte Samples Between COVID-19 Patients Considering Disease Course

Statistical analysis revealed a significant relationship between the severity of the disease and glutathione (GSH) levels, with a p -value of 0.0369. This finding was drawn from data comparing patients experiencing mild, moderate, and severe progressions of COVID-19. It was demonstrated that glutathione levels decreased with the severity of the COVID-19 course (Figure 5).

Antioxidant Levels in Erythrocyte Samples Between COVID-19 Patients, Considering Patients' Age

A significant relationship was found between glutathione (GSH) levels and patients' age ($p = 0.0016$). The highest GSH levels were observed in patients aged <30 years, and the lowest levels were in patients above 60 years (Figure 6).

Table 3 The Comparison Between GSH Concentration and the Activities of GS, R-GSSG in the Study and Control Groups

Parameter	Study Group (I Draw)	Study Group (II Draw)	Study Group (III Draw)	Study Group (VI Draw)	Control Group	p-value
GSH [umol/g Hb]	14,8±57,9 7,7 6,7; 10,2	7,9±3,8 7,1 6,0; 9,3	8,0±3,9 8,1 5,4; 10,1	7,1±2,1 6,9 6,0; 8,1	10,1±2,9 0,01 8,5; 11,5	$p<0,001$
GST [U/g Hb]	0,04±0,1 0,01 0,004; 0,04	0,03±0,04 0,01 0,003; 0,02	0,02±0,03 0,01 0,06; 0,02	0,03±0,1 0,01 0,003; 0,04	0,02±0,03 0,02 0,01; 0,02	$p=0,046$
R-GSSG [U/g Hb]	9,9±7,1 8,3 4,7; 12,9	8,8±7,7 7,7 3,2; 11,6	8,3±6,8 6,4 3,3; 12,3	9,5±14,2 6,0 3,1; 10,9	5,6±5,2 4,5 2,5; 7,1	$p<0,001$

Notes: Mean ± standard deviation, median; lower, upper quartile.

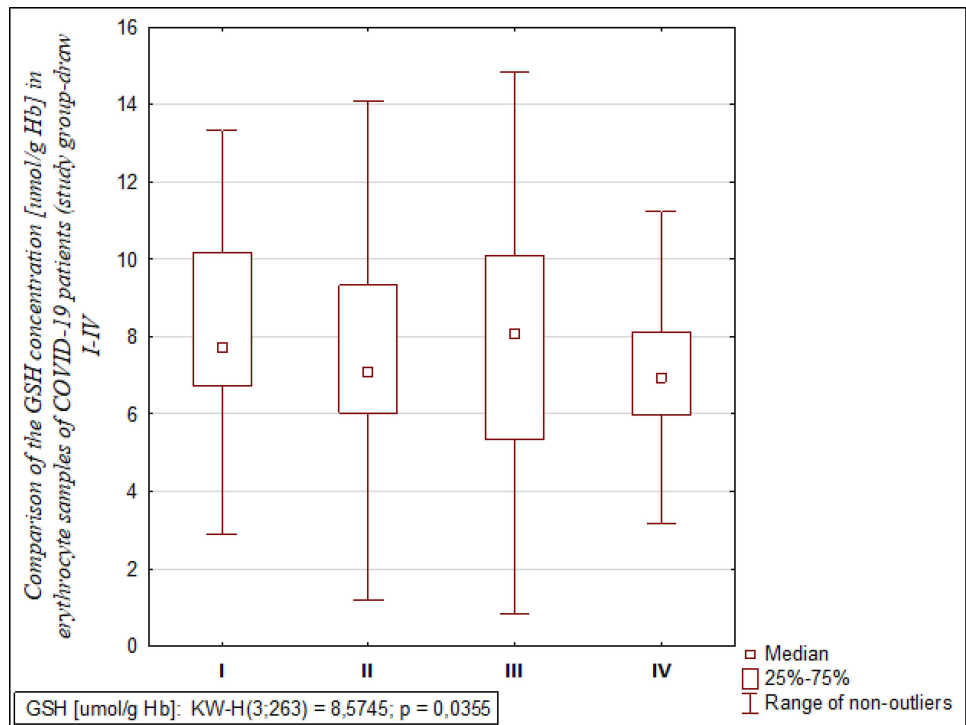


Figure 4 Comparison of the GSH concentration [umol/g Hb] in erythrocyte samples of COVID-19 patients (study group-draw I-IV); p=0,036.

Additionally, a significant correlation was found between R-GSSG activity and patients' age ($p = 0.046$). The enzyme activity appeared the highest in patients >60 years and lowest in those <30 (Figure 7).

Antioxidant Levels in Erythrocyte Samples Between COVID-19 Patients Considering the Occurrence of Patient Death

Notably, a significant dependence was found between GSH concentration ($p = 0.0079$) and GST activity ($p = 0.0446$) and mortality in COVID-19 patients. GSH concentration was lower in patients who died from coronavirus (Figure 8). GST activity tended to be significantly lower in survivors (Figure 9). R-GSSG activity analysis indicated no differential activity, either in survivors or those who died from COVID-19 ($p=0.0844$).

Spearman Rank Correlation Analysis

Spearman's rank correlation coefficients for the test and control groups were used to determine whether there were correlations between the activities of the tested antioxidants.

Table 4 Post-Hoc Analysis Results

Parameter	Compared Groups	p-value
GSH	Draw I vs CG	$p<0,001$
	Draw II vs draw CG	$p<0,001$
	Draw III vs draw CG	$p<0,001$
	Draw IV vs draw CG	$p<0,001$
	Draw I vs CG	$p<0,001$
	Draw II vs draw CG	$p=0,021$

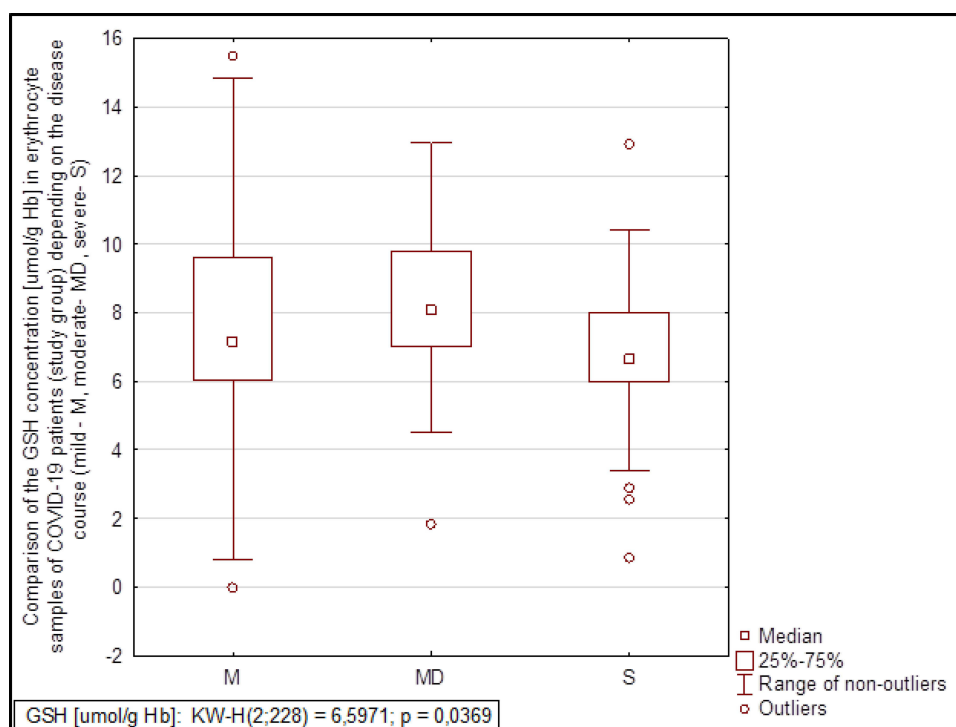


Figure 5 Comparison of the GSH concentration [umol/g Hb] in erythrocyte samples of COVID-19 patients (study group) depending on the disease course (mild - M, moderate- MD, severe- S).

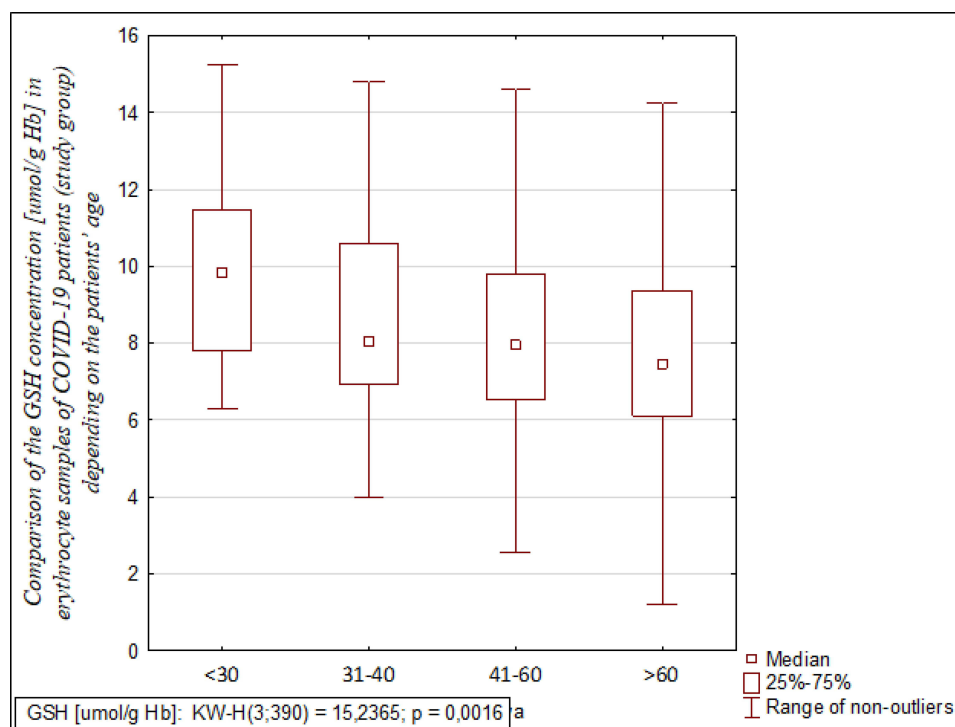


Figure 6 Comparison of the GSH concentration [umol/g Hb] in erythrocyte samples of COVID-19 patients (study group) depending on the patients' age; p=0,016.

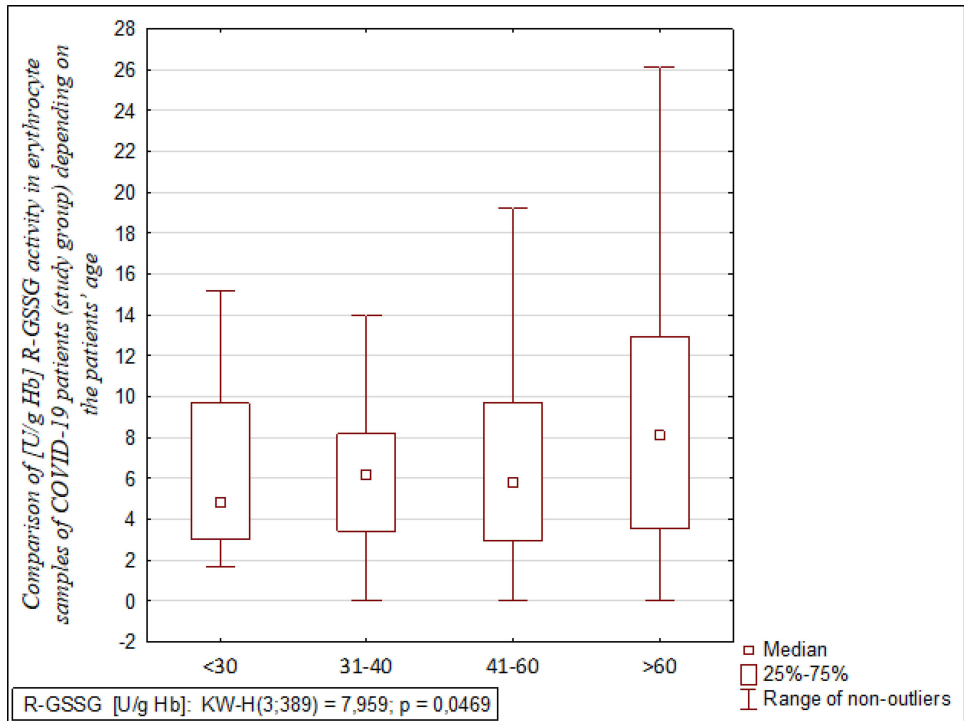


Figure 7 Comparison of [U/g Hb] R-GSSG activity in erythrocyte samples of COVID-19 patients (study group) depending on the patients' age; $p=0,047$.

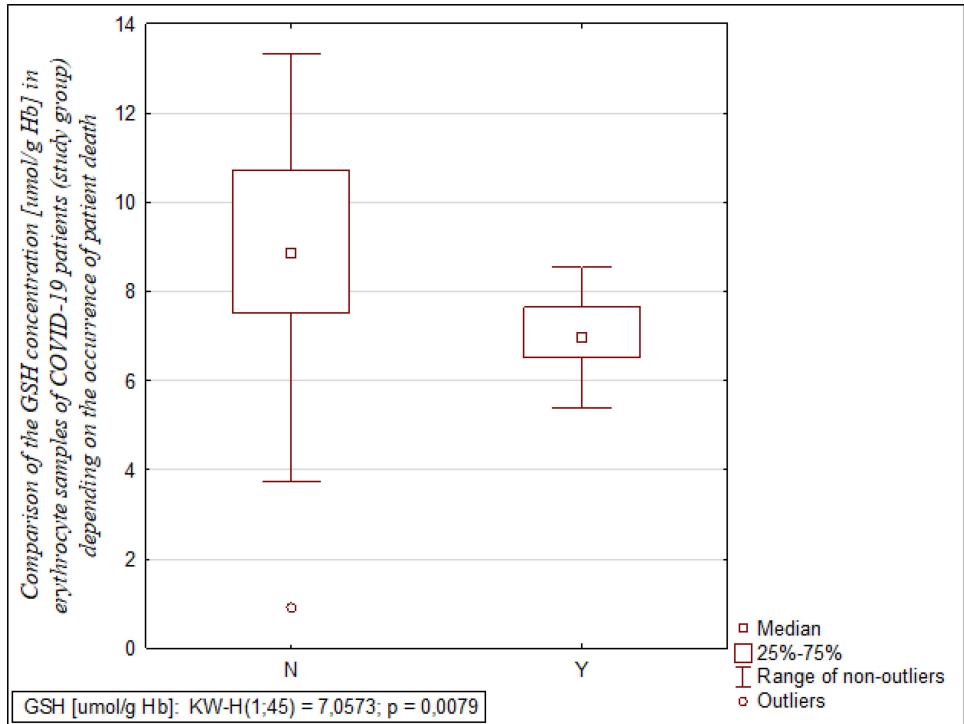


Figure 8 Comparison of the GSH concentration [umol/g Hb] in erythrocyte samples of COVID-19 patients (study group) depending on the occurrence of patient death; $p=0,008$.

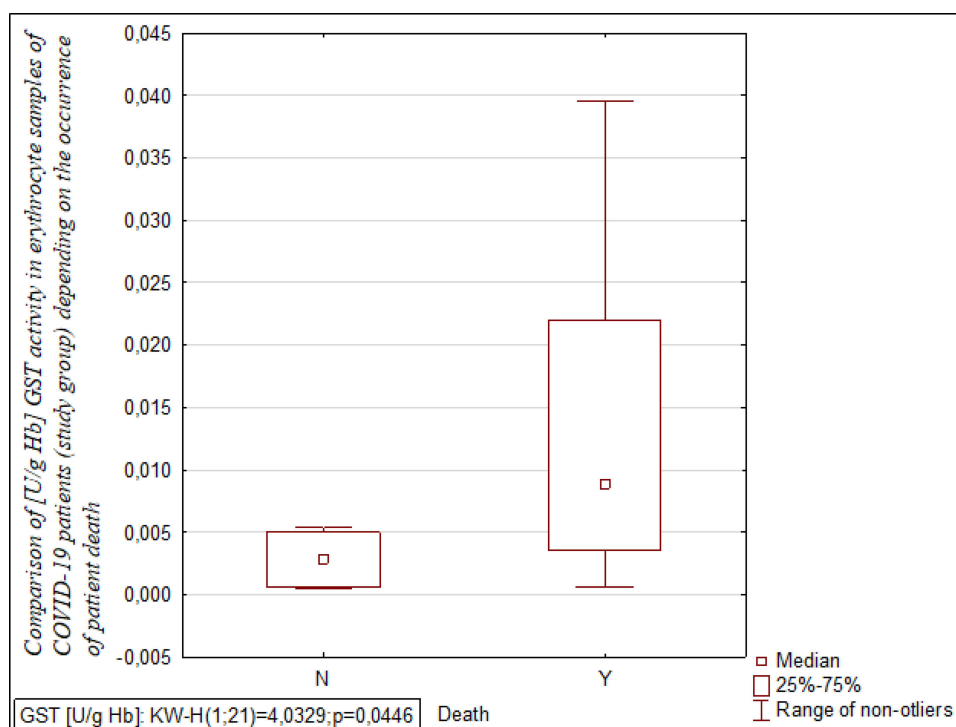


Figure 9 Comparison of [U/g Hb] GST activity in erythrocyte samples of COVID-19 patients (study group) depending on the occurrence of patient death; $p=0,045$.

A positive correlation was found between GSH concentration [$\mu\text{mol/g Hb}$] and R-GSSG activity ($R_s = 0.222$, $p < 0.001$) in the study and control groups. In the case of determining the correlation coefficient solely for the study group, a positive correlation was observed between GSH concentration and GST activity ($R = 0.137$, $p < 0.001$) and between GSH concentration and R-GSSG activity ($R = 0.375$, $p < 0.001$).

A correlation analysis was also performed based on the time elapsed since the disease was detected (draw I–IV). Table 5 presents statistically significant positive correlations between GSH and GST, as well as R-GSSG levels.

GSH concentration and R-GSSG activity were positively correlated with patient death ($R_s = 0.226528$, $p < 0.001$; $R_s = 0.18639$, $p < 0.001$, respectively).

Multivariate Regression Analysis

Multivariate regression analysis examined the effects of independent variables, including age, gender, time since disease detection, survival rate, duration of hospitalization, and disease severity, on the dependent variables: GSH concentration, GST activity, and R-GSSG.

Table 5 Spearman's Rank Correlation Coefficients Between the Concentration/Activity of Individual Antioxidants for the Study Group, Considering Individual Material Intakes

	Draw Number		GSH [$\mu\text{mol/g Hb}$]	GST [U/g Hb]	R-GSSG [U/g Hb]
GSH [$\mu\text{mol/g Hb}$]	I	R_s p-value	-	0,14 $p < 0,001$	0,4 $p < 0,001$
	II	R_s p-value	-	NS	0,4 $p < 0,001$
	IV	R_s p-value	-	0,4 $p < 0,001$	0,4 $p < 0,001$

Abbreviation: NS- non statistical.

Table 6 Results of the Analysis of the Influence of the Studied Parameters on the Level of GSH, GST, and R-GSSG - Multivariate Regression Analysis

	Independent Variables	β	R^2	p	P for model	F
GSH	Time since disease detection	−0,3	0,2	0,04	NS	2,1
	Sex	0,1		0,33		
	Age	0,1		0,33		
	Death	−0,3		0,04		
	Hospitalization duration	−0,3		0,02		
	Disease course	0,4		0,02		
R-GSSG	Time since disease detection	−0,2	0,18	NS	0,04	0,8
	Sex	0,2		NS		
	Age	0,1		NS		
	Death	0,2		NS		
	Hospitalization duration	−0,1		NS		
	Disease course	−0,2		NS		

Notes: β – standardized coefficient in the regression equation, R^2 – coefficient of determination, p – significance factor value.
Abbreviation: NS, non-statistical.

The analysis revealed that GSH levels are approximately 16% influenced by time since disease diagnosis, survival rate, duration of hospitalization, and disease course. It was noted that GSH levels decrease by 0.29 and 0.33 $\mu\text{mol/g}$ hemoglobin (Hb) over time since diagnosis and with prolonged hospitalization. In people who died, the GSH level was lower by an average of 0.25 $\mu\text{mol/g}$ Hb. Moreover, it was determined that depending on the disease course, the GSH concentration changed by approximately 0.38 $\mu\text{mol/g}$ Hb.

R-GSSG activity is influenced by time since disease detection, age, sex, survival, length of hospitalization, and severity of disease course in approximately 18% of cases. No statistically significant relationships were detected for GST. The statistically significant results are shown in Table 6.

Discussion

Since the COVID-19 pandemic began, over 6 million people have died worldwide, and the full consequences of SARS-CoV-2 infection are still not fully understood.⁸ The rapid mutation of the virus complicates treatment, diagnosis, and prevention efforts. At the same time, emerging variants and diverse clinical presentations pose a challenge to the development of effective diagnostic methods, which are crucial for combating the pandemic.^{9,10}

Recent studies have increasingly highlighted the central role of oxidative stress in the pathogenesis of COVID-19 and its complications. Oxidative stress not only modulates immune responses but also amplifies viral replication and tissue injury.^{11,12} Viral infections, such as hepatitis B and C, have previously been associated with decreased antioxidant capacity, as indicated by lower serum levels of glutathione, vitamin C, and vitamin E compared to those of healthy individuals.¹³ These findings underscore the broader relevance of oxidative stress in viral pathologies.

Regarding COVID-19, reactive oxygen species (ROS) may contribute to disease severity by promoting viral proliferation and cell lysis, thereby enhancing viral replication.¹⁴ Clinical observations have consistently reported decreased plasma glutathione (GSH) levels and antioxidant enzyme activities (superoxide dismutase [SOD], catalase [CAT], glutathione peroxidase [GPx]) in patients with severe COVID-19.^{15,16} Importantly, factors associated with

increased oxidative stress, such as advanced age, male sex, obesity, and metabolic dysfunction, also predict worse COVID-19 outcomes and higher mortality.^{17,18}

Given these findings, investigating the activity of antioxidant enzymes as potential prognostic markers of survival in COVID-19 is of particular interest. Endogenous glutathione deficiency and impaired antioxidant defense systems have been increasingly recognized as central to the pathogenesis of many diseases, including COVID-19, through their impact on oxidative stress and inflammatory pathways.^{19,20} Understanding the dynamic changes in antioxidant enzyme activities during SARS-CoV-2 infection may offer valuable insights into disease severity, prognosis, and the development of targeted therapeutic interventions.

A study by Pincemail et al found reduced GSH levels in ICU patients with severe COVID-19 pneumonia, particularly in long-term residents. This deficiency was linked to surfactant abnormalities in the lungs. Normal GSH levels help reduce pro-inflammatory cytokines, and their excess in COVID-19 can trigger an abnormal immune and inflammatory response.²¹ Similarly, Žarković et al observed that patients who died from COVID-19 had decreased levels of reduced glutathione (GSH) and increased oxidized glutathione (GSSG). This imbalance was associated with lower levels of lipid-soluble antioxidants, such as vitamins A and E, exacerbating cellular oxidation, disease severity, and susceptibility to infection.²² Moghimi et al also reported that deceased COVID-19 patients had lower glutathione (GSH) levels and increased reactive oxygen species (ROS) production in their cell nuclei compared to healthy controls. They suggest that SARS-CoV-2 infection may trigger apoptosis through elevated oxidative stress, contributing to tissue damage in cytokine storm scenarios.^{11,23}

The reduced antioxidant barrier in patients with COVID-19 has been further confirmed by Muhammad et al, who found lower levels of GSH, GPx, and SOD in these patients compared to healthy volunteers.²⁴ Antioxidant enzymes play a crucial role in neutralizing oxidative stress, and their diminished levels indicate an excessive production of reactive oxygen species (ROS). Qin et al demonstrated that SARS-CoV-2 infection activates phagocytes, leading to the overproduction of reactive oxygen species (ROS) and further compromising the antioxidant defense system.²⁵

Researchers have observed that COVID-19 has a more severe impact on older individuals. Kumar et al reported that GSH levels decrease with age, with the highest levels observed in individuals aged 21 to 40 and the lowest in those over 60, suggesting a possible link with the severity of infection in the elderly.²⁶ Kryukov et al studied 56 COVID-19 patients hospitalized in a pulmonology department and found that those with more severe disease had lower GSH levels than those with milder symptoms. This reduction affects Na^+/H^+ antiport function, lowering intracellular pH and promoting viral endocytosis and replication, highlighting a key biochemical pathway in COVID-19 severity.²⁷ Polonikov also emphasized that glutathione deficiency worsens the course of COVID-19,¹⁵ while other studies underscore the role of GSH in inhibiting viral replication and supporting immune responses through T-lymphocyte proliferation. Low GSH levels impair the body's immune defense, leading to a more severe disease course.²⁸ GSH scavenges ROS and detoxifies hydroperoxides and lipid peroxides through glutathione peroxidase activity. Franco et al suggest that GSH depletion may promote ROS generation and initiate apoptotic cell death processes.²⁹

Our study confirmed the importance of glutathione (GSH) levels in the context of COVID-19. Participants had lower GSH levels than healthy individuals, consistent with previous findings.^{21,22,30} The observed decrease in GSH may be linked to SARS-CoV-2's impact on respiratory cells and surfactant production.²² GSH is crucial in antioxidant defense and the reduction of pro-inflammatory cytokines. Low GSH levels were associated with more severe disease and higher mortality, with significant reductions seen in severe cases. Regression analysis showed that GSH decreased by 0.29–0.33 $\mu\text{mol/g}$ Hb over time and by 0.25 $\mu\text{mol/g}$ Hb in those who died, with severity correlating to a decrease of 0.38 $\mu\text{mol/g}$ Hb.

Moreover, our findings align with those of Kumar et al²⁶ and other studies, which demonstrate that GSH levels decrease with age, potentially due to reduced synthesis resulting from deficiencies in amino acid precursors, such as glycine and cysteine.³⁰ This deficiency may contribute to oxidative stress, weaken the immune system, and reduce resistance to viral infections.³¹

Recent reviews highlight the crucial role of oxidative stress in determining COVID-19 severity, suggesting that targeting antioxidant systems may hold therapeutic potential. Data indicate a significant GSH deficiency in severe COVID-19 cases, along with reduced activity of antioxidant enzymes such as GPx, SOD, and GR. This leads to an

increased production of reactive oxygen species (ROS), resulting in cellular damage, heightened inflammation, and impaired immunity. GSH deficiency may also trigger a cytokine storm linked to serious complications. Monitoring GSH levels can be vital for assessing risk in COVID-19, and restoring redox balance through GSH precursor supplementation may offer promising treatment options.

Studies conducted by Hosakote et al have shown that during RSV infection, the activity of antioxidant enzymes such as GST, CAT, and GPx decreases, while SOD activity increases. Initially, GST activity rises in infected cells but then declines. The reduced activity of GST, CAT, and GPx, combined with elevated SOD levels, suggests that RSV infection may lead to excessive H₂O₂ production that the body's defense mechanisms cannot neutralize.³²

The study found that GST activity increased as the disease progressed, reaching significantly higher levels 28 days after SARS-CoV-2 infection compared to the first day. Individuals who died from COVID-19 had higher GST activity, which may be related to the overproduction of cytokines and ROS. This increase in GST activity coincided with a decrease in GSH levels, highlighting its role in protecting the body from harmful substances. Glutathione reductase (GR), which reduces oxidized glutathione to glutathione (GSH), plays a crucial role in the glutathione redox cycle and has become a subject of intense global research.³³

A study by Valente et al assessed oxidative stress (OS) and inflammatory biomarkers in individuals with post-COVID-19 syndrome (PASC). Participants were divided into three groups: healthy individuals, patients with acute COVID-19 (symptoms lasting less than 3 weeks), and patients with PASC (symptoms lasting more than 12 weeks). Findings indicated that patients with PASC had elevated levels of IL-6 and IL-8. Both COVID-19 groups showed decreased SOD and CAT activity, while GST activity was reduced only in the acute phase group. The PASC group exhibited higher levels of GGT, GSH, and uric acid, along with elevated protein carbonyls. Correlations between inflammatory markers and OS parameters suggest that PASC individuals experience significant oxidative stress, which may exacerbate disease complications.³⁴

In the study by Labarrere and Kassab, the authors emphasize the significance of glutathione (GSH) deficiency in SARS-CoV-2 infection and its impact on the immune response in severe COVID-19. They note that GSH depletion contributes to increased disease severity and mortality by promoting the production of excessive reactive oxygen species (ROS), activating inflammatory pathways (NF- κ B), and elevating the levels of pro-oxidant enzymes. The authors suggest that therapies targeting increased glutathione (GSH) levels may be crucial for mitigating the severity and mortality of COVID-19.³⁵

Studies conducted by Orlewska et al found that in patients with a prior COVID-19 vaccination, the GSTP1 Ile/Val genotype was linked to an increased risk of developing a severe form of the disease (odds ratio: 2.75; $p = 0.0398$). This suggests that GST gene polymorphisms may influence the progression of COVID-19 in the body's antioxidant response.³⁶

Additionally, an analysis by Coricia et al 2021 revealed that individuals carrying GSTP1 rs1695 and GSTM3 variants have an increased risk of both contracting COVID-19 and experiencing a more severe disease course. This suggests that specific GST gene polymorphisms may influence susceptibility to SARS-CoV-2 infection and its severity.³⁷

Our findings align with existing literature, showing significant changes in antioxidant enzyme activity during SARS-CoV-2 infection. We observed increased GST activity in later disease stages, likely due to the rise in reactive oxygen species (ROS) and cytokines. Decreased GSH levels indicate compromised defenses, potentially leading to cellular damage. Reduced GR activity suggests disturbances in the glutathione redox cycle, which can impact disease progression. Overall, our results underscore the role of oxidative stress in COVID-19 and emphasize the importance of monitoring antioxidant parameters for effective patient care.

Our research, building on the work of Moisejevs et al and Kim et al, has shed light on the complex relationship between R-GSSG activity and disease outcomes. We examined 65 patients with septic shock and found that the 29 deceased individuals exhibited significantly higher plasma R-GSSG activity, indicating an excessive inflammatory response. In contrast, Kim et al observed an opposite trend in a rat model, where lower R-GSSG activity correlated with increased mortality.^{38,39} This discrepancy highlights the complex nature of immune responses and underscores the need for further investigation into this area. The literature provides no clear consensus regarding R-GSSG activity during

infections, with some studies suggesting that Gram-negative bacteria may increase R-GSSG activity and glutathione synthesis. In contrast, others report decreased levels of this enzyme.³⁸

In our study of COVID-19 patients, we observed elevated R-GSSG activity in deceased individuals, which may reflect a systemic and potentially fatal inflammatory response. However, determining a direct relationship between R-GSSG activity and specific pathogens remains challenging due to conflicting research outcomes. For instance, Quaye et al reported that lower GSH and R-GSSG levels in HIV-infected patients enhanced oxidative stress and promoted viral replication. They also demonstrated that increased antioxidant enzyme activity may benefit HIV patients, with antioxidant supplementation reducing lipid peroxidation.⁴⁰ These findings present intriguing possibilities for potential treatments and further research in the fields of immunology and infectious diseases.

Similarly, Naghashpour et al analyzed 52 outpatients with COVID-19 and found significantly lower glutathione reductase activity compared to healthy controls. This finding highlights disrupted redox balance and the accumulation of reactive oxygen species (ROS) in COVID-19, suggesting that the antioxidant system plays a crucial role in mitigating inflammation and preventing organ failure.⁴¹ Our study also demonstrated increased R-GSSG activity in patients infected with SARS-CoV-2. These results underscore the need for further research to fully understand the role of R-GSSG activity in disease progression and recovery.

It is well established that SARS-CoV-2 elevates ROS production and impairs antioxidant defenses, promoting viral replication and exacerbating clinical symptoms. Infected individuals exhibit elevated cytokine and chemokine levels, which can result in a cytokine storm, severe inflammation, tissue damage, and, ultimately, death. In this context, increased R-GSSG activity may represent an effort to regenerate GSH and counter oxidative stress. Ehtiati et al, studying HTLV-1-infected patients, found higher R-GSSG and GSH levels in healthy controls than in infected individuals, suggesting that decreased R-GSSG activity may hinder glutathione regeneration, aligning with our findings.⁴² Moreover, a gradual increase in R-GSSG activity post-infection may indicate a recovery-related restoration of glutathione-reducing capacity and detoxification processes.

Pavlova et al studied 27 hospitalized COVID-19 patients and 30 healthy controls, examining oxidative stress biomarkers, plasma antioxidant activity, and inflammatory markers (CRP, WBC, ESR). They found that COVID-19 is linked to significant oxidative stress and impaired antioxidant balance, which may worsen disease progression and increase mortality risk. The authors noted that biomarkers such as TBARS, AOC, SOD, CAT, and GRA could have both prognostic and diagnostic value in COVID-19.¹⁶

Silvagno et al proposed that glutathione deficiency may increase the risk of severe COVID-19, especially in the elderly and those with conditions like diabetes, cardiovascular disease, and obesity. They suggested that supplementing with glutathione precursors, such as N-acetylcysteine (NAC), could help reduce inflammation and oxidative stress, making it a potentially valuable adjunctive treatment for COVID-19.⁵

In another study conducted at Clinical Hospital Dubrava (Zagreb), elevated GR activity in COVID-19 patients with lung involvement was interpreted as a compensatory mechanism responding to increased oxidative stress, aiming to restore redox homeostasis.²²

Recent studies highlight antioxidant system dysfunction in severe COVID-19 cases, revealing the adverse effects of oxidative stress on disease progression. This includes decreased levels of glutathione enzymes, such as glutathione reductase, and increased cellular oxidation markers. Research on Long COVID (PASC) points to ongoing redox imbalance and mitochondrial dysfunction, which may lead to chronic symptoms like fatigue and neurological issues. Our findings deepen the understanding of oxidative stress's role in COVID-19 and contribute to the scientific community's knowledge.⁴³

Spearman rank correlation analysis revealed a positive relationship between GSH levels and GST activity, confirming their interaction as antioxidant components. GSH is vital for detoxification through the action of GST. We also found a positive correlation between GSH concentration and R-GSSG activity, highlighting the role of glutathione reductase in regenerating reduced glutathione. Our study showed that COVID-19 patients had lower GSH levels but higher GST and R-GSSG activity compared to healthy volunteers, indicating these systems collaborate to protect cells from oxidative stress.⁴⁴

Crucially, the most recent research further supports the key role of antioxidant system dysfunction in severe COVID-19. Aghadei et al demonstrated disrupted extracellular and intracellular redox states in severe COVID-19, with a significant reduction in the GSH/GSSG ratio—a prognostic marker for disease severity and mortality risk.⁴⁵ Moreover, a meta-analysis assessing the effect of N-acetylcysteine (NAC) confirmed its positive impact on key inflammatory parameters (eg, CRP, D-dimers) and improvement in the PaO₂/FiO₂ ratio, suggesting clinical benefits for COVID-19 prognosis.⁴⁶

Research shows that glutathione reductase (R-GSSG/GR) activity increases in COVID-19 patients during both the acute phase and recovery, likely as a response to oxidative stress. In contrast, those with post-COVID syndrome experience reduced total antioxidant capacity and an imbalance in redox status. The interplay between glutathione (GSH), oxidized glutathione (GSSG), and glutathione S-transferase (GST) is crucial for detoxification and the management of oxidative stress. Studies suggest that enhancing antioxidant defenses may benefit both acute COVID-19 cases and their long-term effects.

Conclusions

The results of this study demonstrate that COVID-19 infection disrupts redox homeostasis by markedly decreasing glutathione (GSH) levels, while simultaneously increasing the activity of glutathione reductase (R-GSSG) and glutathione S-transferase (GST). This biochemical profile indicates an adaptive cellular response designed to counteract oxidative stress. The reduction in GSH concentrations appears to contribute to more severe disease progression and higher mortality risk, particularly among older patients, suggesting that GSH depletion may serve as a potential prognostic marker. Furthermore, R-GSSG activity was found to increase with advancing age and showed a positive correlation with mortality, highlighting its relevance as an age-sensitive indicator of disease severity. In parallel, GST activity exhibited a progressive increase over the course of infection and was also associated with poorer outcomes, reflecting the organism's intensified detoxification response under sustained oxidative pressure. The observed relationships between GSH, R-GSSG, and GST highlight the crucial role of the antioxidant system in modulating inflammatory responses and protecting cells from oxidative stress. Taken together, these findings support the potential clinical benefit of antioxidant-based therapeutic approaches, including supplementation with glutathione precursors, as a means of mitigating oxidative damage and improving patient outcomes both during acute illness and in the post-infectious period.

Data Sharing Statement

All data generated or analyzed during this study are included in this published article.

Ethical Statement

All subjects gave informed consent for inclusion before participating in the study. The study was conducted by the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Pomeranian Medical University (No. KB-0012/83/2020).

Author Contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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