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Original article

Variations in levels of ACE2 and TMPRSS2 receptors among COVID-19

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ABSTRACT

Background: Key actors in SARS-CoV-2 entrance into host cells include angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2). Our objective was to study gene expression of those key actors in COVID-19 patients, with especial emphasis on patients having other comorbidities. **Methods:** The Expression of ACE2 and TMPRSS2 genes in nasopharyngeal swabs was assessed in 84 COVID-19 patients in comparison with 30 healthy controls using real-time PCR. We also compared expression levels of ACE2 and TMPRSS2 between different COVID-19 groups; moderate & severe, diabetic & non-diabetic, hypertensive & normotensive individuals, and between individuals belonging to different age groups. **Results:** Our results showed that up-regulation of ACE2 and TMPRSS2 was observed in COVID-19 patients relative to healthy controls, and there was a positive correlation between ACE2 and TMPRSS2 expression levels. TMPRSS2 gene expression level was higher in older age groups and both genes showed higher expression levels in males. On the other hand, no difference was detected in the gene expression level of ACE2 and TMPRSS2 between moderate and severe COVID-19 patients. Also, alterations were detected in the two genes expression levels in patients having comorbidities. **Conclusions:** The ACE2 and TMPRSS2 gene expression levels varied between different patient groups, posing a greater risk of getting SARS-CoV2 infection.

Introduction

At the end of 2019, a new coronavirus known as SARS-CoV-2 surfaced in the Chinese city of Wuhan, causing an outbreak of atypical viral pneumonia. This unique coronavirus disease, also known as coronavirus disease 2019 (COVID-19), has spread rapidly due to its high transmissibility. SARS-CoV2 invades host cells via angiotensin-converting enzyme 2 (ACE2) and trans-membrane serine protease 2 (TMPRSS2). ACE2 has been identified as the primary functional receptor for SARS-CoV2 entry into cells in studies.]1[

Several human tissues, including the lung, gut, heart, and kidney, have been demonstrated to express the ACE homologue as a membrane attached protein. This protein was shown to be expressed on the surface of ciliated bronchial cells, lung alveolar epithelial cells, and endothelial cells [2]. ACE2 not only serves as a cellular receptor for

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SARS-CoV entry, but it also serves as a protective mechanism against lung injury. The expression of ACE2 plays a key role in SARS-CoV infection. As a result, comorbid disorders that regulate the expression of this protein may influence illness severity [3].

The TMPRSS2 is a tool employed by a wide variety of viruses. The 70 kDa serine protease is produced as a precursor protein (zymogen) that is autoproteolytically activated. [4]. TMPRSS2 has been linked to physiological and pathological processes such digestion, tissue re-modelling, blood coagulation, fertility, inflammatory responses, tumour cell invasion, apoptosis, and pain. [5]. Expression of TMPRSS2 gene is developmentally regulated and increases with ageing [6]. Enveloped viruses such as corona or influenza viruses enter host cells via the binding of their envelope glycoproteins (GP) to their cognate receptors. To trigger fusion between the viral and cellular membranes, some viral GPs rely on proteolytic activation. TMPRSS2 is widely expressed in the human airways, and its role in the activation of clinically relevant respiratory viruses such as influenza and coronaviruses is well established [7].

According to recent studies, people with conditions such as diabetes, hypertension, and obesity have the highest prevalence of new coronary pneumonia infections and are at a higher risk of death [8].The aim of our study was to analyze gene expression levels of ACE2 and TMPRSS2 in nasopharyngeal swabs of COVID-19 patients. Also, to compare their levels between different COVID-19 groups (moderate & severe cases, diabetic & non-diabetic, & hypertensive & normotensive individuals) and between individuals belonging to different age groups.

Methods

This cross-sectional study was conducted in the duration from December 2021 to April 2022 in the Molecular Biology Research and Studies Institute laboratories, Assiut University. Study participants included 84 patients with SARS-CoV-2 infection admitted to the Chest Department .Assiut University Hospitals and 30 age and sex matched healthy controls. The study was authorized by the Ethics Committee of the Faculty of Medicine, Assiut University (IRB no 0010947). Patients provided informed consent for sample collection prior to enrollment in the study.

Participants' demographic information and medical histories were recorded, and patients were clinically examined. Baseline laboratory tests included ferritin and liver enzymes. Arterial blood gases were also measured. Chest computed tomography (CT) was used to diagnose COVID-19 pneumonia. Cases were considered severe if they had any of the following: SpO2 < 94% on room air at sea level, PaO2/FiO2 < 300 mm Hg, or a respiratory rate >30 breaths/min. Cases were considered moderate if they did not meet the above criteria. According to the internal policy of Assiut University hospitals in the management of the COVID-19 pandemic, no mild cases were enrolled in the hospital during the study period [9]. Patients were evaluated for the presence of comorbidities such as diabetes mellitus (DM), hypertension, (HTN), and chronic renal and liver failure.

Nasopharyngeal swab samples were taken from all participants. The extraction was done using ABT total RNA Mini Extraction Kit (Applied Biotechnology, Egypt) following the manufacturer's instructions. RNA was reverse transcribed into cDNA using the ABT reverse transcription kit (Applied Biotechnology, Egypt) according to the manufacturer's instructions. Real-time PCR was performed using SYBR Green (WizPure, Korea). After initial denaturation step for 5 min at 95˚C, a three-step cycling procedure (denaturation at 95˚C for 30 sec, annealing according to Ta for primers for 30s, and extension at 72˚C for 30sec) was used for 40 cycles. The sequences of primer sets specific for transcripts of the studied genes are shown in table (1) [10]. The relative expression levels of ACE2 and TMPRSS2 were normalized to the level of β-actin housekeeping gene transcripts and quantified by the 2-ΔΔCT [11].

Statistical analysis:

Date entry and data analysis were done using SPSS version 24 (Statistical Package for Social Science, USA). Data were presented as number, percentage, mean, standard error, median and range. Normality of data distribution was assessed by Sharpio-Wilk test. Chi-square test and Fisher Exact test were used to compare between qualitative variables. Independent Mann-Whitney was used to compare quantitative variables between groups. Correlations between variables were evaluated using Spearman correlation

coefficient. A p-value was considered statistically significant when $p < 0.05$.

Results

Table (2) shows demographic, clinical and basic laboratory data of the patients. In our study 47.6% of patients were males, and more than 50% of patients were above fifty years old. Eighty-one percent of patients showed moderate COVID-19 and 19% had severe COVID-19.Additionally, 47.6% of patients had cough, 45.2% had dyspnea and 23.8% had fever. As regards comorbidities, 39.3% of patients were hypertensive, 31% of patients were diabetic and 27.4% of patients had renal dysfunction. The mean value of PCO2 was 39.6 ± 13.21 mm Hg, PO2 was 67.5 ± 20.08 mm Hg and HCO3 was 26.5 ± 10.10 mEq/L. Ferritin mean level was 1051.14 ± 876.68.

Gene expression levels of ACE2 and TMPRSS2 in patients and controls

Table (3), shows fold changes in ACE2 and TMPRSS2 gene expression levels in patients and control groups. The expression levels of both genes were significantly higher in COVID-19 patients than the control group (p=0.003 and <0.0001, respectively). Moreover, a positive correlation was observed between ACE2 & TMPRSS2 gene expression levels in COVID-19 patients (r= 0.3, p<0.001), as shown in figure (1).

Relations of the ACE2 and TMPRSS2 gene expression levels with the clinical symptoms

Table (4) shows the relation between ACE2 and TMPRSS2 gene expression levels and clinical symptoms. There were no significant differences in either ACE2 or TMPRSS2 between patients showing fever, cough or dyspnea compared with those not having these symptoms.

Relations of the ACE2 and TMPRSS2 gene expression levels with the age, gender, and severity

Table (5) shows the levels of expression of ACE2 and TMPRSS2 genes relative to age, gender, and severity. Only TMPRSS2 was significantly higher in older age groups (p=0.02). The expression levels of both genes were significantly higher in males from similar age groups (p=0.002 and p=0.001, respectively). Unlike ACE2, TMPRSS2 expression level was significantly higher in more severe cases (p=0.04).

Relations of the ACE2 and TMPRSS2 gene expression levels with comorbid diseases

As presented in table (6), while ACE2 gene expression levels were significantly lower in hypertensive, diabetic patients and patients having chronic renal failure, TMPRSS2 gene expression levels were significantly higher in patients having these comorbidities compared to those not having them.

Table 1. The sequences of primer sets specific for the transcripts of the studied genes

Gene		3`-5` Sequences	Annealing temperature
ACE2	Forward	TCCATTGGTCTTCTGTCACCCG	55˚ C
	Reverse	AGACCATCCACCTCCACTTCTC	
TMPRSS2	Forward	CCTCTAACTGGTGTGATGGCGT	56˚ C
	Reverse	TGCCAGGACTTCCTCTGAGATG	
β-actin	Forward	GAAGGTGAAGGTCGGAGT	57 ˚C
	Reverse	GAAGATGGTGATGGGATTTC	

Table 2. demographic, clinical and basic laboratory data of the patients

Item	**Patients (n=84)**
Age (Range)	45-90
Gender [No. (%)]	
Male	40 (47.6%)
Female	44 (52.4%)
Condition [No. (%)]	
Moderate	68 (81%)
Severe	16 (19%)
COVID-19 symptoms [No. (%)]	
Cough	40 (47.6%)
Dyspnea	38 (45.2%)
Fever	20 (23.8%)
Comorbidities [No. (%)]	
HTN	33 (39.3%)
DM	26 (31%)
Renal dysfunction	23 (27.4%)
Blood gases	
PCO2	
Mean ± SD	39.6 ± 13.21
Median (Range)	36.5 (19.0-73.0)
PO2	
Mean ± SD	67.5 ± 20.08
Median (Range)	70.0 (21.0-123.0)
HCO3	
Mean ± SD	26.5 ± 10.10
Median (Range)	24.4 (13.0-56.9)
Basic laboratory data	
AST	
Mean ± SD	48.18 ± 31.09
Median (Range)	39.0 (13.0-144.0)
ALT	
Mean ± SD	41.55 ± 38.40
Median (Range)	26.0 (11.0-195.0)
Ferritin	
Mean ± SD	1051.14 ± 876.68
Median (Range)	990.0 (139.0-4082.0)

HTN (hypertension), DM (diabetes mellitus), PCO2 (The partial pressure of carbon dioxide), PO2 (partial pressure of oxygen), HCO3 (Bicarbonate), AST (aspartate aminotransferase), ALT (alanine aminotransferase)

Table 3. Gene expression levels of ACE2 and TMPRSS2 in patients and controls

Item	**Patients (n= 84)**	**Controls (n= 30)**	***P*-value**
***ACE2*:**			
Mean ± SE	5.01 ± 0.97	1.07 ± 0.05	0.003*
Median (Range)	1.83 (0.01-49.08)	1.30 (0.70-1.30)	
***TMPRSS2*:**			
Mean ± SE	19.17 ± 5.10	0.94 ± 0.05	<0.0001*
Median (Range)	2.65 (0.04-313.46)	0.77 (0.70-1.30)	

Table (4): Relations of the ACE2 and TMPRSS2 gene expression levels with the clinical symptoms:

Clinical symptoms	**Mean ± SD**	**Median (Range)**	***P*-value**
	ACE2		
Fever:			
Yes	4.18 ± 1.80	2.10 (0.04-36.18)	0.757
No	5.27 ± 1.15	1.81 (0.01-49.08)	
Cough:			
Yes	3.96 ± 1.02	2.10 (0.01-36.18)	0.943
No	5.97 ± 1.60	1.74 (0.12-49.08)	
Dyspnea:			
Yes	4.95 ± 1.23	2.14 (0.04-36.18)	0.318
No	5.07 ± 1.46	1.69 (0.01-49.08)	
	TMPRSS2		
Fever:			
Yes	20.73 ± 5.62	2.08 (0.14-313.46)	0.291
No	18.68 ± 4.69	3.10 (0.04-194.30)	
Cough:			
Yes	22.52 ± 8.29	3.78 (0.22-313.46)	0.107
No	16.12 ± 6.23	2.35 (0.04-194.30)	
Dyspnea:			
Yes	26.47 ± 9.35	3.78 (0.04-313.46)	0.291
No	13.14 ± 5.15	2.42 (0.15-194.30)	

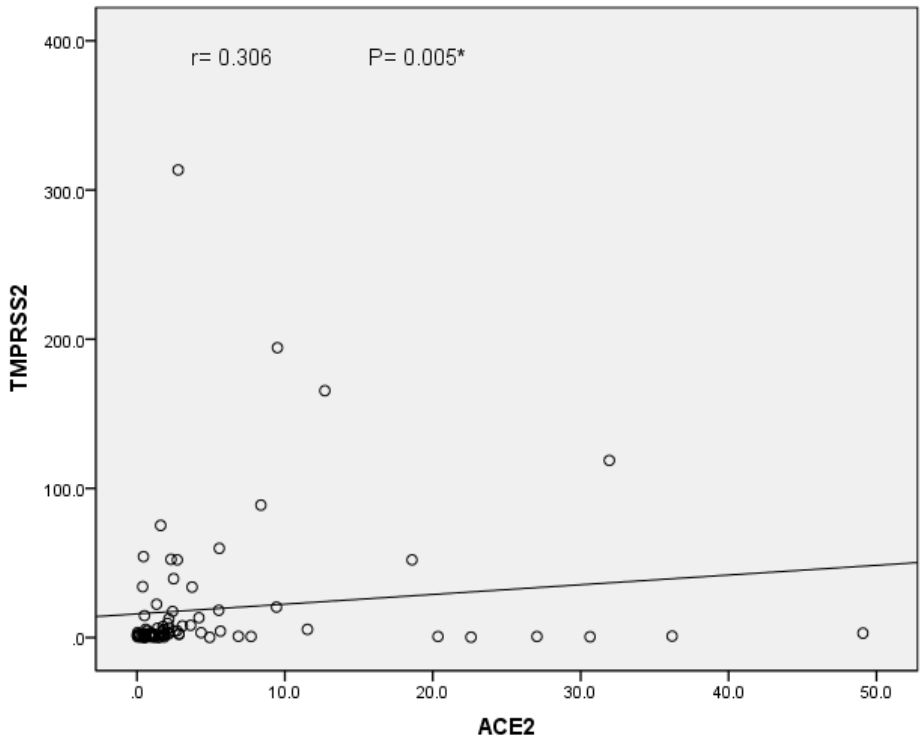
Table (5): Relations of the ACE2 and TMPRSS2 gene expression levels with the age, gender, and severity:

		Age (years)		
	< 60	**60 – 70**	**> 70**	***P*-value**
***ACE2*:**				
Mean ± SE	8.30 ± 3.02	3.31 ± 0.81	4.73 ± 1.49	0.818
Median (Range)	1.66 (0.12-49.08)	1.83 (0.01-27.04)	1.89 (0.04-31.94)	
***TMPRSS2*:**				
Mean ± SE	8.36 ± 3.27	19.35 ± 5.87	25.33 ± 10.88	0.02*
Median (Range)	2.47 (0.20-59.80)	2.42 (0.04-313.46)	4.51 (0.30-118.78)	
		Gender		
	Male		**Female**	***P*-value**
***ACE2*:**				
Mean ± SE	14.62 ± 1.47		8.37 ± 1.29	0.002**
Median (Range)	1.85 (0.01-49.08)		1.77 (0.05-36.18)	
***TMPRSS2*:**				
Mean ± SE	19.95 ± 8.41		10.46 ± 6.13	0.001**
Median (Range)	3.10 (0.14-313.46)		2.41 (0.04-194.30)	
		Condition		
	Moderate		**Severe**	***P*-value**
***ACE2*:**				
Mean ± SE	3.33 ± 1.16		5.67 ± 1.24	0.75
Median (Range)	1.79 (0.04-49.08)		2.23 (0.01-18.60)	
TMPRSS2				
Mean ± SD	17.52 ± 5.77		26.16 ± 10.99	0.04*
Median (Range)	2.63 (0.04-313.46)		3.32 (0.14-165.46)	

Table (6): Relations of the ACE2 and TMPRSS2 gene expression levels with comorbid diseases

	HTN		**DM**		**Renal dysfunction**	
	Yes	**No**	**Yes**	**No**	**Yes**	**No**
***ACE2*:**						
Mean ± SE	3.14 ± 0.69	6.23 ± 1.52	3.38 ± 1.00	5.75 ± 1.32	3.30 ± 1.54	5.66 ± 1.20
Median	1.79	1.89	1.31	1.87	1.12	1.85
(Range)	(0.05-18.60)	(0.01-49.08)	(0.04-22.58)	(0.01-49.08)	(0.12-36.18)	(0.01-49.08)
***P*-value**	0.03*		0.02*		0.01*	
***TMPRSS2*:**						
Mean ± SE	28.06 ± 10.65	13.41 ± 4.75	25.30 ± 13.24	16.42 ± 4.48	22.80 ± 13.67	17.80 ± 4.86
Median	3.42	2.35	2.41	2.87	2.35	3.24
(Range)	(0.15-313.46)	(0.04-194.30)	(0.22-313.46)	(0.04-194.30)	(0.04-313.46)	(0.14-194.30)
***P*-value**	0.001**		0.02*		0.04*	

Figure (1): Correlation between gene expression levels of ACE2 and TMPRSS2 genes



Discussion

SARS-CoV-2, which has had a huge negative impact on the world since the end of 2019, is reported to invade cells by binding to ACE2 receptors on human cells via the spike (S) protein, while TMPRSS2 is the key protease that activates the S protein, greatly facilitating SARS-CoV-2 entry into target cells. [12].

Colonization and invasion of pulmonary tissues was the most significant characteristic for SARS-CoV-2 [13]. However, recent studies have confirmed SARS-CoV-2 can be detected in alveolar lavage fluid, nasal secretions, sputum, urine, feces, and blood samples of COVID-19 patients, which could just explain the respiratory, gastrointestinal and reproductive system symptoms observed in COVID-19 patients [14].

COVID-19 significantly increases morbidity and death when combined with pre-existing risk factors such age and co-morbidities. While the majority of infected people recover, even young and unpredictably healthy people can give in to the sickness. [15]. The Expression of ACE2 and TMPRSS2 genes in nasopharyngeal swabs was assessed in 84 COVID-19 patients in comparison with 30 healthy controls. We also compared the two former gene expression levels between different COVID-19 groups; mild and severe COVID-19, diabetic and non-diabetic & hypertensive and normotensive individuals and between individuals belonging to different age groups.

In the present study, ACE2 and TMPRESS2 gene expression levels were significantly higher in patients' group than controls. Furthermore, TMPRSS2 correlated strongly with ACE2 gene expression level. Peter and coauthors proposed that strong correlations between the two genes in sputum samples indicate that these genes are coexpressed in same cell types [16]. Also, Gkogkou and others deduced that ACE2 and TMPRESS2 genes are widely expressed in human tissues and tend to be co-regulated [17].

There were no significant differences in either ACE2 or TMPRSS2 gene expression levels between patients showing fever, cough or dyspnea compared with those not having these symptoms. Unlike ACE2, TMPRSS2 expression level in our patients was significantly higher in more severe cases. A recent study demonstrated that the entry of SARS-CoV2 into a host cell depends on host protease TMPRSS2 that is expressed throughout the human body but highly expressed in cells of the respiratory tract (the primary target of COVID-19 in humans), gastrointestinal tract, kidneys and prostate. They also described that the difference in TMPRSS2 gene expression levels in respiratory tract among different population groups might be the basis for the discrepancy observed in COVID-19 susceptibility and disease outcomes 18] [.

Meanwhile, data regarding the relation between ACE2 and disease severity are controversial. [19] speculated that this gene ACE2 might be a predisposing factor for COVID-19 case-fatality. On the other hand, a previous study described a protective effect for ACE2 and the angiotensin II type receptor (AT2) against acute lung injury/inflammation in mice [20]. Moreover, Rossi and coauthors found that ACE2 has a protective effect against respiratory distress requiring supplemental oxygen during COVID-19 [21]

Age was described as a major risk factor for the need for oxygen support during SARS-CoV-2 infection [22]. In our study, we found that the expression levels of ACE2 and TMPRSS2 genes in the nasal epithelial cells of the relatively elderly people were higher than those of the relatively younger; suggesting that age related expression of these two genes could help to explain the lower susceptibility to SARS-CoV-2 among young people. Also, the expression of TMPRSS2 gene of males was higher than that of females with similar age. In the same way, higher level of SARS-CoV-2 nucleic acids in male and the elderly COVID-19 patients in the saliva test report was published earlier [23]. Furthermore, more attention should be paid to infected patients who are male and elderly since they may have greater risk of spreading SARS-CoV-2 via saliva [24].

Noteworthy is that the reports indicate slightly increased infection rate and significant higher fatality rate in male patients (> 60% vs < 40% women) [25]. ACE2 expression was 3-fold higher in male in comparison to female lung samples, as shown by Zhao et al. through single-cell RNA-seq [26]. A study done by Dhindsa et al., suggested that low testosterone concentrations may play a mechanistic role in worse outcomes observed in men with COVID-19 [27]. Higher levels of androgens in men were shown to directly upregulate the expression of ACE2 [28] .TMPRSS2 is also regulated by androgenic hormones [29]. In contrast,

estradiol was found to reduce ACE2 and TMPRSS2 mRNA expression levels in human lung epithelial cells [30]. However, in a statistical analysis of the data from the database in a preceding work, there was no statistically significant difference in the expression levels of ACE2 and TMPRSS2 in oral epithelial cells between different genders [31].

In our study, ACE2 gene expression levels were significantly lower in hypertensive, diabetic patients and patients having chronic renal failure, TMPRSS2 gene expression levels were significantly higher in patients having these comorbidities compared to those not having them. According to Ghafouri-Farda and others down regulation of ACE2 was observed in patients having comorbidities as hypertension. Kidney disease also was accompanied by a decrease in ACE2 gene expression due to Increased cortical ACE activity and reduced ACE2 activity in the medulla and cortex and increased plasma and urinary ACE2 activity [32]. Furthermore, TMPRSS2 was found enhanced in exocrine glands of pancreas of healthy individuals which might explain the diabetic complications in COVID-19 [33].

Conclusion

The ACE2 and TMPRSS2 gene expression levels increased in CVID-19 patients and were correlating with each other. Their levels were significantly higher in males and elderly. Also, different COVID-19 comorbidities seem to be influencing the expression level of ACE2 and TMPRSS2 genes, posing a greater risk of getting SARS-CoV2 infection.

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Conflict of interest

Non declared.

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