Medicinski podmladak

Medical Youth

ORIGINAL ARTICLE

THE ASSOCIATION OF COMMON GLUTATHIONE S-TRANSFERASES POLYMORPHISMS WITH INFLAMMATORY AND MULTIORGAN IMPAIRMENT BIOMARKERS IN COVID-19

POVEZANOST POLIMORFIZAMA GLUTATION S-TRANSFERAZA SA BIOMARKERIMA INFLAMACIJE I MULTIORGANSKOG OŠTEĆENJA U COVID-19

Ana Krunić[1], Đurđa Jerotić[1,2], Marija Matić[1,2]

[1] Univerzitet u Beogradu, Medicinski fakultet, Beograd, Srbija
[2] Univerzitet u Beogradu, Medicinski fakultet, Institut za medicinsku i kliničku biohemiju, Beograd, Srbija

Correspondence: ana_krunic@yahoo.de

Abstract

Introduction: Due to the established role of oxidative stress in the pathophysiology of COVID-19, it has been proposed that inter-individual differences in patients' clinical manifestations might be affected by variations in genes encoding antioxidant enzymes, such as glutathione S-transferases (GSTs).

Aim: The aim of this study was to assess the association of polymorphisms in cytosolic GSTs (*GSTA1* rs3957357, *GSTM3* rs1332018 and *GSTP1* rs1695) with inflammatory parameters (leukocytes, lymphocytes, monocytes, C-reactive protein (CRP), fibrinogen, ferritin) and multiorgan impairment biomarkers (urea, creatinine, AST, ALT, LDH) in COVID-19 patients at two-time points.

Material and methods: *GSTM3, GSTA1* and *GSTP1* genotypes were determined in 150 COVID-19 patients by appropriate polymerase chain reaction (PCR) methods.

Results: Inflammatory biomarkers (leukocytes, lymphocytes, monocytes) increased 7 days upon admission to the hospital ($p < 0.001$), while CRP and fibrinogen decreased ($p < 0.001$). Out of five analyzed multiorgan impairment biomarkers, only urea increased significantly 7 days upon admission ($p < 0.007$), while AST showed a statistically significant drop ($p < 0.001$). COVID-19 patients homozygous for variant *GSTM3*C/C* genotype had increased levels of inflammatory biomarkers such as CRP, fibrinogen and ferritin, but the borderline significance was observed only for fibrinogen ($p = 0.057$). COVID-19 patients homozygous for variant *GSTM3*C* allele had the highest levels of ALT ($p = 0.021$) and LDH ($p = 0.045$) upon admission.

Conclusion: Our results on the association between *GSTM3* variant genotype with parameters of systemic inflammation and liver damage in COVID-19 patients can contribute to further understanding of pathophysiological mechanisms underpinning this disease, as well as early recognition of COVID-19 patients prone to worse course of the disease.

Keywords:
COVID-19,
oxidative stress,
glutathione
S-transferases,
polymorphism

The authors declare no conflicts of interest.

doi:10.5937/mp74-39841
Editorial board: podmladak.med.bg@gmail.com
e-ISSN: 2466-5525

Sažetak

Uvod: Imajući u vidu značajnu ulogu koju oksidativni stres ima u patofiziologiji COVID-19, može se pretpostaviti da razlike u kliničkim manifestacijama bolesti kod ovih pacijenata mogu biti posledica varijacija u genima koji kodiraju antioksidantne enzime, kao što su glutation S-transferaze (GST).

Cilj: Cilj ove studije bio je da se ispita povezanost polimorfizama citosolnih GST (*GSTA1* rs3957357, *GSTM3* rs1332018 i *GSTP1* rs1695) sa pokazateljima zapaljenja (leukociti, limfociti, monociti, C-reaktivni protein (CRP), fibrinogen, feritin) i biomarkerima multiorganskog oštećenja (urea, kreatinin, aspartat aminotransferaza (AST), alanin aminotransferaza (ALT), laktat-dehridrogenaza (LDH)) kod COVID-19 pacijenata u dva vremena.

Materijal i metode: Polimorfizmi *GSTM3, GSTA1* i *GSTP1* gena su određeni kod 150 COVID-19 pacijenata odgovarajućim metodama reakcije lančanog umnožavanja DNK (engl. *Polymerase chain reaction* - PCR).

Rezultati: Pokazatelji zapaljenja (leukociti, limfociti, monociti) su porasli 7 dana nakon prijema u bolnicu ($p < 0,001$), dok su CRP i fibrinogen bili smanjeni ($p < 0,001$). Od pet analiziranih biomarkera multiorganskog oštećenja, samo urea se značajno povećala 7 dana nakon prijema ($p < 0,007$), dok je AST pokazala statistički značajan pad ($p < 0,001$). Pacijenti sa COVID-19 homozigoti za varijantni *GSTM3*C/C* genotip imali su povećane nivoe inflamatornih biomarkera kao što su CRP, fibrinogen i feritin, ali je granična značajnost pokazana samo za fibrinogen ($p = 0,057$). Pacijenti sa COVID-19 homozigoti za varijantni *GSTM3*C* alel imali su najviše nivoe ALT ($p = 0,021$) i LDH ($p = 0,045$) u trenutku prijema u bolnicu.

Zaključak: Naši rezultati o povezanosti *GSTM3* varijantnog genotipa sa pokazateljima sistemske inflamacije i oštećenja jetre kod pacijenata sa COVID-19 mogu doprineti daljem razumevanju patofizioloških mehanizama koji su u osnovi ove bolesti, kao i ranom prepoznavanju pacijenata sa COVID-19 sklonim pogoršanju toka bolesti.

Ključne reči:
COVID-19,
oksidativni stres,
glutation
S-transferaze,
polimorfizam

Introduction

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus, discovered in Wuhan in December 2019, has led to a still ongoing pandemic (1). The SARS-CoV-2 belongs to the group of coronaviruses. It enters the body via its Spike protein binding to Angiotensin-converting enzyme 2 (ACE2) which can be found on various cells, such as epithelial cells of the lung and intestine, cardiac myocytes, arterial and venous endothelial cells (2). Hence, respiratory failure, colitis, microvascular injury and inflammation are frequent characteristics of coronavirus disease 2019 (COVID-19) (1). Symptoms of COVID-19 vary from mild (cough, loss of smell and taste) to life-threatening conditions (3). Patients with severe COVID-19 might develop acute respiratory distress syndrome (ARDS), systemic inflammatory response syndrome (SIRS), and/or multiple organ failure, which are accompanied by high mortality (4).

Overproduction of reactive oxygen species (ROS) by the mitochondrial electron transport chain can generate increased oxidative stress favouring viral replication and lung tissue injury, initiating free radical damage of lipids and proteins (5). Elevated ROS levels serve as an early indicator of severe COVID-19 pathogenesis (6). Namely, ROS induce activation of transcription factors and pro-inflammatory genes which triggers immune cells to secrete various cytokines and chemokines, leading to additional ROS generation by immune cells. According to the recent findings of Kosanovic et al. (7), levels of redox biomarkers positively correlate with certain inflammatory and multiorgan impairment biomarkers in COVID-19 patients. In addition to the overproduction of ROS, COVID-19 is characterised by a failure of antioxidant mechanisms (8).

Protection against ROS includes enzymes such as glutathione S-transferases (GST). GSTs are a superfamily of enzymes which detoxify various xenobiotics but also have various antioxidant and anti-inflammatory roles (9–11). Therefore, the impaired function of GSTs increases the susceptibility to ROS and according to the latest reports, susceptibility to COVID-19 (12–14). The most important cytosolic GSTs include mi (GSTM), alpha (GSTA), pi (GSTP), and theta (GSTT) classes. Functional consequences of *GSTM3* A>C (rs1332018) and *GSTA1* (rs3957357) C>T single nucleotide polymorphisms (SNP) are decreased gene expression and consequently, the lower antioxidant protection of these enzymes in individuals carrying variant alleles. Additionally, the SNP in *GSTP1 Ile>Val* (rs1695) has functional relevance in terms of altered catalytic activity towards a variety of substrates and differences in GSTP1-mediated regulation of redox signalling pathways (15, 16). With regard to COVID-19, there are a few reports suggesting the association of GST polymorphisms with susceptibility and severity of COVID-19 (12–14).

Considering that oxidative stress has an important role in the pathophysiology of COVID-19, it is logical to assume that functional polymorphisms in antioxidant genes might influence inter-individual differences in the severity of COVID-19 clinical manifestations. Therefore, the aim of this study was to assess the association of

polymorphisms in cytosolic glutathione S-transferases (*GSTA1* rs3957357, *GSTM3* rs1332018 and *GSTP1* rs1695) with inflammatory and multiorgan impairment biomarkers in 150 COVID-19 patients on admission and seven days upon admission to the hospital.

Material and methods

Study group

The study group included 150 COVID-19 patients (80 (53%) men and 70 (47%) women, with an average age of 52.89 ± 11.89 years) recruited from the Institute of Infectious and Tropical Diseases, University Clinical Centre of Serbia, between July 2020 and February 2021. All patients were ≥18 years old with positive SARS-CoV-2 reverse transcription (RT)-PCR test performed from nasopharyngeal and oropharyngeal swabs. Patients gave their informed consent before they participated in the study. This case study was conducted in accordance with the ethical standards of the Declaration of Helsinki and the study protocol was approved by the Ethical Committee of the Clinical Centre of Serbia (566/01 from July 13, 2020 and 608/01 from August 7, 2020).

DNA Isolation and Glutathione S-Transferases Genotyping

A total DNA was isolated from EDTA-anticoagulated peripheral blood collected from the study participants using PureLink™ Genomic DNA Mini Kit (*ThermoFisher Scientific, United States*) and stored at -20°C.

Real-time PCR was used for the determination of *GSTM3* rs1332018 and *GSTP1* rs1695 polymorphisms on *Mastercycler® ep realplex* (*Eppendorf, Germany*), using TaqMan Drug Metabolism Genotyping assays *(Life Technologies, Applied Biosystems, United States*). Assays' IDs were as follows: C_3184522_30 and C_3237198_20, respectively. The PCR protocol was comprised of an initial denaturation step (95˚C, 4 min), followed by 40 cycles (95˚C for 15 s and 60˚C for 1 min). The results were visualized using *Mastercycler® ep realplex* software (*Eppendorf, Hamburg, Germany*).

GSTA1 rs3957357 polymorphism was determined by PCR–Restriction Fragment Length Polymorphism (PCR–RFLP). The primers sequences were: forward: 5'-GCATCAGCT TGCCCTTCA-3' and reverse: 5'-AAACGCTGTCACCGTCCT-3'. The PCR protocol included initial incubation at 95˚C for 1 min followed by 31 cycles of amplification reaction: 1) Denaturation of DNA sequence for 1 min at 94˚C, 2) Annealing of primers for 1 min at 62˚C, 3) Extension of primers for 1 min at 72˚C. The final elongation took place at 72˚C for 7 min.). Enzymatic digestion of amplified sequence was performed overnight at 37°C using EarI restriction enzyme (*Thermo Fisher Scientific, USA*). The restriction fragments of 481, 385 and 96 base pairs (bp) were separated on 2% agarose gel stained with SYBR® Safe DNA Gel Stain *(Invitrogen, USA*) and visualized on *Chemidoc* (*Biorad, USA*).

Statistical analysis

The statistical analyses were performed using IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA). Differences between the groups were compared using the $\chi 2$ test for categorical variables. All categorical variables were presented using frequency (n, %) counts. After initial testing for data normality, continuous variables were compared using Student's t-test, Mann-Whitney, ANOVA or Kruskal-Wallis, where appropriate. Depending on normality, continuous variables were expressed as mean ± SD or median (Min-Max). The level of statistical significance was set at $p < 0.05$.

Results

The demographic characteristics of 150 COVID-19 patients are presented in **table 1**. The study group consisted of 80 (53%) males and 70 (47%) females with an average age of 52.89 ± 11.89 years. The BMI of the COVID-19 patients was 28.64 ± 4.83. A number of 49 patients had BMI above 30 (33%), 49 patients (55%) had existing hypertension, while the presence of diabetes was observed in 14 individuals (9%). Furthermore, the COVID-19 patients group consisted of 75 (50%) non-smokers, 49 former smokers (33%) and 18 smokers (12%). The distribution of *GSTM3* genotypes were: 59 (39%) *AA*, 42 (28%) *AC* and 49 (33%) *CC*; *GSTA1* genotypes: 48 (32%) *CC*, 67 (45%) *CT*, 23 (15%) *TT* and *GSTP1* genotypes: 71 (47%) *Ile/Ile*, 66 (44%) *Ile/Val*, 12 (8%) *Val/Val*.

Table 1. Baseline characteristic of 150 COVID-19 patients.

Parameter	
Age (years)[a]	52.89 ± 11.89
Gender, n (%)	
Male	80 (53)
Female	70 (47)
Hypertension, n(%)	
No	40 (45)
Yes	49 (55)
Diabetes, n(%)	
No	136 (91)
Yes	14 (9)
BMI (kg/m^2)[a]	28.64 ± 4.83
Smoking, n (%)	
Never	75 (50)
Former	49 (33)
Ever	18 (12)
Obesity, n (%)	
BMI < 30	100 (67)
BMI > 30	49 (33)

[a] Mean ± SD; n - number of individuals in each group.

The levels of the inflammatory biomarkers and multiorgan impairment biomarkers of COVID-19 patients on admission and 7 days upon admission are shown in **table 2**. Inflammatory biomarkers such as leukocytes, lymphocytes and monocytes showed a statistically significant rise 7 days upon admission ($p < 0.001$). Conversely, CRP and fibrinogen decreased significantly ($p < 0.001$) 7 days upon admission. Moreover, out of five analysed multiorgan impairment biomarkers, only urea increased significantly 7 days upon admission ($p < 0.007$), while AST showed a statistically significant drop ($p < 0.001$).

The association between *GSTM3*, *GSTA1* and *GSTP1* gene polymorphisms and levels of inflammatory biomarkers on admission and 7 days upon admission are presented in **table 3**. COVID-19 patients homozygous for variant *GSTM3*C/C* genotype had increased levels of inflammatory biomarkers such as CRP, fibrinogen and ferritin on admission to the hospital, but the borderline significance was observed only for fibrinogen ($p = 0.057$). The other two examined polymorphisms, *GSTA1* and *GSTP1*, did not show an association with the levels of presented inflammatory biomarkers either on admission or 7 days upon admission to the hospital.

The presence of *GSTM3*C/C* genotype was also found to be significantly associated with multiorgan impairment biomarkers, as presented in **table 4**. Namely, COVID-19 patients homozygous for variant *GSTM3*C* allele had the highest levels of ALT ($p = 0.021$) and LDH ($p = 0.045$) on admission to the hospital. Individuals with *GSTM3*C/C* genotype also had higher levels of other examined multiorgan impairment biomarkers than those carrying at least one referent allele, but statistical significance was omitted. Similarly, to before mentioned results that showed a lack of association between *GSTA1* and *GSTP1* polymorphisms with inflammatory parameters, these polymorphisms were not associated with the levels of multiorgan impairment biomarkers as well.

Discussion

The data obtained in this study have shown that, among examined polymorphisms, only *GSTM3* rs1332018 polymorphism was associated with inflammatory and multiorgan impairment biomarkers in COVID-19 patients. COVID-19 patients homozygous for variant *GSTM3*C/C* genotype had increased levels of inflammatory biomarkers such as CRP, fibrinogen and ferritin, but the borderline significance was observed only for fibrinogen on admission to the hospital. Moreover, COVID-19 patients homozygous for variant *GSTM3*C* allele had the highest levels of ALT and LDH assessed on admission to the hospital.

The appearance of SARS-CoV2 in December 2019 in Wuhan, China has led to a still ongoing COVID-19 pandemic (1). While the common cold symptoms are observed in mild cases, COVID-19 is accompanied by multiorgan failure in severe patients (3). Thus in severe cases of COVID-19, apart from the lungs, different organs might be affected: heart, liver, kidneys, as well as gastrointestinal, hematologic and nervous systems (17). The SARS-CoV2 may directly invade the host cells of different organs through the ACE2 receptor (18). On the other hand, activation of the complement system, dysregulated immune responses, cytokine storm, coagulation dysfunction and infiltration of inflammatory cells in SARS-CoV2 infection can induce multiorgan failure in these patients (19). Importantly, routine biochemical and hematological laboratory testing is essential in assessing disease severity, and early recognition of cardiac, renal, and hepatic injury, as well as in monitoring the course of the disease.

Table 2. Levels of the inflammatory biomarkers and multiorgan impairment biomarkers in COVID-19 patients on admission and 7 days upon admission.

Laboratory parameters	On admission	7 days upon admission	p-value
	Inflammatory biomarkers		
WBC count (10^9/L)	6.14 ± 2.26	7.51 ± 3.65	**<0.001**
Lymphocyte count (10^9/L)	1.30 ± 0.61	1.61 ± 0.72	**<0.001**
Monocyte count(10^9/L)	0.47 (0.17 - 1.82)	0.54 (0.13 - 1.37)	**<0.001**
CRP (mg/l)	36.6 (0.60 - 282.2)	5.9 (0.8 - 151.8)	**<0.001**
Fibrinogen (g/l)	3.8 (2.2 - 8.0)	3.1 (2.5 - 4.4)	**<0.001**
Ferritin (μg/L)	467.5 (10.1 - 4937.2)	458.7 (13.30 - 11404.0)	0.129
	Multiorgan impairment biomarkers		
Urea (mmol/L)	5.34 ± 1.86	5.51 ± 2.11	**0.007**
Creatinine (μmol/L)	85.78 ± 18.13	91.51 ± 89.92	0.266
ALT (U/L)	44.0 (11.0 - 173.0)	94.0 (19.0 - 244.0)	0.270
AST (U/L)	34.0 (13.0 - 152.0)	33.0 (12.0 – 145.0)	**<0.001**
LDH (U/L)	233.5 (106.0 - 566.0)	193.0 (80.0 - 530.0)	0.080

WBC-white blood cells; CRP-C reactive protein; ALT-alanine aminotransferase; AST-aspartate aminotransferase; LDH-lactate dehydrogenase; depending on the type of variables and the normality of the distribution, results were presented as mean ± standard deviation or median (Min-Max).

Table 3. The association between *GSTM3*, *GSTA1* and *GSTP1* gene polymorphisms and levels of inflammatory biomarkers on admission and 7 days upon admission.

Genotype	WBC 10^9/L	p	Lymphocytes 10^9/L	p	Monocytes 10^9/L	p	CRP mg/l	p	Fibrinogen g/l	p	Ferritin μg/L	p
Levels of inflammatory biomarkers on admission												
GSTM3												
AA	6.23 ± 2.31		1.37 ± 0.47		0.53 (0.12-1.33)		19.1 (0.5-245.8)		3.4 (2.20-8.0)		326.90 (13.6-2125.3)	
AC	6.00 ± 2.31		1.30 ± 0.60		0.43 (0.14-1.09)		36.6 (1.0-280.50)		3.6 (2.2-8.0)		531.45 (10.10-4937.2)	
CC	6.11 ± 2.38	0.889	1.51 ± 0.79	0.228	0.42 (0.21-1.82)	0.212	43.5 (1.0-282.20)	0.105	4.1 (2.1-8.0)	0.057	458.65 (82.9-3037.9)	0.194
GSTA1												
AA	6.28 ± 1.97		1.49 ± 0.69		0.45 (0.15 – 1.43)		36.6 (0.6-282.2)		3.75 (2.2-8.8)		502.9 (45.5-2001.1)	
AC	6.00 ± 2.48		1.36 ± 0.63		0.47 (0.12-1.82)		26.7 (0.5-280.5)		3.6 (2.1-7.0)		409.1 (13.6-2125.3)	
CC	6.03 ± 2.76	0.843	1.38 ± 0.57	0.536	0.51 (0.14-1.02)	0.745	24.2 (0.9-224.5)	0.579	3.9 (2.5-8.0)	0.978	418.3 (10.1-4937.2)	0.669
GSTP1												
AA	5.98 ± 2.23		1.5 ± 0.61		0.51 (0.17-1.82)		24.4 (0.6-282.2)		3.6 (2.4-8.0)		313.0 (10.1-4937.2)	
AC	6.21 ± 2.36		1.31 ± 0.66		0.43 (0.12-1.43)		29.3 (0.5-280.5)		4.0 (2.1-8.0)		461.9 (13.6-3037.9)	
CC	6.61 ± 2.83	0.647	1.33 ± 0.57	0.189	0.63 (0.24-1.33)	0.074	31.7 (1.6-164.7)	0.632	3.3 (2.2-5.2)	0.186	552.25 (36.7-674.4)	0.193
Levels of inflammatory biomarkers 7 days upon admission												
GSTM3												
AA	7.21 ± 2.83		1.73 ± 0.79		0.56 (0.11-1.65)		5.2 (0.5-81.2)		2.9 (2.0-5.10)		474.7 (19.0-2297.0)	
AC	8.00 ± 3.52		1.67 ± 0.57		0.67 (0.24-1.48)		5.95 (0.5-151.8)		3.2 (2.2-6.2)		671.1 (13.3-11404.0)	
CC	8.60 ± 4.4	0.112	1.88 ± 0.83	0.360	0.57 (0.13-1.97)	0.225	5.0 (0.3-76.60)	0.450	3.0 (2.1-4.0)	0.153	459.95 (101.6-1704.3)	0.165
GSTA1												
AA	7.89 ± 2.99		1.79 ± 0.8		0.61 (0.11-1.65)		4.8 (0.5-81.2)		3.1 (2.1-4.4)		355.3 (53.2-2297.2)	
AC	7.95 ± 3.75		1.52 ± 0.37		0.57 (0.13-1.33)		5.5 (0.3-133.9)		3.0 (2.0-4.7)		527.8 (19.0-1706.3)	
CC	8.07 ± 4.65	0.981	1.81 ± 0.48	0.065	0.56 (0.3-1.97)	0.821	6.1 (0.5-73.2)	0.756	2.9 (2.4-6.2)	0.756	448.3 (3.3-11404.0)	0.732
GSTP1												
AA	8.23 ± 3.67		1.87 ± 0.86		0.56 (0.11-1.65)		5.7 (0.5-133.9)		3.1 (2.1-6.2)		421.25 (13.3-11404.0)	
AC	7.76 ± 3.78		1.64 ± 0.62		0.58 (0.18-1.97)		4.45 (0.3-151.8)		3.0 (2.0-5.1)		494.2 (19.0-1819.30)	
CC	7.11 ± 2.76	0.610	1.71 ± 0.68	1.593	0.64 (0.35-1.09)	0.631	6.0 (1.8-73.2)	0.422	3.05 (2.5-4.1)	0.446	513.2 (74.8-912.8)	0.726

Results are presented as mean ± SD or median (Min-Max).

Herein, available laboratory data of inflammatory and multiorgan impairment biomarkers was presented, obtained from 150 COVID-19 patients at two-time points: on admission and 7 days upon admission to the hospital. Seven days upon admission to the hospital, the number of leukocytes, lymphocytes and monocytes increased, whereas levels of CRP and fibrinogen decreased in COVID-19 patients, compared to the values of these parameters measured on admission to the hospital. Out of five analysed multiorgan impairment biomarkers in

Table 4. The association between *GSTM3*, *GSTA1* and *GSTP1* gene polymorphisms and levels of multiorgan impairment biomarkers on admission and 7 days upon admission.

Genotype	Urea (mmol/l)	p	Creatinine (μmol/L)	p	ALT (U/L)	p	AST (U/L)	p	LDH (U/L)	p
Multiorgan impairment biomarkers on admission										
GSTM3										
AA	5.23 ± 1.7		84.13 ± 20.83		42.0 (11.0-96.0)		27.0 (11.0-113.0)		215.0 (122.0-539.0)	
AC	5.27 ± 2.01		85.93 ± 20.21		40.5 (19.0-109.0)		33.5 (13.0-66.0)		227.0 (106.0-502.0)	
CC	5.81 ± 1.77	0.229	89.22 ± 26.99	0.547	48.0 (20.0-173.0)	**0.021**	34.5 (15.0-152.0)	0.111	231.0 (145.5-566.00)	**0.045**
GSTA1										
AA	5.42 ± 2.09		85.69 ± 19.64		44.0 (11.0-96.0)		36.0 (16.0-67.0)		237.0 (122.0-506.0)	
AC	5.39 ± 1.42		86.5 ± 22.03		42.0 (20.0-173.0)		29.0 (11.0-152.0)		201.5 (106.0-566.0)	
CC	5.51 ± 2.01	0.963	88.38 ± 33.06	0.901	42.0 (21.0-152.0)	0.986	33.0 (18.0-92.0)	0.63	254.0 (125.0-407.0)	0.271
GSTP1										
AA	5.38 ± 1.66		87.58 ± 19.59		43.0 (11-173)		30 (13-152)		210.5 (125-566)	
AC	5.64 ± 2.03		84.75 ± 27.32		42.5 (20-145)		33.5 (11-113)		243 (143-544)	
CC	4.72 ± 1.6	0.289	89.5±15.97	0.710	50.0 (21-78)	0.507	27 (13-62)	0.649	208.5 (106-331)	0.708
Multiorgan impairment biomarkers 7 days upon admission										
GSTM3										
AA	5.46 ± 1.87		83.28 ± 78.41		88.5 (19-301)		37 (12-181)		196.5 (107-530)	
AC	6.03 ± 2.34		74.56 ± 17.07		73 (22-205)		32 (14-99)		220 (80-527)	
CC	6.19 ± 2.0	0.242	73.83 ± 12.17	0.652	95.5 (24-495)	0.631	34.5 (12-185)	0.628	196.5 (124-514)	0.464
GSTA1										
AA	5.73 ± 2.09		86.49 ± 92.87		106.5 (19-446)		37.5 (16-185)		215 (113-514)	
AC	5.66 ± 1.79		75.13 ± 14.7		78.0 (21-357)		30 (12-145)		192 (80-530)	
CC	6.51 ± 2.61	0.332	72.41 ± 14.26	0.565	81.5 (20-495)	0.623	28 (12-112)	0.054	186 (107-527)	0.134
GSTP1										
AA	5.71 ± 1.84		83.43 ± 71.8		93.0 (19-357)		35 (12-181)		196 (113-530)	
AC	6.01 ± 2.28		71.14 ± 14.84		88.5 (21-495)		34(12-185)		202 (80-441)	
CC	5.39 ± 2.27	0.652	84.14 ± 15.46	0.455	43.0 (22-116)	0.159	25 (14-55)	0.639	196 (104-264)	0.708

Results are presented as mean ± SD or median (Min-Max).

this group of patients, only urea increased significantly 7 days upon admission to the hospital, while AST showed a statistically significant drop. Of note, these results are in accordance with the changes in inflammatory and multiorgan impairment biomarkers across two-time points in a Serbian cohort of 58 COVID-19 patients with pneumonia, as previously reported by Kosanovic et al. (7).

As already apostrophised, SARS-CoV2 infection can be followed by mild symptoms but also, by life-threatening conditions. Inter-individual differences in response to the virus might be explained by the presence of several risk factors, such as diabetes, hypertension, obesity or other comorbidities. In addition, recent data pointed to the influence of genetic predisposition to more severe form of COVID-19 disease (14). Due to the established role of oxidative stress in the pathophysiology of COVID-19, it has been proposed that interindividual differences in patients' clinical manifestations might be affected by variations in genes encoding antioxidant enzymes, such as GSTs (12–14, 20). So far, it has been reported that countries with lower frequency of the *GSTT1-null* genotype and higher frequency of *GSTP1* rs1695 *Val* allele exhibit higher COVID-19 mortality (13, 20). In a cohort of 207 COVID-19 patients and 252 respective controls from Serbia, Coric et al. (14) showed that *GSTP1* rs1695 and *GSTM3* rs1332018 polymorphisms influence both COVID-19 occurrence and COVID-19 severity. Other examined GST polymorphisms (*GSTM1*, *GSTT1* and *GSTA1*) in this study did not exhibit such a significant effect (14). In this line, in our study, it was demonstrated that *GSTM3* rs1332018 polymorphism was associated with inflammatory and multiorgan impairment biomarkers in COVID-19 patients. The *GSTM3* belongs to the GST superfamily. As such, GSTM3 is a protective enzyme involved in conjugating reduced glutathione to a wide range of electrophiles (21) but also has the role in maintaining redox homeostasis. Additionally, *GSTM3* rs1332018 A>C polymorphism, located in the promoter region, results in reduced expression of *GSTM3*. To date, the significant role of *GSTM3* polymorphism has been the best established in tumor pathology (22). It has also been connected to chronic obstructive pulmonary disease and lung disease in children with cystic fibrosis (23–25). According to our results, variant *GSTM3*CC* homozygotes had increased levels of inflammatory biomarkers, but the borderline significance was observed only for fibrinogen on admission to the hospital. This finding indicates higher systemic inflammation in these patients. Additionally, COVID-19 patients homozygous for variant *GSTM3*C* allele had higher levels of ALT and LDH assessed on admission to the hospital, than patients carrying at least one referent *GSTM3**A allele. Other researchers have also demonstrated that reduced GSTM3 expression contributes to oxidative stress-mediated liver damage (26).

Conclusion

The limitations of this study are the relatively small sample size and a low incidence of particular polymorphisms. Still, this study may offer valuable direction for future research. The results on the association between *GSTM3* variant genotype with parameters of systemic inflammation and liver damage in COVID-19 patients can contribute to further understanding of pathophysiological mechanisms underpinning this disease, as well as early recognition of COVID-19 patients prone to worse course of the disease. However, further studies are needed to clarify the exact roles of specific glutathione transferases in SARS-CoV-2 infection.

Literature

1. Yuki K, Fujiogi M, Koutsogiannaki S. COVID-19 pathophysiology: A review. Clin Immunol. 2020; 215:108427.
2. Iwasaki M, Saito J, Zhao H, Sakamoto A, Hirota K, Ma D. Inflammation triggered by SARS-CoV-2 and ACE2 augment drives multiple organ failure of severe COVID-19: molecular mechanisms and implications. Inflammation. 2021; 44(1):13–34.
3. Joly BS, Siguret V, Veyradier A. Understanding pathophysiology of hemostasis disorders in critically ill patients with COVID-19. Intensive Care Med. 2020; 46(8):1603–6.
4. Gibson PG, Qin L, Puah SH. COVID-19 acute respiratory distress syndrome (ARDS): clinical features and differences from typical pre-COVID-19 ARDS. Med J Aust. 2020; 213(2):54–6.
5. Fernandes IG, de Brito CA, Dos Reis VMS, Sato MN, Pereira NZ. SARS-CoV-2 and Other Respiratory Viruses: What Does Oxidative Stress Have to Do with it?. Oxid Med Cell Longev. 2020; 2020:8844280.
6. Miripour ZS, Sarrami-Forooshani R, Sanati H, Makarem J, Taheri MS, Shojaeian F, et al. Real-time diagnosis of reactive oxygen species (ROS) in fresh sputum by electrochemical tracing; correlation between COVID-19 and viral-induced ROS in lung/respiratory epithelium during this pandemic. Biosens Bioelectron. 2020; 165:112435.
7. Kosanovic T, Sagic D, Djukic V, Pljesa-Ercegovac M, Savic-Radojevic A, Bukumiric Z, et al. Time Course of Redox Biomarkers in COVID-19 Pneumonia: Relation with Inflammatory, Multiorgan Impairment Biomarkers and CT Findings. Antioxidants. 2021; 10(7):1126.
8. Muhammad Y, Kani YA, Iliya S, Muhammad JB, Binji A, El-Fulaty Ahmad A, et al. Deficiency of antioxidants and increased oxidative stress in COVID-19 patients: A cross-sectional comparative study in Jigawa, Northwestern Nigeria. SAGE open Med. 2021; 9:2050312121991246.
9. Tew KD, Townsend DM. Glutathione-s-transferases as determinants of cell survival and death. Antioxid Redox Signal. 2012; 17(12):1728–37.
10. Menon D, Innes A, Oakley AJ, Dahlstrom JE, Jensen LM, Brüstle A, et al. GSTO1-1 plays a pro-inflammatory role in models of inflammation, colitis and obesity. Sci Rep. 2017; 7(1):1–15.
11. Pljesa-Ercegovac M, Savic-Radojevic A, Matic M, Coric V, Djukic T, Radic T, et al. Glutathione transferases: potential targets to overcome chemoresistance in solid tumors. Int J Mol Sci. 2018; 19(12):3785.
12. Saadat M. The morbidity and mortality of COVID-19 are correlated with the Ile105Val glutathione S-transferase P1 polymorphism. Egypt J Med Hum Genet. 2020; 21(1):1–5.
13. Abbas M, Verma S, Verma S, Siddiqui S, Khan FH, Raza ST, et al. Association of GSTM1 and GSTT1 gene polymorphisms with COVID-19 susceptibility and its outcome. J Med Virol. 2021;93(9):5446-5451.
14. Coric V, Milosevic I, Djukic T, Bukumiric Z, Savic-Radojevic A, Matic M, et al. GSTP1 and GSTM3 variant alleles affect susceptibility and severity of COVID-19. Front Mol Biosci. 2021; 8:747493.
15. Ali-Osman F, Akande O, Antoun G, Mao J-X, Buolamwini J. Molecular cloning, characterization, and expression in

Escherichia coli of full-length cDNAs of three human glutathione S-transferase Pi gene variants: evidence for differential catalytic activity of the encoded proteins. J Biol Chem. 1997; 272(15):10004–12.
16. Di Pietro G, Magno LA V, Rios-Santos F. Glutathione S-transferases: an overview in cancer research. Expert Opin Drug Metab Toxicol. 2010; 6(2):153–70.
17. Tabary M, Khanmohammadi S, Araghi F, Dadkhahfar S, Tavangar SM. Pathologic features of COVID-19: A concise review. Pathol Pract. 2020; 153097.
18. Astuti I. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. Diabetes Metab Syndr Clin Res Rev. 2020; 14(4):407–12.
19. Mokhtari T, Hassani F, Ghaffari N, Ebrahimi B, Yarahmadi A, Hassanzadeh G. COVID-19 and multiorgan failure: A narrative review on potential mechanisms. J Mol Histol. 2020; 1–16.
20. Saadat M. An evidence for correlation between the glutathione S-transferase T1 (GSTT1) polymorphism and outcome of COVID-19. Clin Chim Acta. 2020; 508:213.
21. Thyagaraju K, Hemavathi B, Vasundhara K, Rao AD, Devi KN. Comparative study on glutathione transferases of rat brain and testis under the stress of phenobarbitol and β-methylcholanthrene. J Zhejiang Univ Sci B. 2005; 6(8):759–69.
22. Wang S, Yang J, You L, Dai M, Zhao Y. GSTM3 Function and Polymorphism in Cancer: Emerging but Promising. Cancer Manag Res. 2020; 12:10377.
23. Flamant C, Henrion-Caude A, Boëlle P-Y, Brémont F, Brouard J, Delaisi B, et al. Glutathione-S-transferase M1, M3, P1 and T1 polymorphisms and severity of lung disease in children with cystic fibrosis. Pharmacogenet Genomics. 2004; 14(5):295–301.
24. Çalışkan M, Baker SW, Gilad Y, Ober C. Host genetic variation influences gene expression response to rhinovirus infection. PLoS Genet. 2015; 11(4):e1005111.
25. Van de Wetering C, Elko E, Berg M, Schiffers CHJ, Stylianidis V, van den Berge M, et al. Glutathione S-transferases and their implications in the lung diseases asthma and chronic obstructive pulmonary disease: Early life susceptibility? Redox Biol. 2021; 3:101995.
26. Qi L, Zou Z-Q, Wang L-Y, Gao S, Fan Y-C, Long B, et al. Methylation of the glutathione-S-transferase M3 gene promoter is associated with oxidative stress in acute-on-chronic hepatitis B liver failure. Tohoku J Exp Med. 2012; 228(1):43–51.