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Original Article

Role of ACE2 and TMPRSS2 polymorphisms on COVID-19 outcome and disease severity in adult patients: A prospective cohort study in a tertiary hospital, Indonesia

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Abstract

Coronavirus disease 2019 (COVID-19) has led to a significant number of infections and deaths worldwide, yet its pathogenesis and severity remain incompletely understood. Angiotensin-converting enzyme 2 (ACE2) and transmembrane protease, serine 2 (TMPRSS2), play crucial roles as receptors and molecules responsible for the virus's entry into host cells, initiating the infection process. Their polymorphisms have been extensively studied in relation to COVID-19 severity. The aim of this study was to examine the association of *ACE2* (rs2074192) and *TMPRSS2* (rs12329760) polymorphisms with COVID-19 outcome and severity. A prospective cohort study was conducted in 2022 at Haji Adam Malik Hospital, Medan, Indonesia. We randomly recruited hospitalized adult patients with COVID-19, confirmed by real-time polymerase chain reaction (RT-PCR). The baseline demographic data, disease severity, underlying disease, comorbidities, and COVID-19 vaccination status were collected. The single-nucleotide polymorphism (SNP) was assessed using TaqMan SNP genotyping assay, and the levels of TMPRSS2 and ACE2 proteins were measured using enzyme-linked immunosorbent assay (ELISA). A total of 151 COVID-19 patients were recruited and there were significant associations between age and severity with mortality outcomes. The age, kidney and lung diseases, and vaccination status were associated with severity levels. The results showed the CC genotype of *ACE2* had the highest proportion, followed by TT and CT genotypes among patients, while CT was the most prevalent genotype, followed by CC and TT for *TMPRSS2*. This study did not find a significant association between *ACE2* and *TMPRSS2* genetic variants with disease severity and outcome but highlighted a specific association of TMPRSS2 SNP with mortality within the group. In addition, ACE2 concentration was significant different between mild-moderate and severe-critical COVID-19 groups (p=0.033).

Keywords: Rs2074192, rs12329760, polymorphism, *ACE2*, *TMPRSS2*

Introduction

The coronavirus disease 2019 (COVID-19) pandemic has caused a significant number of infections and mortalities worldwide, and the causative agent, severe acute respiratory syndrome

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coronavirus 2 (SARS-CoV-2), infects not only the human respiratory tract but also other organs [1,2]. Human angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) are the well-known receptors and molecules that are responsible for virus entry into the host cells [3]. The *ACE2* has many polymorphisms, some of which are associated with individual susceptibility to various diseases such as type 2 diabetes and hypertension [4]. The single-nucleotide polymorphism (SNP) variant of *ACE2* (rs2074192) is associated with the incidence of hypertension and the T allele is associated with cardiovascular disease, diabetic retinopathy, and hypertension [5].

TMPRSS2 is a type II transmembrane protein with serine protease activity on the target cells and has an important role in facilitating the process of SARS-CoV-2 protein S breakdown and then being able to fuse into cells [6]. The process of viral fusion begins with proteolytic cleavage of the S1 and S2 of SARS-CoV-2 by furin and subsequently, the S2 site is cleaved to activate the fusion process by TMPRSS2 or cathepsin L [3]. It is hypothesized that the genetic variations in *TMPRSS2* might affect the SARS-CoV-2 infection process [4]. There are numerous SNPs in the *TMPRSS2*, but only 21 SNPs affect functional proteins [7]. The rs12329760 polymorphism, also known as the p.Val160Met variant, has been known to play a role in the risk of prostate cancer, suggesting that this polymorphism has an association with clinical consequences [8].

Since *ACE2* and *TMPRSS2* SNPs are associated with several diseases [9,10] and considering those receptors play a role in enhancing the SARS-CoV-2 propagation within cell targets leading to high viremia, these SNPs could be considered as determining factors in the development of severity and mortality of COVID-19. The aim of this study was to examine the association of *ACE2* and *TMPRSS2* SNPs on the outcomes and severity of COVID-19 patients.

Methods

Study setting and patients

A prospective cohort study was conducted at Haji Adam Malik Hospital, Medan, Indonesia, for one year in 2022. The hospital was responsible for advanced clinical management of emerging or re-emerging diseases, including COVID-19, in the Sumatra Utara province. We randomly recruited hospitalized adult patients with COVID-19, which was confirmed by real-time polymerase chain reaction (RT-PCR). Patients with pregnancy, HIV/AIDS, cancer, and already treated with antiviral drugs were excluded from this study.

Data collection and study variables

The baseline demographic data, disease severity, underlying disease or comorbid/comorbidities, and COVID-19 vaccination status were collected from the patients. Demographic data included sex and age, and comorbidities were categorized into common groups such as diabetes mellitus, hypertension, kidney diseases, cardiac impairment, and lung disease. Vaccination status was categorized as the number of COVID-19 vaccines received by the patients. Disease severity was classified as mild, moderate, and severe according to the World Health Organization (WHO) and Indonesia COVID-19 guidelines [11,12]. The outcomes of the inpatient were then followed up and classified as recovered/self-isolation or death.

We collected 3 mL of blood samples from the eligible patients immediately after COVID-19 diagnosis was established (by RT-PCR) for SNP and enzyme-linked immunosorbent assay (ELISA). Polymorphism of *ACE2* and *TMPRSS2* was identified by deoxyribonucleic acid (DNA) genotyping, and the levels of ACE2 and TMPRSS2 were measured using ELISA at the Laboratory of Universitas Sumatera Utara, Medan, Indonesia.

DNA isolation and genotyping

Genomic DNA was extracted using a Wizard Genomic DNA Purification Kit (Promega Corp., Madison, USA) following the manufacturer's instructions. The resulting DNA was examined for purity and concentration using a Nanodrop. All DNA was diluted to 7 ng/µL using 1X Tris-EDTA buffer (Sigma-Aldrich) prior to PCR genotyping.

Genotyping assay of rs2074192 polymorphism of *ACE2* and rs12329760 of *TMPRSS2* was carried out using TaqMan SNP genotyping assay (Cat. No. 4351379, Thermo Fisher Scientific,

Waltham, USA). The real-time PCR examination was performed following the kit protocol using a 10 µL reaction composition (master mix) and a total of 14 ng gDNA was used as a PCR template.

Real-time PCR was performed in the applied biosystems QuantStudio 5 Real-Time PCR system (Thermo Fisher Scientific, Waltham, USA) for 40 cycles. PCR results were analyzed by using QuantStudio Design & Analysis software (Thermo Fisher Scientific, Waltham, USA) to read the specific amplification. All data were continued to be analyzed by using TaqMan™ Genotyper Software v1.3.1.

The assay assessed the *ACE2* rs2074192 for sequence AGTGTGGAAATGTATAAATGG TTGG[C/T]ATTTATTCATTTGTGACTGCTGT and the *TMPRSS2* rs12329760 for sequence CAGGACTTCCTCTGAGATGAGTACA[C/T]CTGAAGGATGAAGTTTGGTCCGTAG. The SNP rs2074192 was an intron variant, while the SNP TMPRSS2 rs12329760 was a missense mutation. Both SNPs were characterized by the genotypes CC, CT, and TT.

ELISA assay of ACE2 and TMPRSS2

The plasma was collected by centrifuging blood samples collected in EDTA tubes at 3000 rpm for 20 minutes, which separated the plasma from the rest of the blood. The supernatant was collected to be used for ELISA assays. ELISA was carried out to determine ACE2 and TMPRSS2 protein levels in the patient blood using the Human ACE2 ELISA kit (Cat.No. E3169Hu, Bioassay Technology Laboratory, Shanghai, China) and the Human TMPRSS2 ELISA kit (Cat.No. E2625Hu, Bioassay Technology Laboratory, Shanghai, China). These kits contained plates pre-coated with human ACE2 or TMPRSS2 antibodies to bind present protein in the samples. The biotinylate-labeled human ACE2 or TMPRSS2 antibodies were used as secondary antibodies, and then streptavidin-horseradish peroxidase (HRP) was added and bound to the Biotinylated ACE2 or TMPRSS2 antibody. The reaction was measured at 450 nm wavelength.

Statistical analysis

Data entry and statistical analysis were performed using SPSS (SPSS Inc., Chicago, USA). Categorical data were reported as frequencies and percentages and then were compared using Pearson's chi-square test or Fisher's exact test between the groups. Mean differences between groups were compared using the Kruskal-Wallis or Mann-Whitney test. In the second stage, significant predictors with significant odds ratios were analyzed with logistic stepwise regression analysis. The receiver operating characteristic (ROC) curve and area under the curve (AUC) were then determined.

Results

Characteristics of the patients

A total of 151 COVID-19 patients were included in this study and their characteristics are presented in **Table 1**. More than half of the patients were male and the largest age group was the elderly (35.1%). Additionally, 42.4% of patients had no comorbidities, 37.8% had one type of comorbidity, and the rest, 19.6% of patients, had more than one comorbidity, with the most common was diabetes mellitus (21.9%). More than one-third of patients (34.4%) did not receive the COVID-19 vaccine. Most of the patients had a recovery outcome (72.9%), while 27.1% were deceased (**Table 1**).

Factors associated with COVID-19 outcomes

The elderly, the presence of comorbid, having kidney disease or lung disease, vaccination status, and the level of severity at admission were significantly associated with mortality ($p<0.05$) (**Table 1**). Our data indicated that 53.6% of the patients had the CC, 26.5% had the TT, and 19.9% had the CT genotype of *ACE2*. For *TMPRSS2*, 43.1%, 39.7%, and 17.2% had CT, CC, and TT genotypes, respectively. Our study did not reveal a significant difference in outcomes between mutant (CT and TT) and non-mutant (CC) genotypes ($p>0.05$) (**Table 1**). The multivariate analysis showed that the elderly group and those with severe disease have greater risks of mortality (**Table 1**).

Table 1. Factors associated with COVID-19 outcome (n=151)

Variables	Outcomes		Univariate analysis		Multivariate analysis	
	Recovery, n (%)	Deceased, n (%)	Relative risk (95%CI)	*p*-value	Relative risk (95%CI)	*p*-value
Gender						
Female	54 (74.0)	19 (26.0)				
Male	56 (70.8)	22 (28.2)	1.08 (0.64–1.83)	0.764		
Age group (years)						
Adult (18–44)	45 (88.2)	6 (11.8)				
Pre-elderly (45–59)	34 (72.3)	13 (27.6)	2.35 (0.89–6.18)	0.083		
Elderly (>60)	31 (58.5)	22 (41.5)	3.52 (1.43–8.70)	0.006*	2.77 (1.18–6.49)	0.019*
Comorbidity						
No comorbid	54 (84.4)	10 (15.6)				
1 comorbid	36 (63.2)	21 (36.8)	2.34 (1.12–4.89)	0.023*		
>1 comorbid	19 (63.3)	11 (36.7)	2.58 (1.09–6.07)	0.030*		
Diabetes mellitus						
No	90 (75.6)	29 (23.4)				
Yes	20 (62.5)	12 (37.5)	1.54 (0.89–2.66)	0.138		
Hypertension						
No	93 (72.7)	35 (27.3)				
Yes	17 (73.9)	6 (26.1)	0.95 (0.45–2.01)	0.901		
Heart disease						
No	99 (71.4)	39 (28.3)				
Yes	11 (84.6)	2 (15.4)	0.54 (0.15–2.00)	0.318		
Kidney disease						
No	93 (76.2)	29 (23.7)				
Yes	17 (58.6)	12 (41.4)	1.74 (1.02–2.98)	0.044*		
Lung disease						
No	96 (76.2)	30 (23.8)				
Yes	14 (56.0)	11 (44.0)	1.85 (1.08–3.18)	0.038*		
Vaccination status						
Booster	41 (91.2)	3 (6.8)				
2 doses	34 (75.6)	11 (24.4)	3.59 (1.07–12.04)	0.039*		
1 dose	7 (70.0)	3 (30.0)	4.40 (1.03–18.76)	0.045*		
No vaccination	28 (53.9)	24 (46.1)	6.77 (2.18–21.06)	0.001*		
Disease severity						
Mild-moderate	88 (88.9)	11 (11.1)				
Severe-critical	22 (41.3)	30 (57.7)	5.19 (2.60–10.36)	<0.001*	4.25 (2.24–8.07)	<0.001*
Genotype of ACE2 (rs2074192)						
CC	57 (70.4)	24 (29.6)				
CT	24 (80.0)	6 (20.0)	0.93 (0.31–1.49)	0.331		
TT	29 (72.5)	11 (27.5)	0.68 (0.51–1.70)	0.810		
Genotype of TMPRSS2 (rs12329760)						
CC	44 (73.3)	16 (26.7)				
CT	51 (78.5)	14 (21.5)	0.76 (0.41–1.41)	0.383		
TT	16 (61.5)	10 (38.5)	1.36 (0.72–2.56)	0.344		

* Statistically significant at $p<0.05$

The proportion of mild, moderate, and severe-critical COVID-19 cases were 41 (27.2%), 58 (38.4%) and 52 patients (34.4%), respectively. There was a significant association between gender, age of group, the presence of comorbidity, and vaccination status with the degree of COVID-19 severity (p<0.05). Multivariate analysis showed that the elderly group, with the presence of comorbidities, particularly hypertension, kidney and lung disease, and uncompleted vaccination status, were at greater risk of experiencing more severe stages of COVID-19. This study did not find a significant difference between mutant (CT and TT) and non-mutant (CC) genotypes in terms of disease severity (p>0.05) (**Table 2**).

Association between *ACE2* and *TMPRSS2* gene polymorphisms with COVID-19 outcome based on gender

Among females, a significant proportion had CC genotype of *ACE2* in mild COVID-19 (41.5%) and among recovered patients (73.2%). Regarding *TMPRSS2*, the CT genotype was more commonly found in mild (41.9%) and recovered (74.2%) COVID-19 patients. Among males, a substantial percentage showed the CC genotype for *ACE2* in severe COVID-19 (42.5%) and among recovered patients (67.5%). Additionally, the CT gene of the *TMPRSS2* genotype was more frequently observed in moderate (50%) and recovered (82.4%) COVID-19 patients. We found a significant association between *TMPRSS2* genotype to the male group outcome (p<0.05) (**Table 3**).

Association between polymorphisms and the level of ACE2 and TMPRSS2

The highest mean of *ACE2* and *TMPRSS2* was in the CT genotype group at 6.247 ng/µL and 10.488 ng/µL, respectively. However, the mean difference was not statistically significant (**Table 4**). Moreover, the mean values of *ACE2* and *TMPRSS2* were higher in the deceased group compared to the recovered group. However, the difference in mean values was not statistically significant (**Table 5**).

There was a significant mean difference in ACE2 levels between mild-moderate and severe-critical groups (p<0.05), indicating an association between ACE2 and COVID-19 severity. However, there was no association between TMPRSS2 and severity (**Table 5**).

Analysis of ACE2 and TMPRSS2 levels as the predictors of COVID-19 outcomes and severity

The ROC curve analysis was calculated to evaluate the predictive ability of ACE2 and TMPRSS2 levels to COVID-19 outcomes. The cut-off values of ACE2 and TMPRSS2 levels were determined to be 2.707 ng/µL and 6.138 ng/µL, resulting in an area under the curve (AUC) of 0.51 and 0.55, respectively (**Figure 1**).

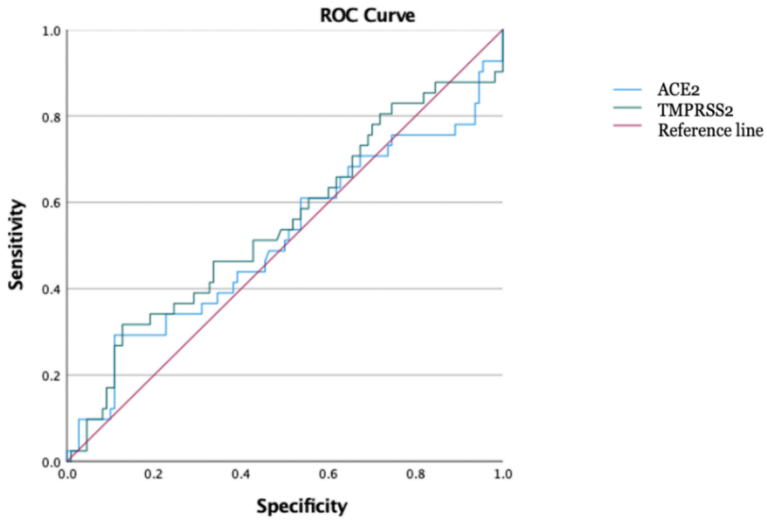

Figure 1. The receiver operating characteristic (ROC) curves of angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) as predictors of COVID-19 outcomes.

Table 2. Factors associated with COVID-19 severity (n=151)

Variables	Disease severity			Univariate analysis		Multivariate analysis	
	Mild, n (%)	Moderate, n (%)	Severe-critical, n (%)	Odds ratio (95% CI)	*p*-value	Odds ratio (95%CI)	*p*-value
Sex							
Female	29 (39.7)	22 (30.1)	22 (30.1)				
Male	12 (15.4)	36 (46.1)	30 (38.5)	2.16 (1.17–3.98)	0.014*		
Age group (years)							
Adult (18–44)	28 (52.9)	13 (25.5)	10 (19.6)				
Pre-elderly (45–59)	10 (21.3)	22 (46.8)	15 (31.9)	3.51 (1.51–8.19)	0.004**		
Elderly (>60)	3 (5.7)	23 (43.4)	27 (50.9)	8.23 (3.57–18.98)	<0.001**	2.33 (1.09–4.95)	0.028*
Comorbidities							
No comorbid	37 (52.1)	21 (29.6)	13 (18.3)				
1 comorbid	2 (3.6)	29 (52.7)	24 (43.7)	6.93 (3.37–14.26)	<0.001**		
>1 comorbid	2 (8.0)	8 (32.0)	15 (60.0)	11.02 (3.91–31.07)	<0.001**		
Diabetes mellitus							
No	38 (31.9)	42 (35.3)	39 (32.8)				
Yes	3 (9.4)	16 (50.0)	13 (40.6)	1.94 (1.04–3.62)	0.038*		
Hypertension							
No	38(29.7)	50 (39.1)	40 (31.2)				
Yes	3 (13.0)	8 (34.8)	12 (52.2)	2.49 (1.08–5.73)	0.032*		
Heart disease							
No	41 (29.7)	52 (37.7)	45 (32.6)				
Yes	0 (0.0)	6 (46.2)	7 (53.8)	3.11 (1.29–7.50)	0.011*		
Kidney disease							
No	41 (33.6)	46 (37.7)	35 (28.7)				
Yes	0 (0)	12 (41.4)	17 (58.6)	4.53 (2.29–8.98)	<0.001*	2.91 (1.30–6.51)	0.009**
Lung disease							
No	41 (32.5)	48 (38.1)	37 (29.4)				
Yes	0 (0.0)	10 (40.0)	15 (60.0)	4.52 (2.18–9.38)	<0.001**	4.12 (1.39–12.20)	0.001**
COVID-19 vaccination							
Booster	28 (63,6)	11 (25,0)	5 (11,4)				
2 doses	9 (20.0)	24 (53,3)	12 (26.7)	5.94 (2.34–15.06)	<0.001**	4.47 (1.79–11.19)	0.001**
1 dose	3 (30.0)	5 (50,0)	2 (20.0)	3.78 (0.93–15.21)	0.061		
No vaccination	1 (1.9)	18 (34,6)	33 (63.5)	28.40 (10.56–76.40)	<0.001**	19.52 (6.91– 55.16)	<0.001**
Genotype of *ACE2* (rs2074192)							
CC	19 (23.5)	34 (42.0)	28 (34.5)				
CT	12 (40.0)	9 (30.0)	9 (30.0)	0.60 (0.26–1.40)	0.240		
TT	10 (25.0)	15 (37.5)	15 (37.5)	1.04 (0.52–2.09)	0.907		
Genotype of *TMPRSS2* (rs12329760)							
CC	19 (31.7)	19 (31.7)	22 (36.6)				
CT	16 (24.6)	26 (40.0)	23 (35.4)	1.14 (0.58–2.24)	0.714		
TT	6 (23.1)	13 (50.0)	7 (26.9)	0.96 (0.44–2.11)	0.920		

* Statistically significant at $p<0.05$
** Statistically significant at $p<0.01$

Table 3. Association of ACE2 and TMPRSS2 polymorphisms to outcome by gender characteristic (n=151)

Variables	Disease severity			*p*-value	Outcomes		*p*-value
	Mild, n (%)	Moderate, n (%)	Severe-critical, n (%)		Recovery, n (%)	Deceased, n (%)	
Female group							
ACE2 genotype							
CC	17 (41.5)	13 (31.7%)	11 (26.8%)	0.596	30 (73.2%)	11 (26.8%)	0.469
CT	10(40.0)	8 (32.0%)	7 (28.0%)		20 (80.0%)	5 (20.0%)	
TT	2 (28.6)	1 (14.3%)	4 (57.1%)		4 (57.1%)	3 (42.9%)	
TMPRSS2 genotype							
CC	11 (37.9)	7 (24.1%)	11 (37.9%)	0.546	20 (69.0%)	9 (31.0%)	0.565
CT	13(41.9)	9 (29.0%)	9 (29.0%)		23 (74.2%)	8 (25.8%)	
TT	5 (38.5)	6 (46.2%)	2 (15.4%)		11 (84.6%)	2 (15.4%)	
Male group							
ACE2 genotype							
CC	2 (5.0)	21 (52.5%)	17 (42.5%)	0.090	27 (67.5%)	13 (32.5%)	0.675
CT	2 (40.0)	1 (20.0%)	2 (40.0%)		4 (80.0%)	1 (20.0%)	
TT	8 (24.2)	14 (42.4%)	11 (33.3%)		25 (75.8%)	8 (24.2%)	
TMPRSS2 genotype							
CC	8 (25.8)	12 (38.7%)	11 (35.5%)	0.352	23 (74.2%)	8 (25.8%)	0.011*
CT	3 (8.8)	17 (50.0%)	14 (41.2%)		28 (82.4%)	6 (17.6%)	
TT	1 (7.7)	7 (53.8%)	5 (38.5%)		5 (38.5%)	8 (61.5%)	

* Statistically significant at $p<0.05$

Table 4. Association of polymorphism with ACE2 and TMPRSS2 serum concentration (n=151)

Genotype distribution	n (%)	Plasma level mean (95%CI)	*p*-value
Genotype of *ACE2* (rs2074192)			
CC	81 (53.6)	3.87 (2.95–4.78)	0.946
CT	30 (19.9)	6.27 (2.94–9.60)	
TT	40 (26.5)	4.41 (2.98–5.84)	
Genotype of *TMPRSS2* (rs12329760)			
CC	60 (39.7)	9.96 (7.48–12.43)	0.982
CT	65 (43.1)	10.48 (7.55–13.42)	
TT	26 (17.2)	9.31 (5.67–12.59)	

Table 5. The mean difference of ACE2 and TMPRSS2 plasma levels to the outcome and severity

Variables	Recovery mean (95%CI)	Deceased mean (95%CI)	*p*-value	Mild-moderate mean (95%CI)	Severe-critical mean (95%CI)	*p*-value
ACE2	4.15 (3.20–5.10)	5.41 (3.17–7.64)	0.803	4.50 (3.50–5.50)	4.48 (2.59–6.37)	0.033
TMPRSS2	9.52 (7.58–11.45)	11.58 (7.89–15.27)	0.352	10.00 (7.99–12.01)	10.29 (6.99–13.44)	0.358

ACE2: angiotensin-converting enzyme 2; TMPRSS2: transmembrane protease serine 2

The ROC curve analysis used ACE2 and TMPRSS2 levels to predict the severity of mild-moderate versus severe-critical COVID-19. The AUCs were 0.61 and 0.55, with cut-off values of 2.798 ng/µL and 6.138 ng/µL, respectively (**Figure 2**).

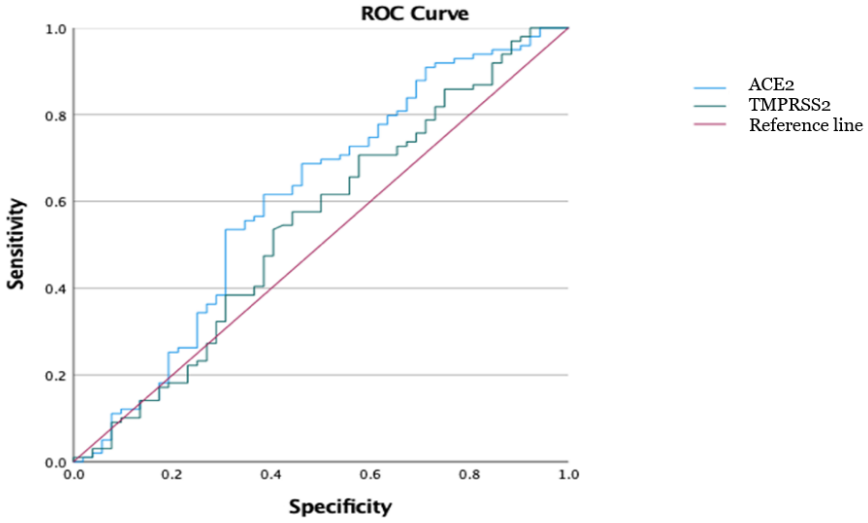

Figure 2. The receiver operating characteristic (ROC) curves of angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) as predictors of COVID-19 severity.

Discussion

Our study results revealed that age, comorbidities, and vaccination status had a significant association with COVID-19 outcomes. Similar to previous studies, elderly patients had a greater risk of getting severe COVID and mortality [13,14]. One systematic review study revealed that patients more than 50 years old, particularly in males with or without comorbidities, showed an increased risk of death significantly [15]. Our study underlined that kidney and lung comorbidities had a higher risk of mortality and severe conditions, while diabetes, hypertension, and cardiac diseases were only related to the degree of severity. In the previous study, diabetes mellitus was the most common comorbid among the subjects [16]. Hyperglycemia is associated with susceptibility to infection due to disruption of the host immune response. Several studies have shown that hyperglycemia suppresses cytokine production, defects of phagocytosis to kill microbes and immune cell dysfunction [16]. In meta-analysis studies, it was demonstrated that chronic kidney disease, hypertension, and diabetes mellitus were associated with COVID-19 mortality and severe outcomes [17,18].

Our study result also revealed that the gender characteristic did not have a significant difference in mortality risk. However, gender differences affected the degree of severity, where males have a higher risk of severity than females. The male and older age infected with COVID-19 tend to have severe symptoms due to poor immune response and a greater risk of thrombus embolism [19]. The latest study highlighted testosterone's influence on COVID-19 severity, suggesting it may protect males from severe cases [20]. Male hypogonadism was identified as a risk factor for COVID-19 hospitalization, with lower testosterone levels associated with higher concentrations of pro-inflammatory cytokines during infection [21]. Additionally, testosterone therapy could potentially mitigate COVID-19 severity [20,21]. A meta-analysis involving 10,000 patients also showed that males and individuals above 50 years of age had a 2.4 higher risk of severe COVID-19 and the presence of comorbidities increases the risk to 3.33 times [22]. A study in 2023 comparing three waves of the COVID-19 pandemic in Canada and Mexico showed that men and older also revealed a greater risk of developing a more severe and deceased [23].

The comorbidities and COVID-19 have a significant association with mortality and severe clinical symptoms. Our study found that one comorbidity had a mortality risk of 2.347 times greater than subjects who did not have comorbidity, while more than one comorbidity had a mortality risk of 2.582 times greater. Patients with comorbidities have higher levels of C-reactive

protein (CRP), neutrophils, leucocytes, and procalcitonin, indicating a strong correlation between the number of comorbidities and inflammation that make an impact on the immune response during the COVID-19 course [24,25].

Vaccination plays an important role in preventing severity and mortality. Data in Indonesia showed that the number of people who were fully vaccinated was 64.27 vaccine doses administered per 100 population, while those who had received only one dose was 75.09 per 100 population [26]. Meta-analysis studies reported that unvaccinated patients with COVID-19 infection are more likely to have bad outcomes compared to those who were vaccinated [27,28]. Our study revealed that the second dose up to booster vaccination has shown better protection than only the first dose. However, a study showed that during the Omicron variant wave in Australia, the effectiveness of booster vaccinations against COVID-19 death in older adults decreased significantly over time, although vaccine effectiveness for more than six months remained above 50% [29,30]. Omicron was well known as the variant of concern (VOC), which has up to 50 protein mutations, including at spike protein, that affect neutralization activity [31].

ACE2 and TMPRSS2 molecules serve as COVID-19 receptors, facilitating the entry of SARS-CoV-2 into host cells at the onset of infection [32]. The interaction of the viral receptor-binding domain (RBD) and ACE2 is believed to influence the clinical findings and case fatality rate (CFR) of COVID-19 in different populations [33]. Therefore, comorbidities associated with higher ACE2 expression may enhance the virus entry and the severity of COVID-19 infection. Many studies have been conducted to explore the associations of SNPs to the severity of COVID-19 disease and the possibility of a predictor of mortality as well [4,32,34]. Our study found that the frequency of *ACE2* rs2074192 CC genotype was higher than CT or TT. Considering the period of our study, whereas the Omicron was the predominant variant, our finding aligns with a previous study that found the Omicron BA.5 variant had a greater frequency of the *ACE2* rs2074192 CC genotype than the other variants [35].

ACE2 SNP rs2074192 and *TMPRSS2* rs12329760 have been identified as polymorphisms associated with several diseases in humans, particularly cardiovascular diseases [36]. Our study did not find an association between the SNPs *ACE2* rs2074192 and *TMPRSS2* rs12329760 to the COVID-19 outcome and severity. However, there was a significant association between male outcomes and *TMPRSS2* SNPs. Several studies showed various results related to this issue [37-40]. A study in 2022 revealed a strong association between rs2074192 and oxygen requirement in the COVID-19 male population [37]. Another study did not find an association between four SNPs with long-term COVID symptoms in previously hospitalized COVID-19 survivors [38]. More about *TMPRSS2* rs12329760 SNP known as Met160Val polymorphism, a study from 2021 also showed no association between the *TMPRSS2* rs12329760 polymorphism with the degree of COVID-19 severity and mortality but correlated with viral load [39]. *TMPRSS2* rs12329760 is previously known as a risk factor for prostate cancer in a population [40]. This corroborates the findings in this study that *TMPRSS2* rs12329760 has an important risk factor for obtaining certain diseases, especially in the male group. It was suggested that the roles of SNPs in *ACE2* and *TMPRSS2* need to be further examined by considering other variables from the host as predictors for COVID-19 outcomes and clinical manifestations.

Expression of *ACE2* and *TMPRSS2* genes has been detected by single-cell ribonucleic acid (RNA)-sequencing analyses on the nasal mucosa, type-2 pneumocytes, and absorptive enterocytes [41]. Consequently, determining the role of ACE2 expression levels in COVID-19 is crucial. Normally, ACE2 exists in its membrane-bound form with very low levels present in the circulation [42]. A study in 2020 showed that elevated plasma ACE2 could be a marker of myocardial structural abnormalities and a predictor of mortality in patients with aortic stenosis [43]. ACE2 and TMPRSS2 plasma levels were examined by assuming that protein expression on the target cell was represented in plasma, and our study showed a significant association between ACE2 level with severity. There was a significant mean difference between the mild-moderate and severe-critical groups. However, there were no significant associations between ACE2 and TMPRSS2 plasma levels with COVID-19 mortality. Despite this, the mean of ACE2 and TMPRSS2 was higher in the deceased group compared to the recovery group. This finding was in line with a study reported that plasma ACE2 in severe COVID-19 patients was higher compared to mild disease. Subsequently, this plasma ACE2 activity is persistently elevated following the acute

infection of SARS-CoV-2 [44]. The ROC curve for severity predictors of ACE2 level showed an AUC of 0.61 and gave a moderate sensitivity and specificity value.

There were several limitations in our study. First, the relatively small sample size may not have been sufficient to detect clinical differences for specific genotypes. Second, it may have been more appropriate to measure ACE2 and TMPRSS2 expression in cells that express these proteins rather than relying solely on blood samples. Third, individuals with cardiovascular and pulmonary diseases were not separately analyzed, making it difficult to conclusively determine the specific effect of ACE2 on COVID-19, given their role in the renin-angiotensin system. Lastly, a limitation of this study is the lack of detailed information on the type of COVID-19 vaccine used.

Conclusion

This is the first prospective cohort study in a tertiary hospital in Medan, Indonesia, to describe the characteristics of *ACE2* and *TMPRSS2* polymorphism and analyze its association with COVID-19 severity and mortality. Age, gender, comorbidities, and COVID-19 vaccination status had a significant correlation to the severity and outcome of COVID-19 in adult patients. There was a wide distribution of rs2074192 and rs12329760 polymorphisms in COVID-19 patients. Our study revealed a significant association of *TMPRSS2* polymorphisms in males with COVID-19 mortality adjusted by gender. However, we did not find a significant association of *ACE2* polymorphisms with severity and outcome. ACE2 could act as a potential biomarker to differentiate mild-moderate and severe-critical COVID-19 patients. Therefore, further study into the genetic association and function of these receptors across populations, ethnicities, and genders is needed.

Ethics approval

This study received ethical approval from the Ethics Commission of Universitas Sumatera Utara No. 1311/KEP/USU/2021. The authors received hospital approval before performing the data collection. Prior to data collection, all patients were informed about the study objectives and provided written informed consent.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

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