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Comparative Study of Angiotensin Convertase Enzyme (ACE2) Gene Expression in Patients with COVID-19 By Molecular Method

Behrooz Farzanegan [1], Naghmeh Bahrami [2,3*], Mohammad Fathi [4], Saba Tahmasebi [4], Mehdi Kazem Pour Dizaji [5], Shadi Shafaghi [6], Farnaz Ahmadi [6], Farnoush Mohammadi [2,7], Seyedeh Zahra Fotook Kiaei [2,8], Mona Mohajeri Tehrani [2], Masoume Farhangiyan [2], Abdolreza Mohamadnia [9*]

1. Critical Care Quality Improvement Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

2. Craniomaxillofacial Research Center, Tehran University of Medical Sciences, Tehran, Iran.

3. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran.

4. Anesthesiology Research Center, Department of Anesthesia and Critical Care, Shahid Modares Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

5. Department of Biostatistics, Mycobacteriology Research Center, National Research Institute of Tuberculosis and Lung Diseases, Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

6. Lung Transplantation Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

7. Department of Oral and Maxillofacial Surgery, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran.

8. Department of Pulmonary and Critical Care, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran.

9. Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran, And Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

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**Corresponding authors:*
Naghmeh Bahrami

Craniomaxillofacial Research Center, Tehran University of Medical Sciences, Tehran, Iran; Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran.

Abdolreza Mohamadnia

Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran, And Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Tel: +98-21-84903746
Fax: +98-21-84903746
Email: mohamadnia.ar@gmail.com, naghmeh-bahrami@gmail.com

ABSTRACT

Introduction: The Covid 19 illness is triggered by a coronavirus known as SARS CoV-2. This particular virus spreads rapidly likely because of the Spike proteins affinity, to the human ACE2 cell receptor causing harm to organs, like the lungs in type 2 pneumocytes that abundantly express this receptor.

Materials and Methods: The research methodology used in this study involved a case control approach. Patients were referred by a specialist following diagnosis. A total of 50 samples, from infectious cases and 50 samples from cases, with mild symptoms were analyzed for statistical purposes. The ACE2 gene expression was assessed using the Realtime RCR method after RNA extraction.

Results: The findings indicate that the average age does not have an impact on individuals experiencing mild symptoms. Moreover, there is a decrease in the expression of ACE2 genes in the blood of patients with severe symptoms hospitalized in intensive care units as compared to those, with mild symptoms, highlighting a substantial disparity.

Conclusion: The numerical value of fold change for ACE2 gene in severe type patients shows a decrease compared to mild patients and it is 2.11 times lower in severe type patients than mild type.

Keywords: COVID-19; ACE2; Realtime RCR.

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Introduction

Coronaviruses (CoVs) belong to the Coronaviridae family and they primarily affect the respiratory system [1,2]. There have been two outbreaks caused by coronaviruses known as severe acute respiratory syndrome coronavirus (SARS CoV) and Middle East respiratory syndrome coronavirus (MERS CoV) [3-5]. Since the new coronavirus, SARS CoV 2 was discovered scientists have been discussing its origin [6,7]. There were speculations that SARS CoV 2 might have been created in a lab and genetically modified. However, the genetic data does not support this idea and it suggests that SARS CoV 2 is actually linked to a virus known before. Analyzing the genome and comparing it with coronaviruses reveals that SARS CoV 2 has characteristics that set it apart from other viruses [8].

During infection coronaviruses attach to the ACE receptor located on host cells using RBD. Then penetrate the cells via endocytosis. To initiate the release of the virus genome, within the cell the S protein undergoes a cleavage by endosomal proteases leading to fusion of the endosomal membrane with both the host cell and virus membranes. Subsequently translation of the virus genome commences, after exiting from its protein coat [9]. Symptoms of the illness typically show up within 2 to 14 days after being exposed. It can still be passed on during this period. Common signs include fever, coughing and difficulty breathing with the possibility of the illness turning fatal [9,10]. According to research findings individuals infected with Covid 19 may experience the illness in either a mild or severe manner.

Studies have indicated that genetic diversity can significantly influence how individuals react to COVID 19 vaccines. This implies that certain individuals may exhibit a stronger immune reaction, to the vaccines, while others might encounter complications due to genetic variations. Those who are more susceptible may experience heightened side effects, from the vaccines [11]. Like other types of coronaviruses, the spike glycoproteins on the virus's outer membrane play a crucial role. They engage with proteins on host cells like ACE2 to adhere to the cells and influence their virulence. This interaction is key in understanding COVID 19s impact, on cells [11,12]. The variation in the expression of ACE2 genes in different people may have a significant effect on virus entry, replication and subsequent disease progression. Investigating the levels of these genes in Covid 19 patients, especially those with severe involvement, can provide valuable insights into the underlying molecular mechanisms of this illness [12]. ACE2 serves as the receptor, for SARS CoV 2. Elevated levels of ACE2 and TMPRSS2 in cells can increase susceptibility to a more severe form of the disease. Assessing the ACE2 marker could provide insights for managing coronavirus patients. Hence this research examines the presence of Angiotensin Convertase Enzyme (ACE2) gene in individuals, with severe COVID 19, compared to the mild type utilizing Realtime PCR analysis.

Materials and Methods

This study is a case-control study. Referral patients are selected by a specialist after diagnosis. For statistical analysis, 40 samples of severe infectious cases and 40 samples of cases with mild symptoms are studied. Blood samples were taken, consisting of 3ml of blood, with EDTA anticoagulant. Following consent and the assigned ethic's code (IR.SBMU.MSP.REC.1402.083) 40 individuals with severe symptoms and 40, with mild symptoms underwent blood collection for analysis. After extracting the RNA, the expression of genes will be checked by Realtime RCR method. RNA extraction, cDNA synthesis and Real-Time PCR (RT-PCR): RNA was extracted using the Qiagen Cat no.52304 RNA Blood Mini Kit following the kits instructions. The purity and concentration of the RNA were assessed using a Nanodrop device. The Viva 2 step RT PCR Kit, with the Cat no. RTPL12 was utilized for generating cDNA and subsequently the cDNA synthesis process was conducted. The quantification of gene expression levels was carried out using the Real time PCR technique. The Real time PCR reactions were executed utilizing the CinnaGreen qPCR Mix, 2X, with the Cat No. MM2041 kit. The experiment was conducted using a master mix volume of 4µl with 1µl each of primer F and R 2µl of cDNA and distilled water added to make a volume of 20µl. A template RNA amount of 5µg was used, with 18srRNA serving as the control. The temperature and time conditions were as follows; denaturation, at 95 degrees Celsius for 5 minutes denaturation at 95 degrees for 15 seconds primer annealing at 56° for 60 seconds and amplification at 72° for 25 seconds (for a total of 40 cycles) followed by an amplification step at 72°, for an additional duration of 5 minutes. Before performing Real-Time-PCR, primers were prepared for ACE2 marker and the characteristics of these primers can be found in Table 1. The genes employed along with their corresponding primers are detailed in the table provided below.

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Statistical Analysis

The data was examined using software known as SPSS Version 22 and the average and deviation were computed. To dissect and examine the variance or association, between gene expression levels the paired t test was employed. A discrepancy is deemed noteworthy at a P value of less than or equal, to 0.05.

Results

This study is a case-control study. Referral patients are selected by a specialist after diagnosis. To carry out the investigation 50 samples of severe infectious cases and 50 samples of cases with mild symptoms are studied. Both groups were similar, in terms of age. The comparison between the groups was done using a t- test based on their ages. There was no difference in the age indicating that age did not pose any issues, in the studied groups. All Real Time PCR reactions were carried out twice. The findings were analyzed based on the melting curve. Expression analysis of studied biomarkers: After analyzing the outcomes of the Real Time PCR test individuals who tested positive for the biomarker expression were identified. In a group of patients, with severe symptoms 21 out of 50 tested positive for the ACE2 marker in their blood. On the other hand, among patients with mild symptoms 29 out of 50 showed a positive result for this marker. A statistical analysis using a paired t-test revealed a difference in the positivity rate of this marker, between the two groups (P value<0.001) (Figure 1). Analyzing the variation in biomarker expression between two groups: Based on the findings, from Real Time PCR analysis the Ct values of the samples were initially established. The comparative variation in ACE2 marker expression between patients with severe symptoms and those with mild symptoms was determined using the ΔΔ Ct 2 formula. Ultimately it was revealed that the level of ACE2 expression in patients experiencing severe symptoms is 2.11 times than that in individuals, with mild symptoms (Figure 2).

Table 1. Sequence and properties of ACE2 gene primers.

	ACE2	*18srRNA*
Forward primer	*CAGGGAACAGGTAGAGGACATT*	*GTAACCCGTTGAACCCCATT*
Length	*22*	*20*
Reverse primer	*CAGAGGGTGAACATACAGTTGG*	*CCATCCAATCGGTAGTAGCG*
Length	*22*	*20*
Favorable temperature Annealing	*60*	*54*

Table 2. Comparison of mean age in two groups.

Main group	*Age (years)*		
	Age range	*average*	*Standard deviation (SD)*
Severe patients (50)	*25-68*	*46.65*	*10.12*
Mild patients (50)	*24-68*	*45.38*	*12.55*
	P-value=0.423		*SD=standard deviation*

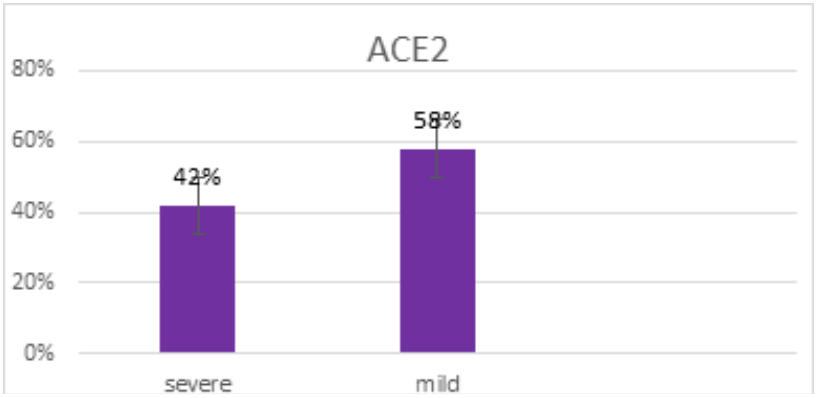

Figure 1. The rate of ACE2 positivity in patients with severe symptoms and patients with mild symptoms.

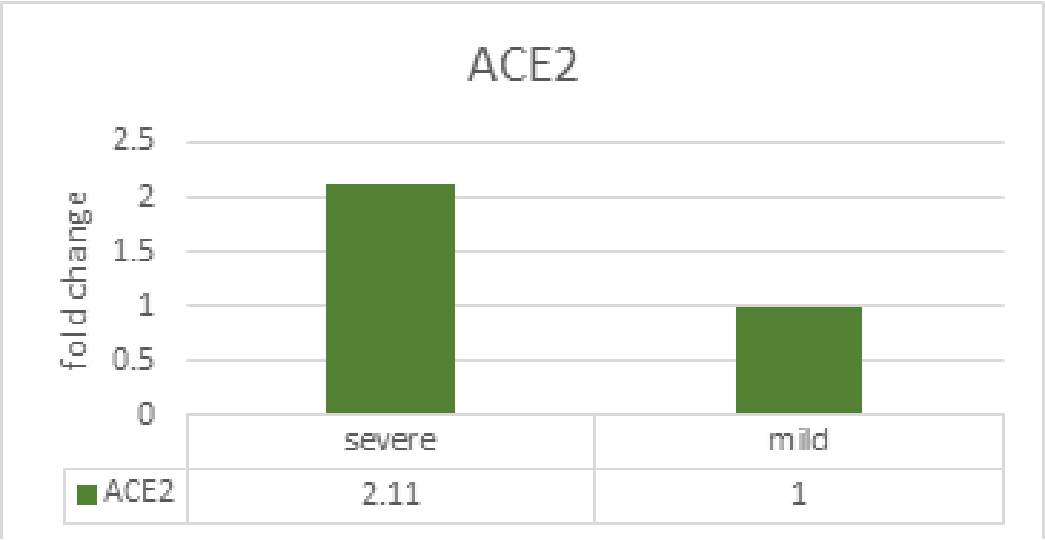

Figure 2. Differences in ACE2 expression in patients with severe symptoms compared to patients with mild symptoms.

Discussion

The study conducted was case-control research aiming to explore the levels of the Angiotensin Convertase Enzyme (ACE2) protease serine 2 (TMPRSS2) gene, in individuals with severe and mild forms of COVID 19 using Realtime PCR. Findings revealed that there was no distinction in age, between the two groups (P value>0.05). The study's findings indicated that the ACE2 gene expression was higher, in samples from severe COVID 19 patients than in those from mild cases as determined by Realtime PCR. These results align with research in this area. This crucial protein is significant, in facilitating the entry of the SARS CoV-2 virus into cells. Increased expression of ACE2 in patients with COVID-19 can be considered as a risk factor for the high sensitivity of the lungs to viral infection and the development of severe pulmonary complications in these patients. ACE2 acts as the main receptor of the SARS-CoV-2 virus, and increasing its expression in lung cells can facilitate the entry of the virus into these cells and its replication [13].

Several studies have shown that ACE2 expression in the respiratory tract varies among individuals. ACE2 expression level may be influenced by age, gender, comorbidities and even genetic factors. A study by Cao et al found that ACE2 expression in the nasal epithelium is higher in males and decreases with age, which may partially explain the observed gender and age differences in the severity of COVID-19 [13]. In the present study, there was no age difference in the studied groups, and the expression of ACE2 gene in the samples of patients with severe type of COVID-19 showed an increase compared to mild type patients. It's worth mentioning that even though ACE2 serves as the receptor, for SARS CoV-2 to enter host cells the level of ACE2 expression alone isn't the only factor influencing vulnerability to COVID 19. Various other elements like the response of the host, viral load and the existence of co receptors such, as TMPRSS2 also significantly contribute [14]. Several studies have investigated the expression of ACE2 and TMPRSS2 in COVID-19 patients compared to healthy individuals. Ziegler et al. showed that the expression of ACE2 is significantly higher in COVID-19 patients compared to healthy people, and it is in line with the study [15].

In general, in the study conducted by Real time PCR method, it was shown that the ACE2 marker notably increases in patients with corona virus (COVID-19) and this increase in patients with more severe clinical symptoms compared to patients with milder symptoms. Understanding how ACE2 functions, in facilitating the entry of the SARS CoV-2 virus into cells can aid in the development of novel treatment approaches, for combating COVID 19. Naturally given the emergence of this illness, further research is necessary to expand our knowledge and achieve outcomes in diagnosing and treating this viral infection.

Conflict of Interest

There is no conflict of interest to declare.

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