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Original Study Article

Association of superoxide dismutase and catalase genetic variants and their gene-gene interactions with the severity of COVID-19

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ABSTRACT

BACKGROUND: Since the outbreak of COVID-19 infection, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), oxidative stress has been proposed as an important player in its severity. This increased the interest in studying antioxidant systems to evaluate their possible role in counteracting disease progression.

AIM: The aim of the current study was to investigate the association of single nucleotide polymorphism (SNP) of superoxide dismutase (SOD) and catalase (CAT) genes with the severity of COVID-19.

MATERIALS AND METHODS: Study subjects were divided into two groups based on the severity of their symptoms. Allele-specific PCR was used for genotyping, and multifactor dimensionality reduction (MDR) analysis was performed to investigate the SNP–SNP interaction models.

RESULTS: The results showed a significant association of *SOD2* rs4880 with the severity of COVID-19 ($p = 0.002$). *SOD2* 47TT genotype was significantly more frequent among patients with severe COVID-19 (OR 4.34; 95% CI 1.72–10.96). The three-locus SNP–SNP interaction model, resulted from MDR analysis, was statistically significant (0.55×10^{-4}, OR 3.81; 95% CI 1.96–7.42). Carriers of *SOD1* 7958G * *SOD2* 47T * *CAT* 262C allele combination had a higher risk of severe COVID-19 ($p = 0.0045$, OR 2.84, 95% CI 1.40–5.78).

CONCLUSIONS: The obtained results contribute to better understanding of COVID-19 pathogenesis and suggest novel potential prognostic biomarkers of the infection.

Keywords: COVID-19; oxidative stress; single nucleotide polymorphisms; *SOD1*; *SOD2*; *CAT*.

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Оригинальное исследование

Ассоциация генетических вариантов супероксиддисмутазы и каталазы и их межгенное взаимодействие с тяжестью течения COVID-19

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АННОТАЦИЯ

Актуальность. С момента вспышки инфекции COVID-19, вызванной коронавирусом тяжелого острого респираторного синдрома 2 (SARS-CoV-2), окислительный стресс был предложен в качестве важного фактора, способствующего ее тяжести. Это повысило интерес к изучению антиоксидантных систем с целью оценки их возможной роли в противодействии прогрессированию заболеваний.

Цель — изучение ассоциации однонуклеотидного полиморфизма (SNP) генов супероксиддисмутазы (SOD) и каталазы (CAT) с тяжестью течения COVID-19.

Материалы и методы. Пациенты были разделены на две группы в зависимости от тяжести симптомов. Аллель-специфическая ПЦР использовалась для генотипирования, а для исследования моделей взаимодействия SNP–SNP был проведен многофакторный анализ снижения размерности (MDR).

Результаты. Результаты показали значительную ассоциацию SOD2 rs4880 с тяжестью течения COVID-19 (p = 0,002). Генотип *SOD2* 47TT достоверно чаще встречался среди пациентов с тяжелым течением COVID-19 (отношение шансов 4,34; 95 % доверительный интервал 1,72–10,96). Модель взаимодействия SNP–SNP с тремя локусами, полученная в результате анализа MDR, была статистически значимой ($0{,}55 \times 10^{-4}$, отношение шансов 3,81; 95 % доверительный интервал 1,96–7,42). Носители сочетания аллелей *SOD1* 7958G * *SOD2* 47T * *CAT* 262C имели более высокий риск тяжелого течения COVID-19 (p = 0,0045, отношение шансов 2,84, 95 % доверительный интервал 1,40–5,78).

Заключение. Полученные результаты способствуют лучшему пониманию патогенеза COVID-19 и предлагают новые потенциальные прогностические биомаркеры инфекции.

Ключевые слова: COVID-19; окислительный стресс; однонуклеотидный полиморфизм; *SOD1*; *SOD2*; *CAT*.

Как цитировать

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BACKGROUND

Oxidative stress (OS) is an imbalance in redox homeostasis, induced by increased reactive oxygen species (ROS) production and/or decreased antioxidant capacity. ROS are mainly produced as byproducts of cellular metabolism. Mitochondrial aerobic respiration is considered as the primary source of ROS in the cell [1]. Some of the main ROS are hydroxyl radicals ($^{\bullet}OH$), Superoxide ($O_2^{\bullet-}$), and hydrogen peroxide (H_2O_2). There are also reactive nitrogen species (RNS), formed from the association of nitrogen molecules with oxygen, such as peroxynitrite ($ONOO^-$), the product of reaction between nitric oxide ($^{\bullet}NO$) and superoxide ($O_2^{\bullet-}$). Moderate levels of ROS are important for normal processes in the cell. For instance, they play a role in intracellular signaling by serving as secondary messengers, therefore mediating cell growth, immune function, autophagy and overall redox regulation [2, 3]. However, OS induced by ROS overproduction has deleterious effects on the cellular components, such as nucleic acids, proteins, and lipids. Thus, it has been associated with a wide range of diseases, including cardiovascular diseases, neurodegenerative diseases, diabetes mellitus, and cancer [4]. To maintain homeostasis and protect the cells against harmful effects of oxidative stress, a complex antioxidant system has evolved. The defense mechanisms include direct ROS scavengers (ex: glutathione, vitamin C), and endogenous antioxidant enzymes (ex: catalase, superoxide dismutase).

The role of oxidative stress in the pathogenesis of viruses has been widely studied due to the emerging evidence of ROS overproduction during viral infections [5]. The infection-induced ROS formation may affect several cellular processes, including inflammatory responses, immune functions, and apoptosis [6]. Recently, studying the role of oxidative stress became of great importance in the quest to fully understand the pathological process of the novel coronavirus disease 2019 (COVID-19). Since the outbreak of COVID-19 infection, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), there have been around 775 million confirmed cases worldwide, including more than 7 million deaths [7].

Patients were grouped based on disease severity into categories such as asymptomatic, mild, moderate, or critical [8]. A wide range of symptoms have been reported, including fever, cough, changes in taste and smell, shortness of breath, and gastrointestinal symptoms [9]. In critical cases, more severe symptoms were observed, including acute respiratory distress syndrome (ARDS) and multiple organ dysfunction. Risk factors for severe COVID-19 include older age, male gender, and existing comorbidities such as diabetes, hypertension, and cardiovascular diseases [10]. In addition, host genetic predisposition is considered an important factor in the variation of symptoms among patients. Oxidative stress is also considered an important factor influencing the severity of COVID-19 disease. Thus, there has been increased interest in the study of antioxidant systems and their possible role in counteracting disease progression.

Superoxide dismutases (SODs) — a family of enzymatic antioxidants protect cells from oxidative stress by neutralizing superoxide anion [11]. Human SODs are divided according to their binding metal cofactors and cellular localization to: cytoplasmic copper–zinc superoxide dismutase (Cu/Zn-SOD or SOD1), mitochondrial manganese-containing superoxide dismutase (Mn-SOD or SOD2), and extracellular superoxide dismutase (EC-SOD or SOD3) [12]. Catalase is an antioxidant enzyme that reduces the formation of ROS, by breaking down hydrogen peroxide. Catalase is present in all aerobic cells. However, the highest levels of this enzyme are found in the kidney, liver, and erythrocytes [13]. Polymorphisms in the genes encoding these enzymes have been associated with various diseases.

In this study, we *aimed* to evaluate the association of *SOD1*, *SOD2*, and *CAT* genetic variants with the severity of COVID-19. In addition, we have studied SNP–SNP interactions and associations of genotype and allele combinations.

MATERIALS AND METHODS

Subjects

A total of 169 COVID-19 patients were included in this study. They were divided into 2 groups: 101 mild and 68 severe cases. Disease severity was determined according to the guidelines of WHO on COVID-19 clinical management [14]. In addition, chest imaging (CT scan) was performed to confirm classification. Enrollment of participants was after excluding all patients with comorbidities (such as diabetes and hypertension) and/or other risk factors (such as smoking) that could influence the severity of symptoms and therefore affect the validity of our results. Age of participants was between 18 and 70 years old. Around 2/3 were females in both mild and severe groups. Blood samples were collected in “Nauka” medical center (Rostov-on-Don, Russia) and analyzed in the post-COVID period, starting at least from 2 months after recovery. All performed procedures were in accordance with the Helsinki declaration (2013) and its later amendments or ethical standards [15]. Informed consents were obtained from all study participants.

Genotyping

Total genomic DNA was extracted from venous blood samples using AmpliSens® RIBO-prep isolation kit (AmpliSens, FBSI “Central Research Institute of Epidemiology”, Rospotrebnadzor, Russia). Quantitative assessment of the isolated DNA was by using NanoDrop (Thermo Fisher Scientific, USA). Candidate genes for the study were selected by using several databases, such as NCBI-PubMed, Google Scholar, CyberLeninka, and eLibrary. The selection was based on their potential role in the antioxidant system. This was determined by exploring the function of each gene in the Genecards human gene database (https://www.genecards.org/). Next, genetic polymorphisms of the selected genes were chosen based on their functional properties.

Databases (Ensembl, SNPedia, and NCBI-SNP) were used to obtain mutant allele frequencies (MAF) of the studied SNPs. *SOD2* rs4880 (C47T) and *CAT* rs1001179 (C262T) SNPs were investigated by real time polymerase chain reaction (RT-PCR) using TaqMan commercial kits (Syntol, Moscow, Russia), and performed on QuantStudio™ 5 RT-PCR System (Applied Biosystems, Waltham, MA, USA). Cycling conditions were: predenaturation for 3 minutes at 95°C, followed by 40 cycles of denaturation for 15 seconds at 95°C, and annealing for 40 seconds at 63°C. *SOD1* rs4998557 (G7958A) was studied using a SNP-specific kit (Lytech Co. Ltd., Russia). Cycling conditions were: predenaturation for 1 minute at 93°C, followed by 35 cycles of denaturation for 1 minute at 93°C, annealing for 10 seconds at 64°C, and extension for 20 seconds at 72°C. The resulted amplified DNA fragments were then analyzed by agarose gel electrophoresis and subsequent staining with ethidium bromide.

Biochemical analysis

Biochemical tests were performed on blood samples obtained from mild and severe patient groups. The plasma was separated by centrifugation at 3000 rpm for 5 min and then stored at –20 °C until analysis. To determine superoxide dismutase activity, a spectrophotometric method was used based on the inhibition of adrenaline autoxidation into adrenochrome. The optical density was measured at a wavelength of 540 nm. Catalase activity was determined by the consumption of a substrate (H_2O_2) forming a yellow colored complex with molybdenum salts. Optical intensity of the samples and controls was measured at a wavelength of 410 nm. Glutathione peroxidase activity (GPA) was analyzed based on the oxidation rate of reduced glutathione in the presence of tertiary butyl hydroperoxide. Optical density was measured at 340 nm. Ceruloplasmin activity was assessed using substrate oxidation, which results in a colorimetric (560 nm) product proportional to the enzymatic activity. The measurements of optical density in all colorimetric tests were performed using a DU-800 spectrophotometer (Beckman Coulter, USA).

Statistical analysis

Data were analyzed using IBM SPSS Statistics 27.0 (IBM, Armonk, NY). Student's *t*-test was used to compare different variables between studied groups. Continuous variables were expressed as mean ± standard deviation. Test for normality was performed using Kolmogorov–Smirnov (K-S) test for a large sample size (>50) to find normal distribution assumptions. Chi-square test was used to assess the differences in allelic variants distribution between the studied groups and $p \leqslant 0.05$ was considered statistically significant. Odds Ratios (OR), with 95% confidence intervals (CI), were calculated to evaluate the risk of COVID-19 severity. Hardy–Weinberg Equilibrium (HWE) was calculated using SNPStats web tool [16]. Multifactor Dimensionality Reduction (MDR) 3.0.2 software (Computational Genetics Laboratory, Institute for Quantitative Biomedical Sciences, Dartmouth, NH, USA) was used to study the possible interactions between the studied genetic variants and evaluate their relation to the risk of severe COVID-19 outcome. Allelic combinations analysis was performed using SNPStats web tool [16]. The most common allele combination was selected as reference. OR and 95% CI were calculated to estimate the degree of association between allele combinations and the risk of severe COVID-19.

RESULTS

Study subjects' clinical characteristics

There was no significant difference in the male/female ratio between mild and severe groups. However, the mean age of patients was significantly lower in mild group. This was confirmed by normality test results that showed no symmetry by age in the studied groups (Kolmogorov–Smirnov statistic 0.092, df = 169, $p = 0.001$). Ceruloplasmin, glutathione peroxidase activity (GPA), CAT, and SOD were all measured as biomarkers for oxidative stress. Of them, SOD activity was significantly higher in mild group (6.72 ± 2.03) than in severe group (5.21 ± 1.37; $p = 0.03$). Furthermore, Lung computed tomography (CT) scan showed that all patients in the mild group were in the CT-1 category (pulmonary parenchymal involvement ⩽25%), while severe group patients were in CT-3 and CT-4 categories (50–75% and ⩾75%, respectively). Clinical characteristics and biochemical analyses of patients are presented in Table 1.

Association of SOD1, SOD2, and CAT genetic variants with COVID-19 severity

Genotypes of *SOD1* rs4998557 (G7958A), *SOD2* rs4880 (C47T), and *CAT* rs1001179 (C262T) SNPs were detected and their genotype distributions were consistent with the Hardy-Weinberg equilibrium (HWE) ($p > 0.05$). The most frequent genotype for each polymorphism was considered the reference group for association studies. Genotype frequencies of *SOD2* rs4880 showed a significant association with COVID-19 severity ($p = 0.002$). In particular, TT genotype was more frequent in severe (33.8%) than in mild group (11.9%). This means that *SOD2* 47TT carriers have a higher risk of severe COVID-19 outcome (OR4.34; 95% CI 1.72–10.96). In addition, T allele was also more frequent in severe patients (55.9%), compared to mild patients (39.1%; $p = 0.003$). However, genotype frequencies of both *SOD1* rs4998557 and *CAT* rs1001179 were not significantly different between the two studied groups ($p = 0.26$ and 0.72, respectively). Same thing applies to their allelic frequencies ($p = 0.82$ and 0.88, respectively). All genotyping data, along with *p*-values, odds ratios, and 95% confidence intervals, are presented in Table 2.

Gene-gene interactions

A three-locus model of SNP–SNP interaction between the studied polymorphisms was established using MDR algorithm. *SOD1* rs4998557 * *SOD2* rs4880 * *CAT* rs1001179

Table 1. Clinical characteristics and oxidative stress biochemical parameters of patients with mild and severe COVID-19 symptoms

Таблица 1. Клиническая характеристика и биохимические параметры окислительного стресса у пациентов с легкими и тяжелыми симптомами COVID-19

Characteristics	Mild (*n* = 101)	Severe (*n* = 68)	*p* value
Clinical characteristics			
Age, years	43.11 ± 13.67	54.16 ± 9.91	4.68×10^{-8}*
Males, *n*	34	22	0.85
Females, *n*	67	46	
Lung CT scan (CT category)	CT-1	CT-3, CT-4	
Oxidative stress biochemical parameters			
Ceruloplasmin, μM/l	1.36 ± 0.41	1.48 ± 0.35	0.09
GPA, units/ml	1.33 ± 1.63	1.58 ± 1.59	0.41
CAT, units/ml	30.17 ± 14.03	31.27 ± 10.22	0.77
SOD, units/ml	6.72 ± 2.03	5.21 ± 1.37	0.03*

*$p < 0.05$, GPA, Glutathione Peroxidase Activity; CAT, Catalase; SOD, Superoxide Dismutase; CT, Computed Tomography. Age and oxidative stress biochemical parameters are presented as Mean ± SD (Standard deviation).

Table 2. Genotype and allele frequencies of *SOD1* rs4998557, *SOD2* rs4880, and *CAT* rs1001179 in mild and severe groups of COVID-19 patients

Таблица 2. Частоты генотипов и аллелей *SOD1* rs4998557, *SOD2* rs4880 и *CAT* rs1001179 в группах пациентов с легкой и тяжелой формой COVID-19

Genotype/Allele	Mild (*n* = 101)	Severe (*n* = 68)	*p* value	OR (95% CI)
SOD1 rs4998557 (7958G>A)				
Genotype				
GG	75 (74.3%)	54 (79.4%)	0.26	Reference
GA	22 (21.8%)	9 (13.3%)		0.57 (0.24–1.33)
AA	4 (3.9%)	5 (7.3%)		1.74 (0.45–6.77)
Allele				
G	172 (85.1%)	117 (86.1%)	0.82	Reference
A	30 (14.9%)	19 (13.9%)		0.94 (0.54–1.64)
SOD2 rs4880 (47 C>T)				
Genotype				
CC	34 (33.7%)	15 (22.1%)	0.002*	Reference
CT	55 (54.4%)	30 (44.1%)		1.24 (0.58–2.63)
TT	12 (11.9%)	23 (33.8%)		4.34 (1.72–10.96)
Allele				
C	123 (60.9%)	60 (44.1%)	0.003*	Reference
T	79 (39.1%)	76 (55.9%)		2.03 (1.28–3.23)
CAT rs1001179 (262 C>T)				
Genotype				
CC	57 (56.5%)	36 (52.9%)	0.72	Reference
CT	36 (35.6%)	28 (41.2%)		1.23 (0.65–2.35)
TT	8 (7.9%)	4 (5.9%)		0.79 (0.22–2.82)
Allele				
C	150 (74.3%)	100 (73.5%)	0.88	Reference
T	52 (25.7%)	36 (26.5%)		1.04 (0.64–1.69)

Note. OR, Odds Ratio; CI, Confidence Interval. *$p < 0.05$.

interaction model was significant (0.55×10^{-4}, OR3.81; 95% CI 1.96–7.42) with a cross-validation consistency — 10/10, accuracy — 67.4%, sensitivity — 52.9%, and specificity — 77.2%. Graphical representation of the resulted interaction model (Figure 1) suggested that carriers of *SOD1* 7958GG * *SOD2* 47TT * *CAT* 262CC and carriers of *SOD1* 7958GG * *SOD2* 47TT * *CAT* 262CT have a higher risk of developing severe COVID-19 symptoms.

Fruchterman–Rheingold graph (Figure 2) showed a high level of redundancy (–1.43%) between *SOD1* rs4998557 and *CAT* rs1001179. A lower level (–0.39%) was noticed between *SOD2* rs4880 and *CAT* rs1001179. The independent effect of *SOD2* rs4880 was the highest (5.15%), in comparison with *SOD1* rs4998557 (1.15%) and *CAT* rs1001179 (0.28%).

Association of studied SNPs allele combinations

In the performed analysis, all allele combinations with frequency more than 1% were included. The reference allele combination was *SOD1* 7958G * *SOD2* 47C * *CAT* 262C. The results showed that *SOD1* 7958G * *SOD2* 47T * *CAT* 262C allele combination was more frequent in severe group, which suggests that it could be associated with a higher risk of severe COVID-19 outcome ($p = 0.0045$, OR2.84, 95% CI 1.40–5.78). Results are presented in Table 3.

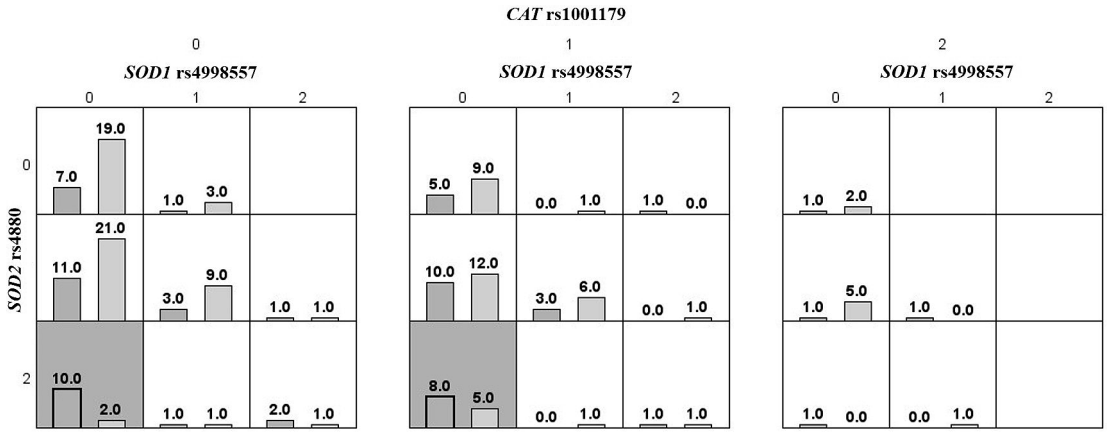

Fig. 1. Multifactor dimensionality reduction (MDR) analysis. The summary of the three-factor model (*SOD1* rs4998557, *SOD2* rs4880 and *CAT* rs1001179). Dark and light backgrounds represent high-risk and low-risk combinations respectively. 0 — homozygous for wild-type allele, 1 — heterozygous, and 2 — homozygous for mutant allele. Right columns are for mild COVID-19 cases, whereas left columns are for severe cases

Рис. 1. Многофакторный анализ снижения размерности (MDR). Краткое изложение трехфакторной модели (*SOD1* rs4998557, *SOD2* rs4880 и *CAT* rs1001179). Темный и светлый фон представляют комбинации высокого и низкого риска соответственно. 0 — гомозиготный по аллелю дикого типа, 1 — гетерозиготный, 2 — гомозиготный по мутантному аллелю. Правые столбцы соответствуют легким случаям COVID-19, а левые столбцы — тяжелым случаям

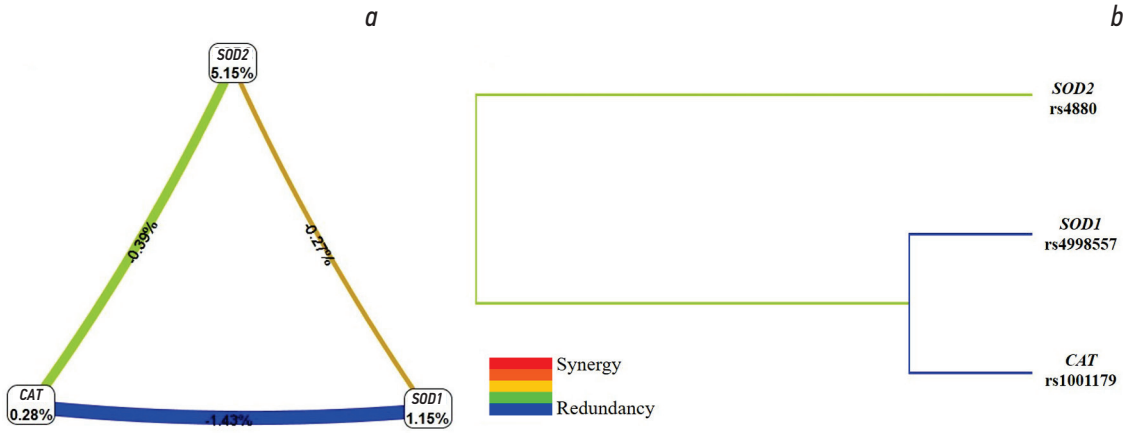

Fig. 2. Fruchterman–Rheingold graph with types of interactions between SNPs. Each SNP node contains entropy value (%) that indicates its independent effect. Colors and values between nodes indicates interaction effects. Positive values represent synergistic effect, while negative ones represent redundancy. The line's color indicates the type of SNP–SNP interaction (*a*). Dendrogram graph, which shows the level of interaction between the studied SNPs (*b*)

Рис. 2. График Фрюхтермана–Рейнгольда с типами взаимодействия между SNP. Каждый блок SNP содержит значение энтропии (%), которое указывает на его независимый эффект. Цвета и значения между блоками указывают на эффекты взаимодействия. Положительные значения представляют синергический эффект, а отрицательные — антагонизм. Цвет линии указывает на тип взаимодействия SNP–SNP (*a*). График дендрограммы, который показывает уровень взаимодействия между изучаемыми SNP (*b*)

Table 3. Association between *SOD1* G7958A, *SOD2* C47T, and *CAT* C262T allele combinations and COVID-19 severity

Таблица 3. Ассоциация между комбинациями аллелей *SOD1* G7958A, *SOD2* C47T и *CAT* C262T и тяжестью течения COVID-19

Allele combination	Frequency	OR (95% CI)	*p* value
SOD1 7958G * *SOD2* 47C * *CAT* 262C	0.38	Reference	–
SOD1 7958G * *SOD2* 47T * *CAT* 262C	0.25	2.84 (1.40–5.78)	0.0045*
SOD1 7958G * *SOD2* 47C * *CAT* 262T	0.12	1.39 (0.54–3.56)	0.49
SOD1 7958G * *SOD2* 47T * *CAT* 262T	0.11	1.72 (0.71–4.14)	0.23
SOD1 7958A * *SOD2* 47T * *CAT* 262C	0.07	2.01 (0.76–5.29)	0.16
SOD1 7958A * *SOD2* 47C * *CAT* 262C	0.04	0.70 (0.12–4.01)	0.69
SOD1 7958A * *SOD2* 47T * *CAT* 262T	0.03	0.93 (0.11–7.95)	0.95

Note. OR, Odds Ratio; CI, Confidence Interval. *$p < 0.05$.

DISCUSSION

Evaluating the association of antioxidant enzymes' single nucleotide polymorphisms (SNPs) with diseases has been the subject of a significant number of studies based on their role in altering enzymatic activity. In fact, several SNPs of antioxidant enzymes have been already linked to the risk of diseases, such as obesity [17], male infertility [18], multiple sclerosis [19], cancer [20], and viral infections [21]. The associations of the genetic variants included in our study with different diseases have been evaluated in previous studies. For example, it was shown that *SOD2* SNP rs4880 might be a risk factor for polycystic ovary syndrome (PCOS) development [22]. Furthermore, *SOD1* 7958A allele was associated with an increased risk of spontaneous abortion in the first trimester [23]. Also, *CAT* rs1001179 (C262T) polymorphism was associated with male infertility in various populations [24].

Based on the pivotal role of oxidative stress in COVID-19 severity, we started investigating the role of antioxidant enzymes' genetic variants with the severity of COVID-19 symptoms in Rostov Region population. Our previous study showed a significant association of paraoxonase 1 *PON1* rs662 (A575G) and nitric oxide synthase 3 *NOS3* rs2070744 (T786C) with COVID-19 severity [25].

In the current study, we investigated the role played by SOD and CAT genetic variants, along with their gene-gene interactions, in the severe outcome of patients with COVID-19. *SOD2* rs4880 TT genotype showed a significant association with COVID-19 severity. *SOD2* SNP rs4880 is a substitution of C for T in the codon 16 of *SOD2* second exon, which causes the change of alanine for valine, and has been associated with a 30040% decrease in SOD2 enzymatic activity [26]. Indeed, our biochemical analysis showed that SOD enzymatic activity is lower in severe group, compared to mild group ($p = 0.03$). According to a previous study of J.-F. Yi et al. [27], *SOD1* mutant allele (A) is associated with increased expression of *SOD1* mRNA, while wild-type allele (G) is related to reduced gene transcription and increase of free radicals in cells as a result. Even though SOD1 was suggested as a predictor for indicating COVID-19 progression [28], our study showed no significant difference between mild and severe groups ($p = 0.26$). However, and to our knowledge, our study is the first to evaluate the possible role of this genetic variant in COVID-19 severity. *CAT* SNP rs1001179 is located in the promoter and consists of C>T substitution that alter *CAT* expression, with T allele being associated with higher levels [29]. The current genotype analysis showed that *CAT* rs1001179 was not associated with COVID-19 severity in our groups of patients ($p = 0.72$). This result is accompanied by our analysis of CAT enzymatic activity that also showed no significant difference between the two groups. Gene-gene interaction analysis showed that the carriers of *SOD1* 7958GG * *SOD2* 47TT * *CAT* 262CC or *SOD1* 7958GG * *SOD2* 47TT * *CAT* 262CT may have a higher risk of developing severe COVID-19. In addition, *SOD2* rs4880 had the highest independent effect among the three studied polymorphisms, therefore the most significant association with the risk of COVID-19 severity. This supports the results of genotype association analysis (Table 1). Furthermore, performed analysis suggested that *SOD1* 7958G * *SOD2* 47T * *CAT* 262C allele combination is associated with a higher risk of severe COVID-19 ($p = 0.0045$), which also supports the findings of MDR analysis.

Some limitations of the current study must be mentioned, such as small sample size and significant age difference between studied groups, since older age may be a potential cause for both reduced SOD activity and COVID-19 severity. Therefore, further studies, with a larger sample size with symmetric age distribution, along with assessing gene expression and performing other biochemical analyses, are required.

CONCLUSIONS

Taken together, our obtained results on the association of certain *SOD1*, *SOD2*, and *CAT* genetic variants with COVID-19 severity, in addition to the evaluation of the combined role of the three variants by studying a three-locus interaction model using MDR algorithms, have shed some light on the possible involvement of genetic factors in COVID-19 severe outcome. Our findings might contribute to better understanding of COVID-19 pathogenesis and suggest novel potential prognostic biomarkers of the infection.

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