Gene Expression Analysis of ACE2, TMPRSS2, and LZTFL1 in COVID-19 Patients: A pilot study

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ABSTRACT

The objective of this research was to assess gene expression levels of ACE2, TMPRSS2, and LZTFL1 in COVID-19 patients from Egypt and investigate their potential association with the severity of the illness. The study involved ninety-six participants, divided into two groups: individuals experiencing severe COVID-19 symptoms and those with milder manifestations. Quantitative polymerase chain reaction (qRT-PCR) was employed to determine the gene expression levels of ACE2, TMPRSS2, and LZTFL1.
In the mild cases, the median age was 35 years, with an age range of 30-41 years, while severe cases had a median age of 65 years, ranging from 58 to 72 years. The male-to-female ratio in the entire group was 47.9% to 52.1%. All participants tested positive for COVID-19 based on nasal swab qPCR tests. Severe cases exhibit a significantly higher mean age. Comorbidities like type 2 diabetes (T2DM) and hypertension are markedly more prevalent in severe cases, while the absence of comorbidities is significantly associated with mild cases. The initial expression levels of all the examined genes were notably higher in severe COVID-19 patients compared to those with mild symptoms. The statistical significance was reflected in the p-values, which were less than 0.001 for ACE2, 0.05 for TMPRSS2, and 0.002 for LZTFL1. ROC curves were employed. The results showed that ACE2 exhibited greater sensitivity (89.58%) and specificity (79.17%) in predicting severe COVID-19 in comparison to LZTFL1 (70.83%) and TMPRSS2 (60.42%). These results propose that ACE2 expression may serve as a profitable prognostic marker for COVID-19. Multivariate analyses further elucidate the complex interplay of age and specific molecular factors, such as TMPRSS2 and LZTFL1, in influencing disease severity.

Key words: COVID-19 - ACE2 - TMPRSS2 - LZTFL1 - Gene expression - Egyptian patients - qRT-PCR - ROC curve.

1. INTRODUCTION:

The global community has grappled with the Coronavirus Disease-2019 (COVID-19) pandemic since late 2019. Individuals infected with COVID-19 exhibit variations in the severity of the infection and their response to treatment. Numerous investigations have been undertaken to examine the factors influencing the severity of COVID-19 infection. Among these factors, the polymorphism of the angiotensin converting enzyme 2 (ACE-2) and the type 2 transmembrane serine protease (TMPRSS2) genes has been a focal point, given their roles in facilitating the virus's entry into host cells (Alaa, *et al*., 2023). Additionally, it has been posited that LZTFL1 mediates genetic susceptibility to SARS-CoV-2 infection and COVID-19-related respiratory failure (Group SC-G, 2020).

ACE2 stands as the essential receptor for SARS-CoV-2, serving as the door for viral entry into host cells. TMPRSS2 (Transmembrane Protease, Serine 2) plays a

critical role in the priming of the viral spike protein, which is a crucial step for efficient infection of host cells (Hoffmann *et al.*, 2020).

The viral spike(S) protein establishes crucial interactions with receptors on the host cell's plasma membrane. The foremost binding partner in this context is ACE2 (Jackson *et al*., 2022). High ACE2 expression was related to higher risk of SARS-COV-2 activity, and development on the early disease phase (Alshahawey *et al*., 2020). Also increased ACE2 expression was correlated with disease severity and poor outcomes in hospitalized patients with COVID-19. (Alobaidy *et al*., 2023). and with cardiovascular diseases and complications in DM patients, which are commonly linked to adverse effects on the clinical progression of COVID-19 (Rahimi *et al*., 2014). ACE2 polymorphism rs2285666 is a frequently examined SNP that may affect severity of COVID-19, is thought to affect the gene splicing (Karakaş Çelik *et al*., 2021).

The efficiency of viral entry into host cells relies heavily on the activity of TMPRSS2, which facilitates the S protein combination with the cell (Prelli Bozzo *et al*., 2021). Egyptian study done by (Alaa *et al*., 2023) showed that there might be a strong correlation between the variability and genetic polymorphism of the ACE-1, ACE-2 and the TMPRSS2 genes and the risk and severity of the COVID-19 infection.

Leucine Zipper Transcription Factor Like 1 (LZTFL1) has garnered attention in the analysis of lung biopsies from COVID-19 patients. These biopsies have revealed indicators accompanying epithelial-mesenchymal transition (EMT), viral response pathways controlled by LZTFL1 (Downes *et al*., 2021).

Our study is dedicated to exploring the intricate relationship between gene expression and COVID-19 severity, with a specific focus on ACE2, TMPRSS2, and LZTFL1. We aim to uncover how variations in the expression of these genes may impact disease outcomes and contribute to our understanding of the underlying genetic factors influencing the clinical course of COVID-19.

2. SUBJECTS AND METHODS:

2.1. Subjects:

In this research, we enrolled 96 Egyptian patients who had recently been diagnosed (after 48 hour of symptoms onset) with COVID-19 via qPCR analysis of nasopharyngeal samples. These patients were divided into two distinct groups based on the world health organization case definition (WHO, 2022), Mild Cases: These patients exhibited mild clinical symptoms and did not show any signs of pneumonia in their lung imaging. Severe Cases: This group included patients who met any of the following severity criteria: a respiratory rate exceeding 30 breaths per minute, resting oxygen saturation levels below 93%, arterial oxygen pressure (PaO2)/inspired oxygen fraction (FiO2) ratio below 300 mm Hg, or those with over 50% of lung lesions detected in imaging within 24 to 48 hours.

The patient's inclusion criteria were that: All of patients had a positive qualitative RT-PCR to detect SARS-CoV-2-RNA.While the exclusion criteria were Patients who have any associated malignancy or viral infection other than COVID-19 were excluded from the study.

All patients underwent comprehensive clinical evaluations, including detailed registrations, chest and general examinations, and extensive laboratory tests to identify any potential complications. The patient recruitment for this study took place between March and May 2021 at Menoufia University Hospital. Written informed consent was obtained from all participants.

2.2. Sample Collection:

Peripheral blood samples were collected from all subjects for routine COVID-19 assessments. These assessments included a CBC, CRP, ALT, AST, prothrombin time assessment, measurement of interleukin-6 (IL-

6) levels, evaluation of lactate dehydrogenase (LDH) levels, analysis of ferritin levels, D-dimer analysis, and kidney function tests using commercially available assays.

2.3. Quantitative Real-time PCR (qPCR):

Blood samples were collected from COVID-19 patients (both mild and severe cases) through venipuncture into EDTA tubes as anticoagulants. The collected whole blood was immediately frozen at -80°C until further analysis. Total RNA was extracted from the whole blood samples using the TriRNA Pure kit, Cat. No. TRPD050 from Geneaid (New Taipei city, Taiwan) following the manufacturer's instructions. The extracted RNA was then reverse transcribed into single-stranded complementary DNA (cDNA) using the TopScriptTM RT DryMix cDNA synthesis kit Cat. No. RT220 from Enzynomics (Daejeon, Korea). For mRNA expression analysis by StepOne™ Real-Time PCR System (applied biosystems by life technologies, USA) gene quantification level was estimated according to the following equation: $2^{-\Delta\Delta ct}$, mixtures were prepared using the WizPureTM qPCR Master (SYBR) Real-time PCR Master Mix Kit, Cat. No. W1721 from Wizbiosolutions (Gyeonggi-do, Korea), following the manufacturer's recommendations. The primer sequences used for qPCR to assess ACE2, TMPRSS2, and LZTFL1 gene expression are as follow for ACE2 Forward- 5'-CGAGTGGCTAATTTGAAACCAAGAA-3'; Reverse-.5'-ATTGATACGGCTCCGGGACA-3' (Xue X. et al, 2021), for TMPRSS2 forward-5'-CCTCTGGTCACTTCGAAGAAC-3'; Reverse 5'-GTAAAACGACGTCAAGGACG-3 (Hermans KG et al., 2006) and for LZTFL1 Forward-5'--CCACTGATAGTGGAGATTGTGCG-3; Reverse-5-CCTCCAGGTATGCCTTCATGTC-3 (Cui Z et al., 2022). GAPDH was utilized as an internal reference gene to normalize gene expression levels.

2.4. Statistical Analysis:

Data analysis for this study was conducted using IBM SPSS software package, version 20.0 (IBM Corp., Armonk, NY). Quantitative data was presented in terms of percentages and visual representations. To assess the normality of data distribution, the Shapiro-Wilk test was employed. Descriptive statistics including the range (minimum and maximum values), mean, standard deviation, and median were used to characterize the quantitative data.

For the comparison of normally distributed quantitative data between the two study groups, the independent t-test was utilized. To evaluate the potential of expression levels as discriminant between mild and severe groups, a receiver operating characteristic (ROC) curve was employed. Furthermore, a combined ROC curve was analyzed using binary logistic regression.

3. RESULTS:

3.1. Demographic data, symptoms and associated comorbidities in the studied population:

Table 1 showed that the mean age of individuals with severe cases (64.19 years) is significantly higher than that of those with mild cases (36.10 years), as indicated by the p-value of <0.001. this underscores the association between older age and increased severity of the disease. However, Gender, did not exhibit significant differences between the mild and severe groups.

Also, the table provides a clear overview of COVID-19 symptoms and associated comorbidities in the studied population, comparing individuals with mild and severe cases. The p-values indicate the statistical significance of the differences between the two groups. Several symptoms, such as cough, dyspnea, fatigue and fever > 38°C are significantly more prevalent in severe cases, yet anosmia and headache are significantly

more prevalent in mild cases. Additionally, comorbidities like type 2 diabetes (T2DM), hypertension, show significant associations with the severity of COVID-19, underscoring their potential as indicators of disease severity, On the other hand, the absence of comorbidities is significantly associated with the mild form of the disease.

3.2. Laboratory Data:

In Table 2, the biochemical and hematological variables of the research groups are presented. Several serum parameters, including ALT, AST, Ferritin, IL6, LDH, WBC count, Prothrombin time, INR, Urea, and Creatinine, were significantly higher in severe patients than in mild patients. Conversely, hemoglobin levels, RBC count, and platelet count were significantly lower in severe patients. Lymphocytic count (Absolute and Relative), CRP, and D-dimers showed no significant differences between the studied groups.

3.3. Expression of ACE2, TMPRSS2, and LZTFL1 Genes:

Table 3 shows the expression levels of ACE2, TMPRSS2, and LZTFL1 in mild and severe COVID-19 cases as detected by qRT-PCR, normalized to the GAPDH (housekeeping gene) as an internal control. It was observed that the expression levels of all three genes were significantly higher in severe cases compared to the mild group. Further, the correlation analysis between gene expression and other laboratory findings in severe COVID-19 cases was explored. In the severe group, there were non-statistically significant correlations observed among the studied genes and the other parameters (Table 4).

3.4. Correlations between gene expression and various parameters in severe cases:

In table 5, It appears that the correlations between gene expression and various parameters in severe cases are generally weak, and many are not statistically significant, indicating that the gene expressions of ACE2, TMPRSS2, and LZTFL1 may not be strongly influenced by these parameters within this specific group of severe cases. However, the table highlights some potential areas of interest, such as the association between gene expression and lymphocyte counts and, to a lesser extent, inflammatory markers.

3.5. Potential Marker for COVID-19 Severity:

ROC curve analysis was conducted to assess the diagnostic performance of the three genes in discriminating severe cases from mild cases *(Table 6, Fig 1&2)*. ACE2 demonstrated the ability to predict severe COVID-19 with a 1.122-fold change, yielding a sensitivity of 89.58% and specificity of 79.17%. TMPRSS2 could predict severe COVID-19 at a level of 5.893, with sensitivity and specificity of 60.42% and 56.25%, respectively. LZTFL1 could predict severe COVID-19 at a level greater than 0.779, with a sensitivity of 70.83% and specificity of 54.17%. These results indicated that ACE2 exhibited superior sensitivity and specificity in predicting severe COVID-19 compared to TMPRSS2 and LZTFL1. Consequently, ACE2 may prove to be an effective diagnostic indicator for COVID-19. Additionally, when the two markers were used together, there was no improvement in sensitivity or specificity compared to using each marker alone.

3.6. Multivariate analysis using logistic regression to examine the parameters influencing the progression from mild to severe cases.

Table 10a presents the results of a multivariate analysis using logistic regression to examine the parameters influencing the progression from mild cases (n=48) to severe cases (n=48). Age demonstrates a noticeable effect (B=0.865, SE=0.565, Sig.=0.126), suggesting a potential association with an increased odds ratio of 2.376 for severe cases.

Other factors such as IL6, LDH, ALT, AST, ACE2, TMPRSS2, and LZTFL1 are also considered, with varying degrees of significance.

Table 10b presents the results of another multivariate logistic regression analysis focusing on the parameters influencing the transition from mild cases (n=48) to severe cases (n=48). ACE2 shows a prevalence of 0.198, but the AOR (0.998) suggests a marginal effect on the odds of transitioning to severe cases, with the 95% CI spanning 0.995 to 1.001. TMPRSS2 and LZTFL1 exhibit statistically significant associations with severe cases, where a one-unit increase in TMPRSS2 is associated with a 0.037 higher prevalence and 1.009 times higher odds of severity, with a 95% CI of 1.001 to 1.019. Similarly, LZTFL1 shows a prevalence of 0.049 and an AOR of 1.388, indicating an increased odds of 1.388 for severe cases, but with a wide 95% CI of 1.0 to 1.929. The AOR is adjusted for Age, IL6, LDH, ALT, and AST, providing a controlled analysis. Overall, these findings suggest that TMPRSS2 and LZTFL1 may have significant roles in predicting the severity of cases, emphasizing their potential importance in understanding disease progression.

4. DISCUSSION:

The world has never been the same since SARS-CoV-2 sparked the global COVID-19 pandemic, leading to a staggering loss of lives across nations (Shereen *et al.,* 2020). The disease's clinical presentation encompasses a broad spectrum, spanning from asymptomatic cases to mild upper respiratory symptoms, and at its most severe, it progresses to bilateral pneumonia with associated respiratory failure, often necessitating advanced life support interventions (Wang *et al.,* 2020). At the molecular level, The SARS-CoV-2 virus's ability to enter host cells is dependent on its spike(S) protein. This intricate process initiates with the virus binding to the zinc metallopeptidase ACE2 on the cell surface of the host, followed by the priming of the S protein by the transmembrane protease serine 2 (TMPRSS2) (Hoffmann *et al.,* 2020 and Lan *et al.,* 2020).

In the course of our investigation, we delved into elucidating the intricate relationship between the expression of ACE2, TMPRSS2, and LZTFL1 genes and their consequential impact on the prognosis of COVID-19 in a cohort of Egyptian patients who were under treatment at Menoufia University Hospital during the period spanning from April 2021 to October 2021.

Within this patient population, we categorized individuals into two distinct groups, differentiating between those with mild and severe manifestations of the disease. Our analysis uncovered that the age of patients with severe COVID-19 was significantly higher in comparison to those with milder cases, aligning with previous research highlighting age as a significant risk factor for severe outcomes in COVID-19 (Cecconi *et al.,* 2020 and Onder *et al*., 2020)

The presented analysis sheds light on the distinct symptomatology and comorbidity patterns associated with the severity of COVID-19 in the studied population. Notably, severe cases exhibited a higher prevalence of acute symptoms such as cough, dyspnea, fatigue and fever > 38°C, compared to mild cases. These findings align with prior studies indicating the predictive value of these symptoms for severe outcomes in COVID-19 (Guan *et al.,* 2020 and Mao *et al.,* 2020).

Furthermore, the association of specific comorbidities with disease severity is evident. Individuals with severe cases were more likely to have comorbidities, particularly type 2 diabetes (T2DM) and hypertension. Similar observations have been reported by other researchers, emphasizing the role of these comorbidities as risk factors for severe COVID-19 outcomes (Zhou *et al.,* 2020 and Yang *et al.,* 2020).

Interestingly, the absence of comorbidities was significantly associated with mild cases, reinforcing the notion that individuals without underlying health conditions may have a more favorable prognosis in the context of COVID-19 that was accepted by other research that emphasized the importance of considering comorbidities as crucial factors in assessing the overall risk and prognosis of COVID-19 patients (Li *et al.,* 2020).

The observed significance of age and comorbidities in severe groups may underscores the intricate relationship between age and health conditions, particularly in the context of COVID-19. where older individuals are more likely to present with comorbidities that may exacerbate the severity of COVID-19 that was supported by studies done by (Wu & McGoogan, 2020).

Furthermore, various serum markers, including ALT, AST, Ferritin, IL6, LDH, WBC count, Prothrombin time, INR, Urea, and Creatinine, exhibited statistically significant elevations in severe cases as compared to mild ones, consistent with prior research findings of (Trofin *et al.*, 2023; Velavan & Meyer, 2020; Zhou *et al.,* 2020). Conversely, severe patients displayed lower levels of hemoglobin, RBCs, and platelets, aligning with observations made by (Abd El-Lateef, *et al.* 2022)

In our unique study, we harnessed a noninvasive technique to evaluate the expression levels of ACE2, TMPRSS2, and LZTFL1 genes within our study cohorts, with the aim of assessing their potential as molecular biomarkers for COVID-19 cases. Our investigation revealed that the mean expression levels of all three genes were significantly higher in severe cases when compared to the mild group, thus reinforcing the observations made in prior research of (Gheware *et al.,* 2022; Ghezelbash *et al.,* 2023; Zheng, 2022). Furthermore, LZTFL1 emerged as a genetic susceptibility locus in COVID-19 patients with respiratory failure, corroborating previous findings of (Group SC-G, 2020)

Remarkably, we did not detect statistically significant correlations between gene expression levels and the various biochemical and hematological parameters within severe COVID-19 cases, underscoring the ongoing challenges in identifying specific laboratory markers for distinguishing COVID-19 severity that was accepted by (Hariyanto *et al.*, 2021).

To gauge the diagnostic potential of the three genes, we conducted ROC curve analyses to discriminate severe from mild cases. Notably, ACE2 exhibited superior sensitivity and specificity at 89.58% and 79.17%, respectively, outperforming TMPRSS2 (60.42%, 56.25%) and LZTFL1 (70.83%, 54.17%). These findings substantiate the notion that ACE2 holds promise as a valuable diagnostic marker for COVID-19, as evidenced by prior studies of *(Fagyas et al.,* 2022; Kassif Lerner *et al.,* 2022).

The multivariate logistic regression analyses provide valuable insights into the parameters affecting the progression from mild to severe cases in the context of the studied population. Age stands out as a potentially significant factor. This finding aligns with previous studies highlighting the impact of age on COVID-19 severity (Hu, *et al.,* 2022).

When comparing the use of these genes for predicting COVID-19 severity with conventional clinical laboratory methods, notable advantages emerge. ACE2 and TMPRSS2 offer valuable molecular insights into the intricate interaction between the virus and host cells, knowledge of their roles may pave the way for the development of specifically targeted therapeutic interventions (Martinez-Diz, *et al.,* 2023). However, these molecular markers exhibit limitations, primarily a confined focus on specific stages of the viral life cycle, potentially overlooking the intricate interplay of host responses and other contributing factors to disease severity. To ensure their clinical utility, rigorous clinical

validation is imperative before their integration into routine clinical laboratory practices for predicting COVID-19 severity (Rossi, *et al.,* 2021).

5. CONCLUSION:

In conclusion, our study yields positive findings concerning the relationship between gene expression and COVID-19 severity. Notably, the expression levels of ACE2, TMPRSS2, and LZTFL1 were significantly elevated in severe cases, suggesting a potential link between these genes and disease severity. The ROC curve analysis revealed that ACE2 has a superior sensitivity and specificity in distinguishing severe cases from mild ones. Additionally, Age discrepancy underscores the well-established observation that older individuals are more susceptible to severe outcomes in COVID-19. Presented multivariate analyses shed light on the intricate interplay of age and specific molecular factors, such as TMPRSS2 and LZTFL1, in influencing the severity of COVID-19 cases. These positive findings underscore the need for further research into the intricate molecular mechanisms driving disease severity.

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7. AUTHOR CONTRIBUTIONS:

The conception and design of this study were collaboratively developed by all authors. F.S. was responsible for the collection of samples, while material preparation and data analysis were conducted by F.S., A.D., and M.S. The manuscript drafting and revisions were equally shared among F.S., A.D., M.S., and H.S. All authors provided valuable input and feedback during the manuscript's evolution, and they collectively approved the final version of the manuscript.

8. COMPLIANCE WITH ETHICAL STANDARDS

8.1.Funding: Not applicable

8.2.Conflict of interest: The authors declare that there are no conflicts of interest**.**

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Table 1. Demographic data, symptoms and associated comorbidities in the studied population.

Variable	Mild (n = 48) No. %	Severe (n = 48) No. %	p-value
Demographic Data			
Age: Range (Mean)	22.0 – 58.0 (36.10)	31.0 – 87.0 (64.19)	<0.001*
Gender: number (%) **Male** **Female**	 24 (50%) 24 (50%)	 23 (47.9%) 25 (52.1%)	NS
Acute symptoms			
Anosmia	33 (68.8%)	14 (29.2%)	<0.01*
Cough	22 (45.8%)	39 (81.3%)	<0.01*
Diarrhea	8 (16.7%)	15 (31.3%)	0.07
Dyspnea	10 (20.8%)	43 (89.6%)	<0.01*
Fatigue	33 (68.8%)	45 (93.8%)	<0.01*
Fever > 38°C	16 (33.3%)	33 (68.8%)	<0.01*
Hemoptysis	0	5 (10.4%)	0.29
Headache	34 (70.8%)	25 (52%)	0.02*
Sore throat	22 (45.8%)	22 (45.8%)	0.86
Rhinorrhea	26 (54.1%)	18 (37.5%)	0.07
Comorbidities			
Autoimmune disease	0	1 (2.1%)	0.22
Chronic kidney disease	0	1 (2.1%)	0.22
COPD	0	1 (2.1%)	0.22
Coronary disease	0	2 (4.1%)	0.49
T2DM	1 (2%)	10 (20.8%)	<0.01*
Hypertension	2 (4.2%)	13 (27%)	<0.01*
Immunodeficiency	0	2 (4.1%)	0.49
No comorbidities	45 (93.8%)	18 (37.5%)	<0.01*

** Statistically significant, p-value < 0.05.*

Ns, non-significant

COPD, Chronic obstructive pulmonary disease

T2DM, Type 2 diabetes Meletus

Table 2. Biochemical, and hematological characteristics of the studied groups

Variables	Mean ± SD		P Value
	Mild cases (n = 48)	Severe cases (n = 48)	
Biochemical parameters			
CRP: number (%)			
Positive (> 10 mg/L)	36 (75 %)	40 (83.3 %)	NS
Negative	12 (25 %)	8 (16.7%)	
IL6 (Pg/ml)	28.28 ± 9.09	36.64 ± 19.78	0.049*
LDH (u/l)	196.59 ± 39.46	351.21 ± 307.41	0.001*
Ferritin (ng/ml)	85.51 ± 75.07	211.41 ± 114.75	<0.001*
D-dimer: number (%)			
Positive (>250 ng/ml)	0	6 (12.5%)	NS
Negative	48 (100%)	42 (87.5%)	
ALT (up to 40 U/L)	25.52 ± 6.99	53.75 ± 40.75	<0.001*
AST (up to 40U/L)	27.29 ± 7.03	57.19 ± 28.84	<0.001*
Creatinine (0.6-1.3 mg/dl)	0.97 ± 0.12	1.18 ± 0.45	0.003*
Urea (20-45 mg %)	31.69 ± 4.78	46.10 ± 15.50	<0.001*
Hematological parameters			
HB	12.88 ± 1.80	12.11 ± 1.57	0.028*
RBCs	4.89 ± 0.45	4.60 ± 0.67	0.015*
WBCs	3.44 ± 0.90	10.17 ± 5.19	<0.001*
Lymphocytes			
Absolute (1000-4000)	628.94 ± 251.98	761.96 ± 375.33	0.056
Relative (20-45 %)	13.53 ± 4.52	11.90 ± 5.90	0.139
Platelets	304.27 ± 95.66	144.33 ± 79.07	<0.001*
Prothrombin time (11-13.5 Sec)	12.04 ± 0.71	14.59 ± 1.26	<0.001*
INR (.8-1.1	0.96 ± 0.09	1.23 ± 0.24	<0.001*

Groups bearing different numbers are significantly different from each other at $P \leq 0.05$.
* Show statistical significant difference.
CRP (C-Reactive protein), IL6 (Interleukin 6), LDH (Lactate dehydrogenase), ALT (Alanine transaminase), AST (Aspartate Transaminase), HB (Hemoglobin), RBCs (Red blood cells), WBCs (White blood cells), INR (International normalized ratio).

Table 3. Expression of ACE2, TMPRSS2, and LZTFL1 Genes.

Gene	Mean ± SD		P Value
	Mild cases (n=48)	Severe cases (n=48)	
ACE 2	38.12 ± 245.66	147.49 ± 436.33	<0.001*
TMPRSS 2	60.38 ± 171.51	234.26 ± 413.20	0.05*
LZTFL 1	2.57 ± 4.52	16.90 ± 38.58	0.002*

ACE 2 (angiotensin-converting enzyme 2), **TMPRSS 2** (type 2 transmembrane serine protease), **LZTFL 1** (leucine zipper transcription factor like 1).

Table 4. correlation between ACE 2, TMPRSS 2, LZTFL 1 and different parameters in severe cases group**.**

Variables	ACE 2		TMPRSS 2		LZTFL 1	
	r_s	P	r_s	p	r_s	P
Age (years)	0.049	0.742	-0.045	0.763	-0.127	0.389
HB	-0.084	0.572	0.184	0.211	-0.053	0.718
RBCs	0.018	0.904	0.271	0.062	-0.057	0.699
Platelets	-0.205	0.163	-0.209	0.155	-0.190	0.195
WBCs	-0.209	0.154	-0.211	0.149	-0.207	0.158
Lymphocytes						
Absolute	0.121	0.414	0.233	0.110	0.205	0.162
Relative	0.263	0.071	0.078	0.596	0.282	0.052
Prothrombin						
Time	-0.125	0.395	-0.121	0.411	0.126	0.395
INR	-0.046	0.758	0.086	0.561	0.150	0.308
IL6	0.258	0.076	0.271	0.062	-0.045	0.763
LDH	0.167	0.258	0.189	0.199	0.132	0.371
Ferritin	0.206	0.160	0.165	0.263	0.141	0.339
Liver function						
AST	0.140	0.342	0.147	0.320	0.090	0.544
ALT	0.102	0.491	0.123	0.405	0.134	0.363
Kidney function						
Urea	0.166	0.259	0.132	0.370	-0.106	0.474
Creatinine	-0.011	0.941	0.132	0.372	-0.133	0.368

rs: Spearman coefficient.
IL6 (Interleukin 6), **LDH** (Lactate dehydrogenase), **ALT** (Alanine transaminase), **AST** (Aspartate Transaminase), **HB** (Hemoglobin), **RBCs** (Red blood cells), **WBCs** (White blood cells), **INR** (International normalized ratio).

Table 5. Correlation between Expression (qPCR) and different parameters in severe cases group (n = 48)

	Expression (qPCR)					
	ACE 2		TMPRSS 2		LZTFL 1	
	rs	p	rs	p	rs	p
Age (years)	0.049	0.742	-0.045	0.763	-0.127	0.389
HB	-0.084	0.572	0.184	0.211	-0.053	0.718
RBCs	0.018	0.904	0.271	0.062	-0.057	0.699
Platelets	-0.205	0.163	-0.209	0.155	-0.190	0.195
WBCs	-0.209	0.154	-0.211	0.149	-0.207	0.158
Lymphocytes						
Absolute	0.121	0.414	0.233	0.110	0.205	0.162
Relative	0.263	0.071	0.078	0.596	0.282	0.052
Prothrombin						
Time	-0.125	0.395	-0.121	0.411	0.126	0.395
INR	-0.046	0.758	0.086	0.561	0.150	0.308
IL6	0.258	0.076	0.271	0.062	-0.045	0.763
LDH	0.167	0.258	0.189	0.199	0.132	0.371
Ferritin	0.206	0.160	0.165	0.263	0.141	0.339
Liver function						
AST	0.140	0.342	0.147	0.320	0.090	0.544
ALT	0.102	0.491	0.123	0.405	0.134	0.363
Kidney function						
Urea	0.166	0.259	0.132	0.370	-0.106	0.474
Creatinine	-0.011	0.941	0.132	0.372	-0.133	0.368

rs: Spearman coefficient**, ACE 2** (angiotensin-converting enzyme 2), **TMPRSS 2** (type 2 transmembrane serine protease), **LZTFL 1** (leucine zipper transcription factor like 1), **IL6** (Interleukin 6), **LDH** (Lactate dehydrogenase), **ALT** (Alanine transaminase), **AST** (Aspartate Transaminase), **HB** (Hemoglobin), **RBCs** (Red blood cells), **WBCs** (White blood cells), **INR** (International normalized ratio).

Table 6. Diagnostic performance of the three genes in discriminating severe cases (n=48) from mild (n=48)

	AUC	P	95% C. I	Cut off	Sensitivity	Specificity	PPV	NPV
ACE 2	0.892	<0.001*	0.825 – 0.958	>1.122	89.58	79.17	79.6	88.1
TMPRSS 2	0.615	0.05*	0.502 – 0.729	>5.893	60.42	56.25	58.0	58.7
LZTFL 1	0.680	0.002*	0.574 – 0.786	>0.779	70.83	54.17	60.7	65.0
ACE 2 + TMPRSS 2	0.699	0.001*	0.594 – 0.804	----	72.92	47.92	59.0	65.7
ACE 2 + LZTFL 1	0.727	<0.001*	0.627 – 0.826	-----	85.42	52.08	63.5	75.8
TMPRSS 2+ LZTFL 1	0.693	0.001*	0.588 – 0.798	-----	70.83	50.0	58.6	63.2

AUC (Area Under a Curve), **CI (**Confidence Intervals), **NPV** (Negative predictive value), **PPV** (Positive predictive value)

Table 7a. Multivariate analysis Logistic regression for the parameters affecting Severe cases (n= 48) from mild cases (n= 48)

	B	SE	Sig.	OR	95% CI	
					LL	UL
Age	0.865	0.565	0.126	2.376	0.785	7.194
IL6	0.023	0.276	0.935	1.023	0.595	1.758
LDH	0.520	0.429	0.225	1.683	0.726	3.900
ALT	0.200	0.168	0.233	1.221	0.879	1.696
AST	-0.001	0.006	0.873	0.999	0.987	1.011
ACE 2	-0.007	0.005	0.171	0.993	0.983	1.003
TMPRSS 2	0.024	0.019	0.191	1.025	0.988	1.063
LZTFL 1	0.050	0.074	0.499	1.051	0.909	1.216

B: Unstandardized Coefficients SE: Estimates Standard error OR: Odds ratio
CI: Confidence interval LL: Lower limit UL: Upper Limit

Table 7b. Multivariate analysis Logistic regression for the parameters affecting Severe cases (n= 48) from mild cases (n= 48)

	p	AOR (LL – UL 95%C. I)
ACE 2	0.198	0.998(0.995 – 1.001)
TMPRSS 2	0.037*	1.009(1.001 – 1.019)
LZTFL 1	0.049*	1.388(1.0 – 1.929)

AOR: adjust Odd`s ratio by Age, IL6, LDH, ALT and AST
C.I: Confidence interval LL: Lower limit UL: Upper Limit
*: Statistically significant at $p \leq 0.05$

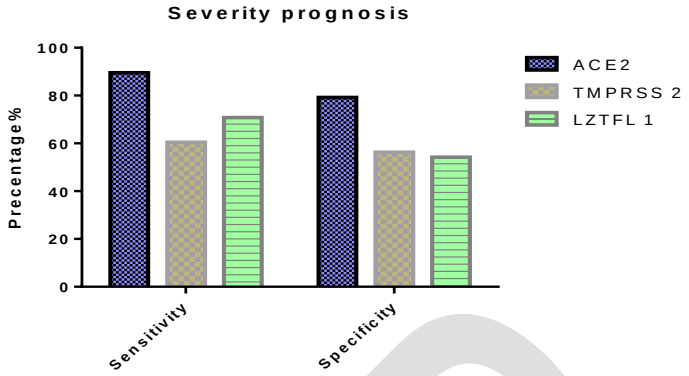

Fig. 1. Diagnostic performance for Expression to discriminate severe cases.

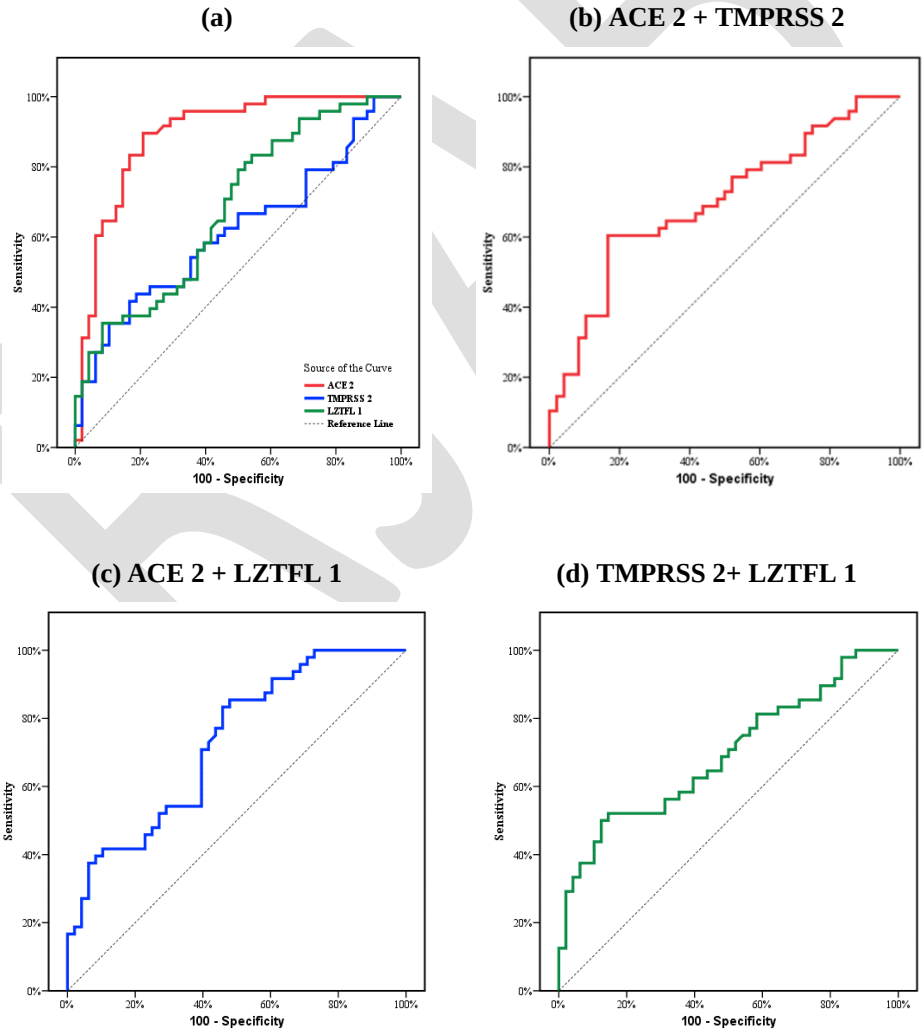

Fig. 2. ROC curve for Expression to discriminate severe cases from mild.