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Original article

TMPRSS2 rs2070788 and rs383510 polymorphisms and laboratory markers as predictors for severity and mortality among COVID-19 patients: A single center study

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ABSTRACT

Background: The COVID-19 pandemic caused a worldwide health crisis and put healthcare systems on the verge of collapse. We aimed to investigate whether genetic variations in the *TMPRSS2* gene namely rs2070788 and rs383510, along with other relevant indicators, can predict the severity of COVID-19 outcomes. **Materials**: This study included 100 COVID-19 patients confirmed by PCR between June to December 2021. Fifty patients were classified as severe COVID-19 cases, while the remaining had mild symptoms. Two specific single nucleotide polymorphisms (SNPs) within the *TMPRSS2* gene were analyzed using real-time PCR. Demographic data, laboratory tests, and clinical symptoms were gathered. **Results**: The study revealed a high prevalence of the *TMPRSS2* rs383510 CC genotype and C allele among COVID-19 patients, particularly in those with severe presentation (P=0.029). Also, that genotype and allele was dominated among severe patients who developed complications or did not survive. For rs2070788, the A allele was more common in patients with severe complications or non-survivors. Cut-off for IL-6 ≥ 62.0 pg/mL and ≥85.0 pg/mL respectively exhibited the highest specificity and positive predictive value for predicting progression to complications and mortality. **Conclusion**: We found a significant association between the *TMPRSS2* rs383510 CC genotype and C allele and severe COVID-19 cases (P=0.029). This finding adds to the growing body of evidence suggesting a role for *TMPRSS2* genetic variations in determining COVID-19 severity. Additionally, the study demonstrated a correlation between elevated levels of inflammatory markers (CRP, ferritin, and IL-6) and the progression to critical illness in COVID-19 patients.

Introduction

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) has much larger global spread and has affected hundreds of millions of people than SARS-CoV and MERS-CoV (Middle East Respiratory Syndrome Coronavirus) [1, 2].

Corona Virus disease 2019 (COVID-19) exhibits a multitude of clinical presentations and is stratified into asymptomatic or presymptomatic

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infection, as well as mild, moderate, severe, and critical cases. The disease gives rise to a range of complications, encompassing hypercoagulability and an augmented propensity for thrombosis, as well as complications affecting the cardiovascular, renal, neurological, and various other systems [3, 4].

Studies have documented inter-individual variability in the incidence, severity, and mortality rate of COVID-19. This may be attributed to environmental disparities between countries, including variations in healthcare accessibility, prevalence of chronic diseases, and the demographic composition of the population. Human genetics may also play a role across different populations. Consequently, the timely identification of individuals at high risk is an imperative medical priority [5, 6].

Membrane bound transmembrane protease serine 2 *(TMPRSS2)* and angiotensin-converting enzyme 2 *(ACE2)* have been implicated in the pathogenesis of influenza, SARS–CoV, and SARS-CoV-2 too. *ACE2* is considered the main receptor for viral (S) protein spike, while *TMPRSS2* is important for priming of the virus's (S) protein and further fusion of the viral with the host cell [7].

Amino acid substitutions at key positions on receptor-binding domain (RBD) have substantially augmented the affinity of SARS-CoV-2 RBD in a 10 to 15-fold increase compared to SARS-CoV. Other systems as cardiac endothelium and kidney have expressed *TMPRSS2* and *ACE2* that explained why these organs were target for SARS-CoV-2 infection [8].

More severe clinical pictures of influenza A and acute respiratory distress syndrome (ARDS) were highlighted among patients with rs383510/T and rs2070788/G genotypes (alleles) of *TMPSRSS2*. Tempting to extrapolate this single nucleotide polymorphism (SNP) influence to SARS-CoV-2 infectivity [9].

The aim of the study was to compare the demographic information, clinical, and laboratory characteristics of COVID-19 cases with different severity. Assess the influence of *TMPRSS2* gene polymorphisms rs2070788 and rs383510 and other laboratory markers as predictor for severity and outcome of COVID-19.

Patients and Methods:

Ethics Approval:

Approval was obtained from the ethics committee of faculty of medicine, Cairo University. The procedures used in this study adhere to the tenets of the Declaration of Helsinki (Ethics approval number: MD-110-2021.)

Consent to participate:

Written informed consent was obtained from all individual participants included in the study.

Study design:

This study was done as single-center, cross sectional analytic study and included a total of 100 COVID-19 patients without any previous history of comorbidity admitted to Kasr Alainy Hospitals ICU and outpatient clinics, Cairo University from June 2021 to December 2021 with confirmed positive PCR for SARS-Cov-2. Fifty of these cases were diagnosed as severe COVID-19 case, and the remaining are mild cases.

COVID-19 cases with chronic diseases/comorbidities were excluded from the study. So, we can study the genetic effect of *TMPRSS2* gene beside demographic characteristics on disease severity.

Severe cases exhibiting respiratory manifestations and low oxygen saturation ($SpO_2 < 90\%$) were admitted to the intensive care unit (ICU). These patients were closely monitored throughout their entire hospital stay to document any complications and assess the outcome of their cases. Conversely, mild cases were managed through home isolation and were monitored for a duration of two weeks to identify any potential complications or the necessity for hospitalization.

Sample size

100 cases of COVID-19 patients. Using Clincal sample size calculator for analytic study; with 0.05 alpha error and power of the study 0.80. to calculate minimal sample size needed to detect of assess TMPRSS2 alleles rs2070788 and rs3835210 as risk factors affecting susceptibility, severity and outcome of COVID-19. ; According to literature the rs2070788 and rs383150 both are 40.7% in H7N9 influenza patients compared to 28% in control (Cheng et al., 2015), the genes expression with COVID-19 patients is high according to literature (expected to be 50% or more) (Strope et al., 2020). The total sample size calculated is 100 persons 50 COVID-19 patients and 50 control.

Study procedure:

All patients were followed up to observe complication, outcome among the studied groups, and subjected to the following:

a. History taking.
b. Laboratory tests: D-dimer, C-reactive protein (CRP), alanine transaminase (ALT), aspartate transaminase (AST), serum creatinine, ferritin, and Interleukin-6 (IL-6) assay was done for hospitalized cases from serum samples. Complete blood counts (CBC) and EDTA blood sample was frozen at -20°C to be used later for DNA extraction, for detection of *TMPRSS2* gene polymorphisms rs2070788 and rs383510 by Real-Time PCR according to manufacturer instructions.
c. Radiological: Chest CT scan for hospitalized cases.

Molecular techniques:

We conducted a study on two specific single nucleotide polymorphisms (SNPs) within the *TMPRSS2* gene, namely rs383510 and rs2070788. These SNPs have garnered significant interest due to their potential association with severe forms of H1N1 and H7N9 influenza A virus infections, as indicated by previous research findings [10, 11, 12]. Molecular testing of *TMPRSS2* gene polymorphisms was performed at the Central Molecular Laboratory, Clinical Pathology Department, National Research Center.

***A.* DNA Extraction:**

Frozen whole blood samples were thawed on day of testing and DNA was extracted using Thermo Scientific GeneJET Whole Blood Genomic DNA purification kit **(Thermo Fisher, Massachusetts, U.S.)** (catalog No. K0781) according to manufacturer instructions. DNA concentration was determined using the NanoDrop Spectrophotometer (UV spectrophotometer Q3000, Quawell Technology, Inc., United States). The purified DNA was stored at -80°C.

***B.* SNP genotyping:**

rs2070788 and rs383510 were detected via real-time PCR using the Rotor Gene Q (QIAGEN, GmbH- Germany). Polymorphism allele discrimination was performed using the Kompetitive allele specific PCR (KASP) genotyping protocol (LGC Genomics, Beverly, MA, USA). KASP utilizes a unique form of competitive allele-specific PCR combined with a novel, homogeneous, fluorescence-based reporting system for the identification and measurement of genetic variation occurring at the nucleotide level to detect SNPs or inserts and deletions (InDels) [13].

Minor allele frequencies (MAFs) for the rs2070788 G-allele are higher in Europeans (0.46) than East Asians (0.36) or Africans (0.27). The MAF of the rs383510 T-allele is about one and a half times higher for Europeans (0.49) than for East Asians (0.36) or Africans (0.33) [14].

Statistical Methods:

The data were coded, tabulated, and analyzed using IBM SPSS Statistics software version 28.0. Quantitative data were tested for normality using the Shapiro-Wilk test, and then described using mean, standard deviation, minimum, and maximum values. Quantitative data were also compared using the independent t-test (two independent groups) and ANOVA test (three independent groups). Qualitative data were described using number and percentage, and compared using the Chi-square test and Fisher's Exact test for variables with small expected numbers. The level of significance was set at P-value <0.05.

Results:

Mild COVID-19 cases were characterized by polymorphic clinical manifestations, such as fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, and loss of taste and smell. However, these patients did not exhibit acute respiratory distress symptoms such as shortness of breath, dyspnea, low oxygen saturation, or abnormal chest imaging findings. Additionally, they did not develop any adverse events during a 2-week follow-up period. These patients were considered the control group for disease severity.

Severe COVID-19 cases were defined by admission to an intensive care unit (ICU) with hypoxia (SpO2 <90%). These patients were monitored throughout their hospitalization for complications and outcomes.

A. Mild COVID-19 group:

Demographic information:

The majority of them (58%) were men, and 30% smokers. They had statistically significant lower ages than severe ones (P <0.001); with age ranging from 18-57 years and mean 32.3 years.

Clinical presentation:

All cases with mild COVID-19 were presented with oxygen saturation SpO2 97-99%.

The most widespread complaints (72%) were fever, fatigue and myalgia. (**Table 1**)

During a 2-week follow-up period, no complications emerged in mild cases. Aside from the persistence of fatigue in 29 (58%) patients and the persistence of anosmia and ageusia in 11 (22%) patients without the need for hospitalization, no mortality was found in mild scenarios.

Laboratory findings:

CBCs were generally normal in patients with mild COVID-19, with the exception of mild anemia (7/50; 14%), thrombocytopenia (1/50; 2%), and lymphopenia (22%) in some patients. Total leukocyte counts (TLCs) were normal in all cases apart from elevation among (2/50; 4%) of patients and decreased TLCs in 4% of patients.

D-dimer levels were normal in all patients except one (0.7 mg/L), which was significantly lower than levels observed in severe COVID-19 patients ($P > 0.001$).

Liver function tests showed elevated ALT in 2 patients, while AST and creatinine were within normal limits in all patients. Ferritin was elevated in 40% of patients. CRP was elevated in all patients, but levels were significantly lower than those observed in severe COVID-19 patients ($P > 0.001$). (**Table 2**)

TMPRSS2 polymorphisms among Mild COVID-19 Cases:

We studied the two single nucleotide polymorphisms of *TMPRSS2* gene, rs383510 and rs2070788, trying to find an association between certain genotypes/ alleles and the illness severity and/or development of complications.

Regarding single nucleotide polymorphisms of *TMPRSS2* gene rs383510, The C allele was the predominant allele in mild cases (94%) and genotypes were distributed as follows; Most of the cases 45 (90%) were of CC genotype, 4 (8%) were CT genotype and 1 case (2%) was TT genotype.

There were no statistically significant changes in demographic features, clinical presentation, nor laboratory findings within the mild group based on rs383510 genotypes. D-dimer levels were considerably higher in mild COVID-19 cases with the rs383510 CT genotype (mean± SD =0.46 ±0.20 mg/L, P= 0.001). (**Tables 3** and **4**)

On the other hand, rs2070788; the most common genotype in mild COVID-19 cases was GA (52%), followed by GG (26%) and AA (22%). The G allele was the prevalent allele, accounting for 52% of cases, while the A allele accounted for 48%.

There was no significant association between *TMPRSS2* polymorphism of rs2070788 genotypes and demographic features, clinical presentation, nor laboratory findings except for a higher frequency of abdominal pain among patients with the AA genotype ($P = 0.003$).

Additionally, there was no significant association between *TMPRSS2* gene polymorphisms rs383510 and rs2070788 or their alleles in mild COVID-19 cases.

B. Severe COVID-19 group:

Demographic information:

Within this cohort, the male population constituted the majority, accounting for (35/50; 70%). The age ranged from 24 to 56 years with significantly higher mean of age 41.8 years ($P< 0.001$) and a higher prevalence of smokers (58%) ($P =0.005$, OR=3.22) compared to the mild group.

Clinical presentations:

Upon presentation, the severe group exhibited oxygen saturation levels ranging from 84% to 90%, which were significantly lower than those observed in the mild group ($P< 0.001$). In comparison to the mild COVID-19 cases, severe cases demonstrated a higher incidence of fatigue and myalgia ($P < 0.001$), fever (94%, $P = 0.003$), cough, and dyspnea (90%, $P < 0.001$). (**Table 1).**

Laboratory findings:

Regarding laboratory findings, a comparison of CBC revealed significantly higher total leukocyte counts among 22 cases with lymphopenia, while platelet counts were notably lower in severe cases (P <0.001, P =0.022, respectively).

D-dimer levels were elevated in 34 (68%) severe COVID-19 cases, which was significantly higher than in mild cases ($P > 0.001$). Elevated levels of ALT and AST were observed in 56% and 30% of severe cases, respectively. CRP levels were elevated in all cases. Ferritin levels were elevated in 46 cases (92%). Serum creatinine showed mild elevation in 5 cases upon admission (ranging from 1.35-1.4 mg/dL), and all of these cases later developed renal failure. IL-6 levels were measured only in the severe group and were found to be elevated in 49 cases (98%). (**Table 2)**

TMPRSS2 polymorphisms among severe COVID-19 Cases:

Regarding single nucleotide polymorphisms of *TMPRSS2* gene rs383510, all the severe cases were of CC genotype. Consequently, the C allele was the predominant and only allele. It was statistically significant more frequent in severe group *(P=0.029).*

Regarding SNPs of rs2070788, the most common genotype in severe COVID-19 cases was GA (21/50; 42%), followed by AA genotype (20/50; 40%) and (9/50; 18%) were of GG genotype. The A allele was the prevalent allele, accounting for 61% of cases, while the G allele accounted for 39%. **(Table 5)**

By applying multivariable analysis for different demographic and genetic characterizations, we found that only age ≥33.0 years was an independent factor that increase the likelihood/risk of severe COVID (*P<0.001*, 95% CI=3.59–42.57).

C. Complications among COVID-19 Severe Cases:

Complications occurred in 15/50 (30%) of severe COVID-19 cases. The most common complication (9/15; 18%) among the 50 severe cases was ARDS, while 8 of the severe cases (16%) developed pleural effusion. Renal failure was seen in 5 patients (10%). Among three cases vascular thrombosis (pulmonary artery and mesenteric) was observed as the least common (6%) complications.

There were no statistically significant variations in demographic information or clinical presentation between the complicated and non-complicated subgroups. Complicated cases showed significantly higher serum levels of ferritin (*P* =0.007), CRP (*P* =0.007), IL-6 (*P* < 0.001), and creatinine (*P* < 0.001) when compared with the non-complicated cases. (**Table 6**)

Among our severe cohort, we highlighted that cut point of IL-6 ≥62.0 pg/mL, CRP ≥88.0 mg/L and ferritin ≥470.0 mg/dL had significant performance in predicting the development of complications in COVID19 cases (*P*<0.001, 0.003 and 0.014 respectively). IL-6 ≥ 62.0 pg/mL had highest specificity (91.4%) and positive predictive value (PPV) (78.6%).

We discovered no difference in rs383510 genotypes and alleles between complicated and non-complicated subgroups by comparing genotypes and alleles of *TMPRSS2* polymorphisms as all severe cases were of CC genotype.

Regarding rs2070788, the GA genotype and A allele were more common in complicated scenarios. However, the differences between complicated and non-complicated subgroups were not statistically significant (**Table 7).**

D. Mortality among COVID-19 Severe Cases:

The mortality rate reached up to (6/50; 12%). All the deceased cases (N=6) had ARDS, and four of them (66.7%) had pleural effusion (*P* < 0.001 and 0.004, respectively). A single patient suffered renal failure, and none developed vascular thrombosis, with no statistically significant difference between those who died and those who survived. (**Table 8)**

Laboratory data revealed that the non-survived cases had statistically significant higher levels of ferritin (mean±SD= 706±197.3, *P*<0.001), IL-6 (mean±SD=107.5±56.1, *P* <0.001) and CRP (mean±SD=172.3±91.7, *P* =0.044).

It was noticed that the same predictors (ferritin, CRP and IL-6) of complication also could predict mortality but with higher cut point (≥475.0 mg/dL, ≥193.0 mg/L, and ≥85.0 pg/mL respectively). IL-6 ≥85.0 pg/mL had highest specificity (97.7%) and PPV (80%) for mortality.

In severe COVID-19 cases, there was no significant difference in the frequency of *TMPRSS2* genotype rs383510 between survivors and non-survivors, as all cases in both subgroups were of the CC genotype. However, there was a trend towards a higher frequency of *TMPRSS2* genotype rs2070788, with AA genotype, and A allele in non-survivors, although this difference was not statistically significant.

Multivariable logistic regression analysis of demographic and genetic characteristics revealed that only pleural effusion was an independent predictor of mortality in severe COVID-19 cases (*P=0.005*, 95% CI=2.96–392.23).

Power of the study

Using PASS 11th release (Hintze J. 2011) the power is 85.7% based on the significant (p<0.050) difference between severe and mild regarding C alleles 100.0% vs. 94.0% respectively with sample size 50 and 50 respectively.

Table 1. Clinical characteristics of the included mild and severe COVID-19 patients.

OR (95% CI)	P-value	Mild (N=50)	Severe (N=50)	Variables	
	General presentation				
	<0.001*	97.7±0.6	87.2±1.6	**Mean±SD**	**Oxygen saturation %**
		97.0–99.0	84.0–90.0	**Range**	
NA	**<0.001***	36 (72.0%)	50 (100.0%)	**Fatigue & Myalgia**	
6.09 (1.63–22.82)	**0.003***	36 (72.0%)	47 (94.0%)	**Fever**	
4.93 (1.50–16.16)	**0.005***	35 (70.0%)	46 (92.0%)	**Headache**	
6.52 (2.21–19.21)	**<0.001***	29 (58.0%)	45 (90.0%)	**Cough & dyspnea**	
0.61 (0.27–1.36)	0.224	24 (48.0%)	18 (36.0%)	**Anosmia**	
	GIT presentation				
0.83 (0.36–1.93)	0.668	17 (34.0%)	15 (30.0%)	**Anorexia**	
0.83 (0.36–1.93)	0.668	17 (34.0%)	15 (30.0%)	**Diarrhea**	
0.75 (0.32–1.77)	0.517	17 (34.0%)	14 (28.0%)	**Nausea**	
0.85 (0.28–2.57)	0.779	8 (16.0%)	7 (14.0%)	**Abdominal pain**	
0.65 (0.10–4.09)	0.999	3 (6.0%)	2 (4.0%)	**Vomiting**	

*Statistically significant values are in boldface type.
OR: Odds ratio. CI: Confidence interval

Table 2. The Laboratory data of the included COVID-19 patients.

Variables		Severe (N=50)	Mild (N=50)	P-value
Hemoglobin (gm/dL) RR: 12.9-14.2	**Mean±SD**	12.4±1.1	12.7±1.3	0.140
	Range	9.9–14.2	9.9–15.1	
TLC (x10^3/mL) RR:3.7-10.1	**Mean±SD**	11.9±3.1	7.0±2.3	**<0.001***
	Range	4.4–20.6	3.2–11.8	
Lymphocytes (x10^3/mL) RR:1-4.8	**Mean±SD**	1.58±0.97	1.83±0.89	0.178
	Range	0.51–4.03	0.60–4.25	
Platelets (x10^3/mL) RR:150-450	**Mean±SD**	221.3±76.4	255.5±70.9	**0.022***
	Range	129.0–394.0	147.0–410.0	
ALT (IU/L) RR: up to35	**Mean±SD**	40.4±17.6	21.3±10.2	**<0.001***
	Range	10.0–94.0	7.0–52.0	
AST (IU/L) RR: up to37	**Mean±SD**	29.7±13.1	17.6±6.6	**<0.001***
	Range	10.0–65.0	8.0–38.0	
Creatinine (mg/dL) RR:0.4-1.3	**Mean±SD**	0.89±0.28	0.62±0.29	**<0.001***
	Range	0.30–1.50	0.20–1.10	
Ferritin (mg/dL) RR:13-150	**Mean±SD**	434.3±203.9	161.2±158.4	**<0.001***
	Range	52.5–906.5	7.1–521.2	
D-dimer (mg/L) RR:0-0.55	**Mean±SD**	0.83±0.46	0.26±0.13	**<0.001***
	Range	0.25–1.96	0.08–0.70	
CRP (mg/L) RR: up to 6.0	**Mean±SD**	84.5±59.9	15.3±5.9	**<0.001***
	Range	19.4–254.0	7.0–31.0	
IL-6 (pg/mL) RR:0-4.4	**Mean±SD**	47.3±40.2		
	Range	3.7–159.6		

*Statistically significant values are in boldface type. SD=standard deviation, RR=reference range, TLC= total leucocytic count, ALT=alanine transaminase, AST=aspartate transaminase, CRP=C-reactive protein, IL-6-interleukin-6.

Table 3. Comparison according to rs383510 in mild group regarding clinical presentation clinical presentation.

Variables	CC (N=45)	CT (N=4)	p-value
General presentation			
Oxygen saturation	**97.7±0.6**	**97.8±1.0**	**^0.959**
Fatigue	**32 (71.1%)**	**3 (75.0%)**	**§0.999**
Myalgia	**32 (71.1%)**	**3 (75.0%)**	**§0.999**
Fever	**32 (71.1%)**	**3 (75.0%)**	**§0.999**
Headache	**31 (68.9%)**	**3 (75.0%)**	**§0.999**
Cough	**25 (55.6%)**	**3 (75.0%)**	**§0.625**
Sore throat	**20 (44.4%)**	**2 (50.0%)**	**§0.999**
Nasal congestion	**19 (42.2%)**	**2 (50.0%)**	**§0.999**
Anosmia	**23 (51.1%)**	**1 (25.0%)**	**§0.609**
GIT presentation			
Anorexia	**14 (31.1%)**	**2 (50.0%)**	**§0.588**
Diarrhea	**15 (33.3%)**	**1 (25.0%)**	**§0.999**
Ageusia	**18 (40.0%)**	**1 (25.0%)**	**§0.999**
Nausea	**14 (31.1%)**	**2 (50.0%)**	**§0.588**
Abdominal pain	**8 (17.8%)**	**0 (0.0%)**	**§0.999**
Vomiting	**3 (6.7%)**	**0 (0.0%)**	**§0.999**

^Independent t-test. §Fisher's Exact test.

Table 4. Comparison between laboratory results among genotypes of rs383510 in mild COVID-19 cases.

Variables	**CC (N=45)**	**CT (N=4)**	**p-value**
Hemoglobin (RR: 12.9-14.2gm/dL)	12.6±1.2	13.8±1.1	0.071
TLC (RR: 3.7-10.1x10^3/mL**)**	6.9±2.3	8.0±1.9	0.349
Lymphocytes (RR:1-4.8x10^3/mL)	1.83±0.88	1.46±0.52	0.411
Platelets (RR:155-366x10^3/mL)	252.5±69.8	309.3±67.5	0.125
ALT (RR: up to35 IU/L)	20.5±9.5	30.0±16.4	0.079
AST (RR: up to37 IU/L)	17.6±6.6	18.0±8.5	0.905
Creatinine (RR:0.4-1.3 mg/dL)	0.59±0.30	0.80±0.18	0.180
Ferritin (RR:13-150 mg/dL)	165.2±163.2	100.1±111.3	0.441
D Dimer (RR:0-0.55mg/L)	0.24±0.11	0.46±0.20	**0.001***
CRP (RR: up to 6.0 mg/L)	15.5±6.1	12.3±3.9	0.307

^Independent t-test. *Significant, SD=standard deviation, RR=reference range, TLC= total leucocytic count, ALT=alanine transaminase, AST=aspartate transaminase, CRP=C-reactive protein.

Table 5. Comparison between Allelic and Genotypic distribution of *TMPRSS2* polymorphisms rs383510 and rs2070788 among COVID-19 cases.

Variables		**Severe (N=50)**	**Mild (N=50)**	**p-value**
rs383510				
Genotypes	**CC**	50 (100.0%)	45 (90.0%)	§0.056
	CT	0 (0.0%)	4 (8.0%)	
	TT	0 (0.0%)	1 (2.0%)	
CC		50 (100.0%)	45 (90.0%)	§0.056
Alleles	**C**	100 (100.0%)	94 (94.0%)	**§0.029***
	T	0 (0.0%)	6 (6.0%)	
rs2070788				
Genotypes	**AA**	20 (40.0%)	11 (22.0%)	#0.144
	GA	21 (42.0%)	26 (52.0%)	
	GG	9 (18.0%)	13 (26.0%)	
AA		20 (40.0%)	11 (22.0%)	#0.052
Alleles	**A**	61 (61.0%)	48 (48.0%)	#0.065
	G	39 (39.0%)	52 (52.0%)	

#Chi square test. §Fishers Exact test. *Significant.

Table 6. Laboratory results in complicated and non-complicated subgroups of severe COVID-19 cases.

Variables	Complicated (N=14)	Non-complicated (N=36)	p-value
Hemoglobin (gm/dL) (RR: 12.9-14.2gm/dL)	12.3±1.4	12.4±1.0	0.919
TLC (x10^3/mL) (RR: 3.7-10.1x10^3/mL)	11.4±2.8	12.2±3.3	0.462
Lymphocytes (x10^3/mL) (RR:1-4.8x10^3/mL)	1.4±1.0	1.7±1.0	0.345
Platelets (x10^3/mL) (RR:155-366x10^3/mL)	207.9±78.2	227.0±76.0	0.424
ALT (IU/L) (RR: up to35 IU/L)	40.7±19.4	40.3±17.1	0.945
AST (IU/L) (RR: up to37 IU/L)	30.5±15.4	29.4±12.2	0.776
Creatinine (mg/dL) (RR:0.4-1.3 mg/dL)	1.14±0.24	0.78±0.23	**<0.001***
Ferritin (mg/dL) (RR:13-150 mg/dL)	550.1±237.8	384.6±167.9	**0.007***
D-dimer (mg/L) (RR:0-0.55 mg/L)	0.92±0.61	0.79±0.38	0.440
CRP (mg/L) (RR: up to 6.0 mg/L)	132.3±83.3	64.0±29.5	**0.007***
IL-6 (pg/mL) (RR:0-4.4 pg/mL)	84.7±45.0	31.2±24.7	**<0.001***

^Independent t-test. *Significant, SD=standard deviation, RR=reference range, TLC= total leucocytic count, ALT=alanine transaminase, AST=aspartate transaminase, CRP=C-reactive protein, IL-6-interleukin-6

Table 7. Distributions of rs383510 and rs2070788 genotypes and alleles among included complicated versus non-complicated COVID-19 cases.

Variables		Complicated (N=15)	Non-complicated (N=35)	P-value
rs383510				
Genotypes	**CC**	15 (100.0%)	35 (100.0%)	NA
	CT	0 (0.0%)	0 (0.0%)	
	TT	0 (0.0%)	0 (0.0%)	
Alleles	**C**	15 (100.0%)	35 (100.0%)	NA
	T	0 (0.0%)	0 (0.0%)	
rs2070788				
Genotypes	**AA**	5 (33.3%)	15 (42.9%)	0.562
	GA	8 (53.3%)	13 (37.1%)	
	GG	2 (13.3%)	7 (20.0%)	
Alleles	**A**	18 (60.0%)	43 (61.4%)	0.893
	G	12 (40.0%)	27 (38.6%)	

NA: Not applicable.

Table 8. Complications among non-survivors versus survivors of the included severe COVID-19 patients.

Complications	Non-Survivors (N=6)	Survivors (N=44)	P-value	OR (95% CI)
ARDS	6 (100.0%)	3 (6.8%)	**<0.001***	NA
Pleural effusion	4 (66.7%)	4 (9.1%)	**0.004***	20.00 (2.75–145.48)
Renal failure	1 (16.7%)	4 (9.1%)	0.487	2.00 (0.19–21.62)
Vascular thrombosis	0 (0.0%)	3 (6.8%)	0.999	NA

*Statistically significant values are in boldface type.
ARDS: Acute respiratory distress syndrome. OR: Odds ratio. CI: Confidence interval. NA: Not applicable.

Discussion

Coronavirus disease 2019, the largest pandemic of the twenty-first century thus far, is characterized by a significant mortality and complication rate. The severity and underlying mechanisms of the disease vary among individuals, influenced by factors such as pre-existing risk factors and chronic illnesses [15].

Regarding demographic data, many studies agreed with us that old age and smoking were highly prevalent among COVID-19 cases especially severe illness as ***Liu et al.*** [16]. This could be explained by a positive association of the *TMPRSS2* in lung tissue among older age, with decrease immunity, subsequently increase viral cell entrance and illness severity [17, 18].

The present study showed high prevalence of COVID-19 among male patients but declared that severity of illness was not affected by gender. This was in concordance with ***Orman et al.*** [19]. However other studies declared that males were more affected as ***Liao et al.*** and ***Ghweil et al.*** [20, 21].

COVID-19 associated mortality rate also differed obviously between studies and countries. Total mortality reached (6/50; 12%) among our involved patients. Also, 30% of severe cases developed complications with 40 % mortality.

Mortality estimates for COVID-19 were widely varied according to different reports, ranging from 3.4% to 88.3% [5, 22- 27]. Another multicenter Egyptian study conducted by ***AbdelGhaffar et al.*** reported mortality rate 24.2% among 3721 hospitalized patients [28].

This variation could be explained by nature of the investigated population, patient risk factors, older age of non-survivors, a history of co-morbidities as diabetes and chronic kidney disease, health system qualities, therapeutic context and by the lack of resources.

According to the severity of the illness, the most frequently experienced symptoms ranged from fever, cough, sore throat, malaise, headache, muscle pain and up to various systems complications according to case severity.

Clinical presentation in different studies revealed that fever, fatigue and lower oxygen saturations were remarkable signs and deteriorated according to illness severity [16, 19, 29].

There was agreement with other conducted studies that pulmonary complications especially ARDS was one of the commonest complications [30- 32].

Renal failure was observed among 5 severe COVID-19 cases in our study. Kidney injury was reported in other studies as ***Liu et al.*** and ***Singh et al.*** [33, 34]. Although the precise mechanism of renal involvement is not completely understood. Researchers have revealed that kidney cells exhibit high levels of cellular components necessary for the virus to enter, including angiotensin-converting enzyme 2, cellular transmembrane serine protease 2, and cathepsin L (CTSL). The severity of the injury varied and was influenced by other factors which included age, co-morbidities such as diabetes, hypertension, and a history of chronic renal disease [35, 36].

During the conducted study we tried to investigate *TMPRSS2* polymorphisms and some laboratory markers as reliable indicators for assessing the severity of COVID-19 during the ongoing pandemic. The goal of our research was to enhance medical decision-making processes and facilitate the efficient allocation of resources, such as hospital beds, medical equipment, and personnel. This in turn would help optimize patient care and alleviate the strain on healthcare systems, ultimately

contributing to better outcomes for individuals affected by COVID-19.

By analyzing *TMPRSS 2* and SNPs rs383510 and rs2070788, we found that rs383510 C allele dominated among severe COVID-19 cases with statistically significant difference (*P*=0.029). The genotype CC was dominated among complicated and non-survivors. It was observed that a trend towards a higher frequency of *TMPRSS2* genotype rs2070788, with A allele, and AA genotype among non-survivors. While GA genotype among severe complicated COVID-19 cases. Although this difference was not statistically significant regarding COVID-19 development of complications nor associated mortality.

The function of *TMPRSS2* polymorphisms in the pathophysiology of SARS-CoV-2 infection is still unknown and debated. Our findings were partially consistent with a German study which reported that the CC genotype of rs383510 was associated with a 1.73-fold greater risk of susceptibility to SARS-CoV-2 infection [14].

Data from other studies conducted among patients from different countries showed different associations between *TMPRSS2* polymorphisms including rs383510 and rs2070788 and more severe course of the disease/case fatality rate (CFR) of COVID-19. This could be explained by variation in genetic makeup across different populations, multiple non genetic reasons may affect the studies structure like the presenting symptoms, the prevalence of complications, and ethnicities [37-39].

In our study we highlighted that liver enzymes, creatinine, laboratory markers as CRP, ferritin, and D- dimer showed statistically significance regarding the disease severity. Also, we reported according to our cohort group the cut off value for Ferritin, CRP and IL-6 to help physicians predict mortality and complication among severe COVID-19 cases.

Many studies findings were in consistent with us and declared that CRP and ferritin are acute-phase reactants and serve as early markers of infection and inflammation, and their higher levels in severe COVID-19 patients are mediated by proinflammatory cytokines as IL-6 [40- 45].

A "cytokine storm," characterized by the excessive release of IL-6 and other inflammatory mediators, has been identified as a crucial factor in the development of respiratory failure, shock, multi-organs dysfunction, and the severity and mortality of COVID-19 [46- 47].

This in consistence with our finding that a significant higher levels of IL-6 among complicated cases (mean was about 2.5 folds higher than non-complicated cases) and in non-survivors, and that IL-6 had the highest specificity and positive predictive value in predicting both complications and mortality at cut-offs of 62 and 85 pg/mL respectively.

Limitation of the study, that the total sample size was relatively small and from a single center. So, further multicenter studies are recommended to get more information on Egyptian population. Also, there is a possibility that the observed relationship between each *TMPRSS2* polymorphism, laboratory parameters, and the prognosis of COVID-19 may have been influenced by bias or confounding factors. Despite these, we believe that our findings highlight the possible role of *TMPRSS2* polymorphisms in COVID -19.

Conclusions:

Genetics and specific genetic polymorphisms in certain genes play a significant role in understanding variations in increased susceptibility and unexpected outcomes in COVID-19 infections.

The frequency of *TMPRSS2* rs383510 C allele could be potentially considered when assessing COVID-19 severity, complications and mortality in our population, while we observed a trend towards a higher frequency of *TMPRSS2* rs2070788 A allele and AA genotype among non-survivors. While GA genotype among severe complicated COVID-19 cases.

However, validating these findings with larger sample sizes and in different ethnic groups is required. Moreover, it is highly encouraged to explore the potential involvement of other genetic factors in determining the severity and prognosis of COVID-19 patients.

Our study demonstrated the association of (CRP, ferritin, and IL-6) with critical illness in COVID-19. However, there is a need for a different approach to infection analysis due to the observed inter-individual variability in terms of disease severity, complications and mortality among COVID-19 cases.

Statements and Declarations

The authors report no conflicts of interest.

All authors have approved the final article.

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Data availability

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

Authors' contribution

All authors contributed to the study conception and design, AMS and HEG designed the study. MAG and HMA provided us with the documents, collected samples and the data. HMA and MAG carried out the study analysis. RNY and HMA carried out the molecular work. RME prepared the statistical analysis of the study. AMS, RNY and MAG revised and edited the final version of the manuscript. All authors read and approved the submitted final manuscript.

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