ACE2 Gene Polymorphism Association COVID-19 Patient

Ahmed Ali Salman[1], Anwar Salih Al-Kinani[2]
[1,2] Department of Microbiology, College of Medicine, University of Al-Qadissiyah, Iraq.
Email ID: aaazzz919191@gmail.com

Abstract
Background: Many SNPs in ACE2 gene polymorphism have been identified and linked COVID-19 in human. Objective: Study the prevalence of rs4646116, rs2285666, rs143936283 gene polymorphism in an Iraqi population. Materials and methods: Amplification-refractory-mutation -system (ARMS-PCR) technique was used to identify rs4646116, rs2285666, rs143936283genotypes in all 86 patients. Results: The genotypes of the targeted SNP were distributed as following: (rs4646116: CC (17.3%), C/T (34.6%)), TT (48.1%), (rs2285666: AA (15.4%), A/G (25.0%), GG (59.6%)), (rs143936283: CC (25.0%), C/T (26.9%) TT (48.1%)). Conclusion: ACE2 single nucleotide polymorphism of rs4646116, rs2285666 and rs143936283 showed no significant variation between co-infection and cases of pure COVID-19 infection however, several genotypes were more frequent than others and can play a risk role in susceptibility to COVID-19. And these SNPs had no correlation with severity of disease
Keywords: ACE2 gene, polymorphism, genotype, COVID-19

1. Introduction

The global pandemic corona-virus infectious disease 2019 "COVID/19"is recently identified infectious illness, result due to severely acute respiratory syndrome virus 2 "SARS/CoV-2", it is rapidly spread all over the world and became an important public health issue, and considered one of the unprecedented challenges among infectious disease in recent history because of high morbidity and mortality rate.SARS-COV2 causing a different clinical manifestations range between milder pulmonary disease to sever acute respiratory distress (SARD) and eventually causing death, even some infected people are asymptomatic (Alimoradi et al., 2022).

The virus may have been described in the tonsil stromal cells and viral transmission via the respiratory droplets has been proposedThe host genetic differences are well recognized to contribute to variable immune response and are important in affecting susceptibility and/or severity of clinical manifestation of the disease.one of the most useful approach in severity and prognosis of COVID-19 is the assessment of innate immune response in association with genetic variation. A particular immune cell and such profile of cytokine production may provide an important information regarding the clinical outcomes and give a hint to choose a suitable therapy protocol that help in management of patients. are depicted in a schematic diagram. In ARDS, angiotensin II may have a role in the pathophysiological processes that lead to pulmonary edema, pulmonary fibrosis, pulmonary inflammation, and parenchymal cell death. SARS-CoV2 spike proteins may cause ACE2 expression to be down regulated, which would raise local angiotensin II levels and exacerbate lung damage. Acute respiratory distress syndrome (ARDS), angiotensin-converting enzyme 2 (ACE2), and severe acute respiratory syndrome coronavirus (SARS-CoV2) (Wang, et. al. (2020).

2. Material and Method

A cross sectional study was designed for 52 patients (both sexes and different ages) divided into four groups depending on the severity of COVID-19 infection.the study included 26 samples from mild cases for COVID-19 infection in private clinics, 26 samples from severe cases in intensive care units were admitted to the hospital and clinic private during December 2020 to March, 2021 in Al-wasit and Baghdad provinces. Also, patients' groups are divided into all ages.

Blood samples were drawn by venipuncture and collected using EDTA tubes. Amplification-refractory-mutation-system (ARMS-PCR) technique was used for detection of the ACE2 gene SNP. The period of samples collection was from November 2021to April 2022.

Genomic DNA from blood samples were extracted by using gSYAN DNA extraction kit (frozen Blood) GENEaid/ USA and done according to manufacturer instruction.

The extracted DNA form blood samples was checked using (Nanodrop spectrophotometer/ THERMO-USA), which measured DNA concentration (ng/µl) to check the DNA purity by reading absorbance at (260/280nm).

ARMS-PCR master mix was prepared using GoTaq® G2 Green Master Mix Kit and this master mix was used to execute two reactions for each sample (one for the wild type allele and the other for the mutant type allele) according to the manufacturer instructions. The mix consisted of 5µltof template DNA, 2µltof forward primer (for each reaction), 2µl of the common reverse, 12.5µltof G2 Green master mix, and 3.5µlt of PCR water.

Thermocycler conditions were 95°C/5 min pre-denaturation for a single cycle, 95°C/30 sec denaturation, 55°C/30 sec annealing/extension,

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72°C/30 sec extension each for 35 cycles, and 72°C/5 min for final extension.

PCR master mix were transferred into Exispin vortex and centrifuged at 3000rpm for 3 minutes, then placed in PCR thermocycler (BioRad/USA).

The PCR product was electrophoresed on 2% agarose gel with ethidium bromide stain at 100 volt and 80 AM for 1 hour and was visualized using Ultraviolet transilluminator (ATTA/Korea).

3. Results

Genotype study

ACE2 (rs4646116)

Table 3.1 Comparison of genotypes frequency distribution of ACE2 (rs4646116) between patients with COVID-19 according to severity of disease					
ACE2 (rs4646116)	Mild COVID-19 *n* = 25		Severe COVID-19 *n* = 27		*p*
	n	%	n	%	
CC	3	12.0	6	22.2	0.365 C NS
C/T	10	40.0	8	29.6	
TT	12	48.0	13	48.1	

ACE2 (rs2285666)

Table 3.2 Comparison of genotypes frequency distribution of ACE2 (rs2285666) between patients with COVID-19 according to severity of disease.					
ACE2 (rs2285666)	Mild COVID-19 *n* = 25		Severe COVID-19 *n* = 27		*p*
	n	%	n	%	
AA	4	16.0	4	14.8	0.773 C NS
AG	8	32.0	5	18.5	
GG	13	52.0	18	66.7	

ACE2 (rs143936283)

Table 3.3 Comparison of genotypes frequency distribution of ACE2 (rs143936283) between patients with COVID-19 categorized according to severity of disease.					
ACE2 (rs143936283)	Mild COVID-19 *n* = 25		Severe COVID-19 *n* = 27		p
	n	%	*n*	%	
CC	4	16.0	9	33.3	0.658 C NS
CT	8	32.0	6	22.2	
TT	13	52.0	12	44.4	

4. Discussion

Genotype study

ACE2 (rs4646116)

In the current study(table3.1) we found no significant difference in the frequency of ACE2 (rs4646116) SNP genotypes and alleles between patients with COVID-19 and patients with co-infection of HBV and SARS-CoV-2. In addition, there was no significant difference in rate of genotypes and alleles with respect to severity of COVID-19; however, we found that genotype TT and allele T were the predominant ones

ACE2 (rs2285666)

(Table 3.2) according to (Alimoradi et al., 2022) there was significant association between ACE2 (rs2285666) gene polymorphism and susceptibility to infection with SARS-CoV2; however, there was no significant association between ACE2 (rs2285666) gene polymorphism and disease severity. Our results are in line with (Alimoradi et al., 2022) in that there is no significant association between ACE2 (rs2285666) gene polymorphism and disease severity, but unfortunately, we could not assess the association between ACE2 (rs2285666) gene polymorphism and susceptibility to infection with SARS-CoV2 because we were unable to gather a healthy control group with no history of COVID-19 infection

ACE2 (rs143936283)

(table 3.3) p. Glu329Gly/c.986A>G (rs143936283) of ACE2 were also among the majorly noticed functional variations which potentially alters major protein–protein interactions with viral S-protein (Senapati et al., 2021). Two specific ACE2 alleles (i.e., rs73635825 and rs143936283) exhibited a relatively low binding affinity for the spike protein of SARS-CoV-2, which might imply a lower likelihood of viral attachment and potential resistance to infection (Lippi et al., 2020).

Of note, the ACE2 alleles, rs73635825 (S19P) and rs143936283 (E329G), produce proteins demonstrating weaker binding affinity and missing key residues for SARS-CoV-2 S protein complex formation. These findings might indicate an intrinsic resistance mechanism to SARS-CoV-2 infection (Hussain et al., 2020).

(Hussain et al.,2020) has shown significant variation in ACE2 (rs143936283) genotypes between patients with COVID-19 in comparison with control people and the allele T was a risk factor and in our observation genotype TT was more frequent and therefore we agree with (Hussain et al.,2020) in this regard

5. Conclusion

ACE2 single nucleotide polymorphism of rs4646116,

rs2285666 and rs143936283 showed no significant variation between co-infection and cases of pure COVID-19 infection however, a number of genotypes were more frequent than others and can play a risk role in susceptibility to COVID-19. And these SNPs had no correlation with severity of disease

References

Djukic, T.; Stevanovic, G.; Coric, V.; Bukumiric, Z.; Pljesa-Ercegovac, M.; Matic, M.; Jerotic, D.; Todorovic, N.; Asanin, M.; Ercegovac, M.; et al. GSTO1, GSTO2 and ACE2 Polymorphisms Modify Susceptibility to Developing COVID-19. J. Pers. Med. 2022, 12, 458. https://doi.org/10.3390/jpm12030458

Lee, I. H., Lee, J. W., & Kong, S. W. (2020). A survey of genetic variants in SARS-CoV-2 interacting domains of ACE2, TMPRSS2 and TLR3/7/8 across populations. Infection, genetics and evolution: journal of molecular epidemiology and evolutionary genetics in infectious diseases, 85, 104507. https://doi.org/10.1016/j.meegid.2020.104507

Wang, J., Xu, X., Zhou, X., Chen, P., Liang, H., Li, X., Zhong, W., & Hao, P. (2020). Molecular simulation of SARS-CoV-2 spike protein binding to pangolin ACE2 or human ACE2 natural variants reveals altered susceptibility to infection. The Journal of general virology, 101(9), 921–924. https://doi.org/10.1099/jgv.0.001452

Alimoradi N, Sharqi M, Firouzabadi D, Sadeghi MM, Moezzi MI, Firouzabadi N. SNPs of ACE1 (rs4343) and ACE2 (rs2285666) genes are linked to SARS-CoV-2 infection but not with the severity of disease. Virol J. 2022;19(1):48. Published 2022 Mar 19. doi:10.1186/s12985-022-01782-6

Srivastava A, et al. Genetic association of ACE2 rs2285666 polymorphism with COVID-19 spatial distribution in India. Front Genet. 2020; 11:1163.

as having PML

Wu YH, et al. The ACE 2 G8790A polymorphism: involvement in type 2 diabetes mellitus combined with cerebral stroke. J Clin Lab Anal. 2017;31(2): e22033. [PMC free article] [PubMed] [Google Scholar]

Möhlendick B, et al. ACE2 polymorphism and susceptibility for SARS-CoV-2 infection and severity of COVID-19. Pharmacogenet Genom. 2021; 31:165–171. [PMC free article] [PubMed] [Google Scholar]

Senapati, S., Banerjee, P., Bhagavatula, S., Kushwaha, P. P., & Kumar, S. (2021). Contributions of human ACE2 and TMPRSS2 in determining host-pathogen interaction of COVID-19. Journal of genetics, 100(1), 12. https://doi.org/10.1007/s12041-021-01262-w

Lippi, G., Lavie, C. J., Henry, B. M., & Sanchis-Gomar, F. (2020). Do genetic polymorphisms in angiotensin converting enzyme 2 (ACE2) gene play a role in coronavirus disease 2019 (COVID-19)?. Clinical chemistry and laboratory medicine, 58(9), 1415–1422. https://doi.org/10.1515/cclm-2020-0727

Hussain M, Jabeen N, Raza F, Shabbir S, Baig AA, Amanullah A, et al. 2020; Structural variations in human ACE2 may influence its binding with SARS-CoV-2 spike protein. J Med Virol. 10.1002/jmv.25832. DOI: 10.1002/jmv.25832. PMID: 32249956. PMCID: PMC7228372.

Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med. 2020;8(5):475-481.

Zhao Q, Meng M, Kumar R, et al. Lymphopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: a systemic review and meta-analysis. Int J Infect Dis. 2020; 96:131-135.

Anwar Salih Saihood, Talib A, Ahmed R, (2017). Molecular Identification of JC Virus in Blood Samples of Iraqi Patients Diagnosed Clinically and Radiologically