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SARS-COV-2 (COVID-19) Incidence and Severity among Smokers According to Angiotensin Converting Enzyme-2 Serum Level

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Abstract:

Introduction: A pandemic has been declared by the World Health Organization (WHO) for Corona Virus Disease of 2019 (COVID-19) on March 11, 2020. It is known that smoking inhibits pulmonary immunity and is a risk factor for contracting other infectious diseases. SARS-CoV2 is transmitted to humans by the angiotensin-converting enzyme-2 (ACE2) receptor.

Aim of the study: To determine whether there were any differences in serum levels of ACE2 and COVID-19 patients between non-smokers and smokers, as well as to investigate the relationship between the level of ACE2 and the severity of the disease.

Subjects and Methods: This case-control analytical study included 50 COVID-19 patients (25 patients were smokers and 25 patients were non-smokers) compared to 25 healthy control subjects. Quantitative detection of ACE2 serum levels was performed using the Enzyme-Linked Immunosorbent Assay method.

Results: The ACE2 serum level was statistically significantly higher in COVID-19-positive patients compared to the control group (p-value <0.001). ACE2 serum level could be used for the diagnosis of COVID-19 disease with a cut-off value (2.91). Among COVID-19-positive subjects, gender, smoking history, and disease severity were statistically insignificant in ACE2 serum levels. Different disease severity groups showed no statistically significant difference in the level of ACE2 between smokers and non-smokers.

Conclusion: This study revealed that the ACE2 serum level was higher in COVID-19 positive compared to the control group and could be used for diagnosis of COVID-19-positive cases. Meanwhile, it showed that smoking has no statistically significant effect on the levels of ACE2 in COVID-19-positive.

Keywords: ACE2; Smokers; COVID-19 disease.

1. Introduction

Coronaviruses cause diseases ranging from mild to severe, including Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS) [1]. The first case of COVID-19 was detected in Egypt on February 14, 2020. A polymerase chain reaction is used to detect viral ribonucleic acid in respiratory samples [2]. Despite the widespread recognition of heart disease, diabetes, and old age as COVID-19 risk factors, many other factors remain subject to controversy and need empirical study. Among these risk factors is smoking. The epidemiology research on the association between smoking and COVID-19 is inconclusive and confusing [3].

According to Olds and Kabbani [4] reported that those with COVID-19 are at a raised risk for nicotine use. However, Changeux et al. [5] propose that COVID-19 infection is influenced by nicotinic receptors, which could represent a target for prevention and control. The SARS-CoV-2 virus competes with nicotine for receptors as a nicotinic agent.

Angiotensin-converting enzyme-2 (ACE2) receptors bind to SARS-CoV-2 in humans. Lung epithelium, specifically oral mucosa, nasal epithelium/ciliated cells, and goblet cells, express abundant angiotensin-converting enzyme-2 [6]. ACE2 converts angiotensin-2 (Ang-2) into metabolites that exert vasodilatory effects. However, ACE2 has been extensively studied in the context of COVID-19 since its onset. It is still unclear whether the modulation of ACE2 activity or its expression levels affects COVID-19 disease outcomes infected with SARS-CoV-2 [7].

Cells infected with SARS-CoV showed high levels of pro-inflammatory cytokines (PICs) and monocyte chemoattractant protein-1 (MCP-1), indicating local immune damage caused by the virus [8].

Our study aimed to establish whether COVID-19 patients who smoked and those who did not smoke had different ACE2 levels, as well as how the disease progresses based on differences in ACE2 levels among genders.

2. Subjects & Methods

2.1. Study design

This analytical case-control study was carried out at Fayoum University Teaching Hospitals during a period extending from January 2021 to December 2021 to determine the ACE2 level of 75 individuals classified into 2 groups:

- **Group 1:** consisted of 50 patients with positive RT-PCR of COVID-19. The positive patients were divided into two subgroups according to smoking history. Smokers: formed of 25 subjects, and non-smokers: formed of 25 subjects.

- **Group 2:** Control group: 25 healthy control subjects were formed to be compared with the two other groups. All patients were subjected to Personal history including demographic data (sex and age), clinical manifestations, and Chest CT.

2.2. Sample collection and laboratory analysis

Five milliliters of whole blood were taken and left to clot at room temperature for ten to twenty minutes in sterile red-topped simple vacutainers. Serum was isolated after centrifuging blood for 20 minutes at 2000–3000 rpm. Until they are needed, serum samples are kept at -80°C. ELISA technique Cat. No. E3169Hu (Bioassay Technology Laboratory firm, China) (BT LAB) was used for all samples to detect the presence of both ACE2 in the blood according to the manufacturer's instructions.

2.3. Statistical Analysis

The data was gathered and organized to enable efficient data processing and then inputted into Microsoft Access. The data analysis was conducted using SPSS software version 22, developed by SPSS Inc. in Chicago, IL, USA. The study was done on a Windows 7 operating system. Basic quantitative analysis, including numerical and percentage representation of qualitative data. The research first assessed the normality of the quantitative data in each study group using the One-Sample Kolmogorov-Smirnov test. Subsequently, appropriate inferential statistical tests were used.

3. Results

Table 1: Distribution of demographic characteristics among COVID-19-positive patients compared to the control group.

Variables		OCP	Trachoma	*P*- value	OR (95% CI)
Sex	Male	36 (72%)	13 (52%)	0.08	2.2 (0.68-7.4)
	Female	14 (28%)	12 (48%)		
Age (years)		58.7 ±14.9	49.2 ±12.7	0.052	_____

*OR=odds ratio; *Significant P-value<0.05; 95% CI=95% confidence interval.*

Table 2: The Clinical characteristics among COVID-19-positive patients.

Variables			Frequency
Disease symptoms	**Dry cough**	Yes	50 (100%)
		No	0 (0%)
	Fever	Yes	46 (92%)
		No	4 (8%)
	Dyspnea	Yes	42 (84%)
		No	8 (16%)
	Myalgia	Yes	49 (98%)
		No	1 (2%)
	Fatigue	Yes	49 (98%)
		No	1 (2%)
	Headache	Yes	48 (96%)
		No	2 (4%)
	Productive cough	Yes	34 (68%)
		No	16 (32%)
	Nausea and vomiting	Yes	25 (50%)
		No	25 (50%)
GGO		No	9 (18%)
		Unilateral	11 (22%)
		Bilateral	30 (60%)

GGO: Ground-glass opacities.

Table 3: Serum ACE2 level in the studied groups.

Variables	COVID-19 positive (N=50)	Control (N=25)	*P*-value
ACE2 (ng/mL)	2.4 (0.75-5.9)	1.3 (0.85-2.2)	**<0.001 HS**

HS: Highly Significant P-value<0.001. ACE2= Angiotensin-converting enzyme-2

Table 4: Serum ACE2 level in COVID-19-positive patients smokers compared to non-smokers.

Smoking habit	ACE2 Level (ng/mL)	*P*-value
Smoker (N=25)	2.4 (1.2-5.9)	0.3
Non-smoker(N=25)	2.4 (0.75-4.6)	

ACE2= Angiotensin-converting enzyme-2

Table 5: Serum ACE2 level in both genders among COVID-19 positive

Gender	ACE2 Level (ng/mL)	*P*-value
Female (N=21)	2.5 (1.6-5.1)	
Male (N= 36)	2.3 (0.75-5.9)	0.9
Female (N=14)	2.4 (1.6-4.6)	

ACE2= Angiotensin-converting enzyme-2.

Table 6: Serum ACE2 level according to smoking history in different disease severity groups among COVID-19-positive patients.

Smoking habit	ACE2 Level (ng/mL)		*P*-value
	Median	**Range**	
Mild to moderate severity degree (**N=18)**			
Smoker (N=6)	2.64	1.5-3.4	0.62
Non-smoker(N=12)	2.45	1.26-3.9	
Sever to critical severity degree (**N=33)**			
Smoker (N=19)	2.33	1.26-5.9	0.34
Non-smoker(N=13)	2.04	0.75-4.6	

ACE2=Angiotensin-converting enzyme-2

Table 7: Specificity and Sensitivity of ACE2 in diagnosis of COVID-19.

Variable	Sensitivity	Specificity	AUC	Cut off point	p-value (95% CI)
ACE2 (ng/mL)	80%	66.7%	68.1%	2.91	**0.03*** (0.52-0.84)

*AUC=area under the curve. *95% CI=95% confidence interval. Significant P-value<0.05.*

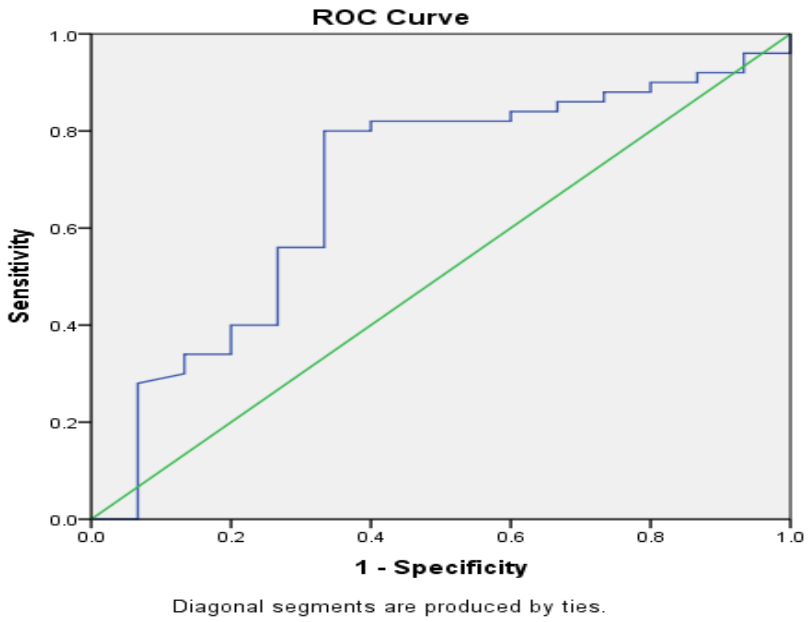

Figure 1: ROC curve of serum ACE2 level for diagnosis of COVID-19 cases. ROC curve: Receiver operating characteristics curve.

4. Discussion

On March 11, 2020, the World Health Organization declared COVID-19 a pandemic, leading to unprecedented research efforts into its complicated pathophysiology [9].

ACE2 plays a role in the transmission of SARS-CoV-2. Viral infection begins when the virus enters the host's cells. A specific receptor on the membrane of host cells can bind to the spike glycoproteins of Coronavirus. In previous studies, ACE2 is a functional SARS-CoV receptor [10].

In this study, ACE2 levels in serum and COVID-19 infection were compared between non-smokers and smokers, and the relation between ACE2 and COVID-19 severity was investigated.

COVID-19 patients had a significantly older average age than controls, based on the demographic data of the study population. This finding aligns with the research conducted by Cortis [11] on Chinese patients. In contrast, the research by Al-Hakeim et al. [12] on Iraqi patients found that age did not have a statistically significant impact. According to Shahid et

al. [13], a study conducted in the United States found that older adults who have other health conditions, like diabetes, chronic renal disorders, and hypertension, are more likely to be at a higher risk of contracting and experiencing a more severe form of infection with the SARS-CoV-2 virus.

Among the clinical presentations, this study found that 100% of patients had dry cough and 98% had myalgia upon admission. Çalıca Utku et al. [14] also reported a similar result in their study. Among COVID-19-positive patients, Alimohamadi et al. [15] found that fever (81.2%) was the most common symptom.

On initial imaging, COVID-19 predominantly involves both lungs, as opposed to MERS and SARS, in which initial chest imaging abnormalities are more commonly unilateral. The study by Palabiyik et al. [16] found that unilateral lung affection (55%) was more common among COVID-19 patients than bilateral lung affection (45%).

ACE2 levels statistically rose significantly among COVID-19-positive patients compared to a control group in this study.

COVID-19 positives had a significant rise in ACE2 activity compared to healthy controls. Similarly, Al-Hakeim et al. [12] found that COVID-19 patients significantly increased levels of ACE2 and controls.

Due to the high level of expression of ACE2 in lung alveolar epithelial cells [17], in SARS-CoV-2 patients, it does not only act as a receptor for the virus, but it also plays a role in post-infection control, including the immune response, viral genome replication, and the production of cytokines [18]. The presence of higher levels of soluble ACE2 may also prevent lung damage by delaying SARS-CoV-2's entry into cells [19].

A statistically significant effect of smoking on the levels of ACE2 in COVID-19-positive patients was not found in this study. Similarly, there was no significant difference in ACE2 expression levels between smokers and non-smokers, according to Voinsky and Gurwitz [20]. There are fewer smokers among hospitalized COVID-19 patients with chronic smoking than among the general population. This study suggests that smokers are less likely to experience a "cytokine storm" after infection with SARS-CoV-2.

Kaur et al., [21] found that COVID-19 patients with a non-smoking history exhibited higher ACE2 activity than smokers. Furthermore, Lutchman [22] study demonstrated increased expression of ACE2 in smokers' airways.

COVID-19-positive males and females showed no statistically significant differences in ACE 2 levels. In the research performed by Maza et al. [23], no association was discovered between ACE2 levels and the sex of the patient or recovered group.

In contrast, research by Kaur et al. [21] revealed that Male patients showed higher ACE2 activity than female patients.

ACE2 levels among COVID-19-positive patients were not statistically significantly different between smokers and non-smokers in different severity groups, similar to the study by Purkayastha et al. [24].

In this study, there was no statistically significance difference in ACE2 levels between smokers and non-smokers in different severity groups among COVID-19-positive patients, which was similar to the study by Purkayastha et al. [24]. ACE2 expression was associated with smoking in rodent and human research, contrary to Smith et al. [25]. In line with this, COPD patients' lungs contain higher levels of ACE2 transcript [25]. Researchers have found an increased risk of severe COVID-19 among those with chronic respiratory diseases, particularly COPD (mainly caused by smoking) [26].

As a result of the difference in methodology and sample type between this study and previous studies, the discrepancy could be explained. This study used ELISA to analyze serum samples from humans, while Gebel et al. [27] used molecular (PCR) techniques on rodent animal samples, and Hung et al. [28] used fluorescent techniques on rodent animal samples.

A serum level of ACE2 could be used as a biomarker for diagnosis of COVID-19 infection with statistically significant sensitivity and specificity, similar to Liu et al. [29] study that recommended ACE2 as a biomarker. The virus particle enters the cell through ACE2, which serves as the cell membrane entrance. It is most common for virus particles to attack epithelial cells-lined ducts because epithelial cells express high levels of ACE2 [30]. The human body elevates ACE2 levels in the

serum to prevent viral entry through the cell membrane. Therefore, serum ACE2 may be used as an indicator of viral load during infection diagnosis [29].

According to the study by Maza et al. [23], As the high level of ACE2 in COVID-19 is associated with a lower susceptibility to infection with COVID-19, ACE2 serum levels cannot be used as biomarkers for COVID-19 diagnosis.

Limitations of the Study: Our study was a single-center observational study that only included attendants of Fayoum University Teaching Hospital in Fayoum City. A retrospective observational design prevented us from testing causal relationships. Furthermore, a small sample size may limit statistical power to achieve significant results.

5. Conclusion

Based on the current study results; There was no statistically significant difference in ACE2 serum level in relation to COVID-19 patient's gender and smoking history. In COVID-19 patients with different severity groups, there was no statistically significant difference in ACE2 serum level. ACE2 serum level cut-off value could be used to diagnose positive COVID-19 cases.

Further studies are needed to investigate the observations in a larger group of patients with more detailed demographic and health information about the control group.

The affection of intensity and duration of smoking on ACE2 levels and COVID-19 outcomes is better to be studied deeply. The study of confidence intervals and effect sizes would be helpful to understand the clinical relevance. The mechanistic pathways through which smoking might affect ACE2 expression or COVID-19 severity should be studied deeply. Additionally, investigating the molecular mechanisms linking ACE2, smoking, and COVID-19 severity could provide deeper insights. Further studies should be done to certify the use of ACE2 for diagnosis of COVID-19 infection.

Ethical approval and consent to participate: According to the Declaration of Helsinki guidelines, the study was conducted after the approval of the local ethics committee of Fayoum University's Faculty of Medicine (ethical approval number: M545). Before consent was obtained, all study subjects were informed of the study's nature.

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