Archives of Razi Institute, Vol. 78, No. 1 (2023) 9-14

DOI: 10.22092/ARI.2022.358871.2323

Original Article

The Association of Angiotensin-Converting Enzyme 2 Polymorphism and SARS-CoV-2 Infection in the Iraqi Population

AL-Mudhafar, M. A[1*], Nima Mezher, M[2], Noaman, I. M[3]

1. Department of Microbiology, Faculty of Veterinary Medicine, University of Kufa, Kufa, Iraq
2. Department of Biology, Faculty of Science, University of Kufa, Kufa, Iraq
3. Department of Physiology, Faculty of Veterinary Medicine, University of Kufa, Kufa, Iraq

Received 16 May 2022; Accepted 7 June 2022
Corresponding Author: parsa.pashaie.2@gmail.com

Abstract

The quick and advancing prevalence of the new coronavirus SARS-CoV-2 produced a global crisis surge with a profound impact on human health and worldwide economic constancy. The virus is known as one strain of coronavirus, which causes the respiratory infection responsible for the current pandemic of COVID-19. The virus spike protein has a high binding affinity to human ACE2, depending on crystallization analysis and biochemical interaction studies. Studies consistently reveal that rs2285666, a polymorphism found in ACE2, diverse significantly between Europeans and Asians, changing ACE2 expression. The alternating allele TT of rs2285666 SNP increased gene expression to 50%; thus, it may have a role in SARS-COV-2 infection vulnerability. This study aimed to investigate rs2285666 SNP association with SARS-CoV2 infection as a first report in the Iraqi population. Fifty (20 Male/30 Female) Covid-19 patients with severe symptoms with mean age (of 41.5±10.7) and 50 (20 Male/30 Female) healthy people as a control group with mean age (of 41.5±10.7) were included in this study. Sample of a patient tested as a mutant genotype (TT) by RFLP assay. The results reveal a MAF value of 0.3 for this gene in Iraqi samples, more than Europeans (0.2) and less than East Asians (0.55). The codominant model had significant OR of both alleles CT and TT (OR=4.26 & 6.7; *P*-value=0.012 & 0.023 respectively). In conclusion, there is an association between increased severity of SARS-Cov-2 infection and rs2285666 polymorphism of the codominant genotype model of the Iraqi population. However, several other factors may affect disease severity, such as ethnic group differences, sex, comorbidity, virus strain, and others.

Keywords: Angiotensin-Converting Enzyme 2, Polymorphism, SARS-CoV-2

1. Introduction

The quick and advancing prevalence of the new coronavirus SARS-CoV-2 produced a global crisis surge with a profound impact on human health and worldwide economic constancy. Severe Acute Respiratory Syndrome CoronaVirus-2 (SARS-CoV-2), known as one strain of coronavirus, causes the respiratory infection responsible for the current pandemic of COVID-19 (1). It is a zoonotic disease with close genetic similarity to bat coronavirus, proposed to emerge from a bat-borne virus (2). Research continues to originate that SARS-CoV-2 is directly spread from bats or through an intermedia host. Found many resemblances between SARS-CoV-2 and the original SARS-CoV where Xu, Chen (3) found 76.5% identical spike proteins 3-D structure of both proteins the receptor-binding do viruses. The spike protein has a high binding affinity to human Angiotensin-Converting Enzyme 2 (ACE2) depending on crystallization analysis and biochemical interaction

studies (4). ACE2 is vital in this virus's entry into the host cell, causing ultimate infection (5). As a receptor, ACE2 is a transmembrane zinc-containing metalloenzyme that acts as carboxypeptidase and has homology with ACE, a key enzyme in the Renin-Angiotensin system (RAS). The gene *ACE2* at the X chromosome extends ~2,4 kb and includes 18 exons (6).

Additionally, some analysis studies proposed that SARS-CoV-2 binds to human ACE2 more effectively than SARS-CoV, raising the SARS-CoV2 ability for transmission from one person to another (7). Overexpression of human ACE2 increased viral host cell entry, and later replication aggravates disease fatality, as shown in the SARS-CoV mouse model infection, which explains that the viral entry is a critical point (8). Injecting SARS-CoV spike in mice cells increases lung injury, but this damage is weakened by obstructive RAS pathway and varies with ACE2 expression. Therefore, ACE2 is not a virus entry receptor for SARS-CoV only but grants protection from virus lung injury (9).

Zhou, Yang (2) proposed that ACE2 overexpression in different species of HeLa cells, including humans, pigs, and civets, endorsed infection and replication of SARS-CoV2. In this manner, SARS-CoV2 directly uses ACE2 as a cell entry receptor. In addition, they explained that spikes of SARS-CoV2 do not bind effectively with other coronavirus receptors, such as dipeptidyl peptidase 4 and aminopeptidase N (2). However, most ACE2-expressing cells are represented by Type 2 alveolar epithelial cells (AECII), so these cells can function as a pool for viral invasion. Furthermore, gene analysis revealed that these ACE2-expressing AECII cells possess high viral process-related genes, involving genes for viral life span, assembly, and genome replication. This suggests that AECII cells that express ACE2 receptors make for easy coronavirus invasion and reproduction in the lung (10).

Recent studies that include large samples have examined the genome variations introduced in global populations (11, 12). Studies consistently reveal that rs2285666, a polymorphism found in ACE2, is significantly diverse between Europeans and Asians (11, 13). The rs2285666 polymorphism is located at the fourth base of the third intron next to the exon—this SNP is represented by C to T nucleotide substitutes that alter the splicing process and change ACE2 expression. Experiments of gene expression suggested that the alternating allele TT of rs2285666 SNP increased gene expression to 50%. Thus, it may have a role in SARS-CoV2 infection vulnerability (14). This study aimed to investigate rs2285666 SNP association with SARS-CoV2 infection as a first report in the Iraqi population.

2. Materials and Methods

2.1. Study Design and Participants

Total of 50 (19 Males/ 30 Females) Covid-19 patients with severe symptoms with mean age (of 41.5±10.7) and 50 (20 Males/ 30 Females) healthy people as a control group with a mean age of 41.5±10.7 were included in this study. The diagnosis of patients was made using a specific SARS-CoV-2 primer in RT-PCR, according to the World Health Organization (15). The patients were classified into positive infection based on Real-time RT-PCR Viral threshold-value results. Ethical issues were approved according to the Iraqi Ministry of Health, and all sampling processes were performed at the Public Health Laboratory in Al-Najaf Province.

2.2. Nucleic Acids Extraction and Genotyping

Samples were collected from nasopharyngeal swaps and treated with a viral transport medium composed of Hank's balanced salt solution at pH 7.4, containing 1% BSA, amphotericin, penicillin G, and streptomycin. Genomic DNA was isolated using an ELK DNA extraction kit (cat. No. EP007-50T). The ACE2-DNA segment that includes investigated rs2285666 SNP was amplified using specific NCBI-designed primers. The forward primer: ATGTCCTTGCCCTTATAGTTCC and Reverse: CTAAATACAATGAGCACCATCTACAG. The procedure carried out according to manufacturer instruction GoTaq® G2 Green Master Mix (Promega,

US). The reactants were placed in a thermal cycler (Biorad, USA) and the following program steps a; initial denaturation one step at 94 ºC for 5 minutes; 35-cycles represented as denaturation at 94 ºC for the 30s, annealing at 50 ºC for 30s, and extraction at 72 ºC for 1 min. The final extension step is at 72 ºC for 5 min. The PCR products of 308b were digested using AIuI endonuclease (BioEngland®, UK) and electrophoresed in 2% gel with Red-Safe DNA dye (Intron, Korea). The wild CC type gives one band (308b), the hetero-variant (CT) gives three bands, 308b, 221b, and 87b, and the homo-variant (TT) gives two bands, 221n and 87b, as shown in figure 1. Also, the sample of a patient was tested as a mutant genotype (TT) by RFLP assay, as shown in figure 2.

The PCR product of this sample was digested using the restriction enzyme (AluI). The wild type (WT) genotype (CC) would have only one band (308 bp). The hetero-genotype (CT) is confirmed once three bands of (308 bp, 221 bp, and 87bp) are shown in the gel. Finally, the mutant genotype (TT) is detected once the gel expresses only two bands (221 bp and 87 bp).

The gel was 2%, and the DNA dye was RedSafe (Intron, Korea). V: 90, Time: 45 minutes. M: DNA ladder.

2.3. Statistical Analysis

The Control group tested for deviation from Hardy Weinberg Equilibrium by chi-square analysis, which was within Hardy principles. Statistical analysis was performed using SPSS V.25.0. Software (SPSS Inc., Chicago, IL, USA). Differences between groups for continuous variables were examined using Student's t-test. The risk factor for SARS-CoV-2 infection with genotype alleles is expressed as an odds ratio (OR) with 95% CI and indices <0.05, which is determined by logistic regression analysis. The genotype results adjusted for gender and age as cofounders.

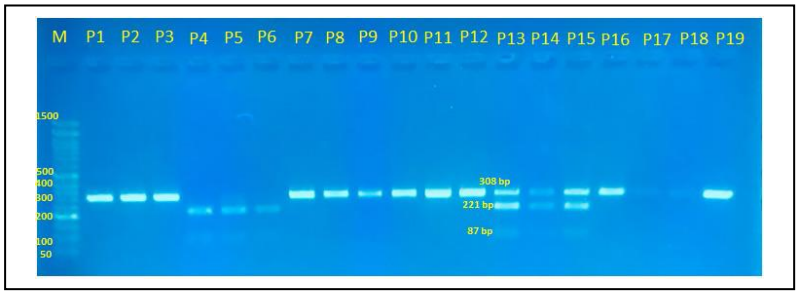

Figure 1. RFLP bands of SNP rs2285666 (C/T) of gene *ACE-2* of *Homo sapiens*

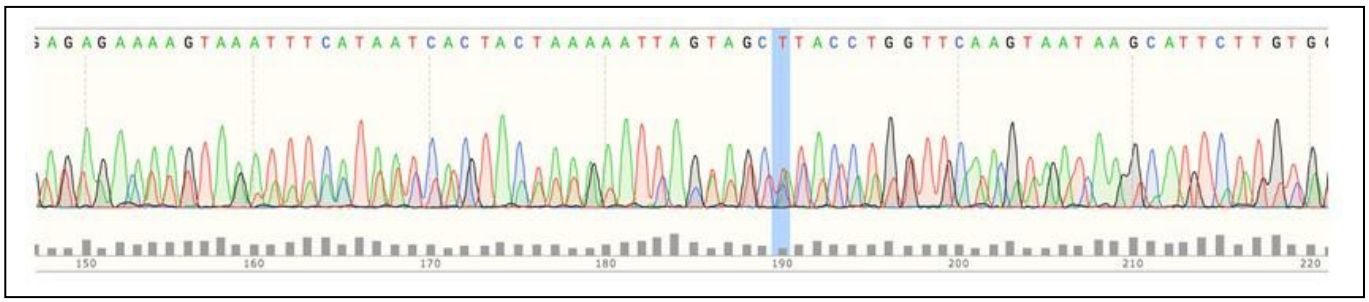

Figure 2. A blast of the DNA sequence of a PCR product of sample P13 against a RefSeqGene of ACE-2 deposited in the NCBI. This sample was tested as a hetero-genotype (C/T) by RFLP assay

3. Results and Discussion

Studies have revealed that SARS-CoV-2 used ACE2 as a receptor for its cellular entry (16). It has been exposed that the human-soluble form of ACE2 impedes the growth of SARS-CoV-2 and breaks off the early stages of infections (17). The diversity of the SARS-CoV-2 infection vulnerability may be related to specific genomic polymorphisms within *the ACE2* gene, which modify its expression and function. Some recent studies on common *ACE2* variants reported population-dependent minor allele frequency dissimilarities for rs2285666 SNP (11, 13, 18). This study reveals 0.3 MAF of this gene in our Iraqi samples, more than Europeans (0.2) and less than East Asians (0.55) (11, 13).

The results in table 1 shows that the MAF of rs2285666 SNP and the odd ratio of genotypes are risk factors for SARS-CoV-2 infection severity. The codominant model had significant OR of both alleles CT and TT (OR= OR=4.26 & 6.7; *P*-value=0.012 & 0.023 respectively).

However, rs2285666 has been approved as a potential risk factor for type 2 diabetes, essential hypertension and heart diseases (19). Thus, it may be an affecting factor linked with the comorbidities detected in SARS-CoV-2 patients. A study also exposed that rs2285666 genotypes associated with ACE2 concentrations were measured, with TT alleles having more levels than CC alleles by 50% (Li, 2012). Recently, it has been shown that the alteration of C to T polymorphism is predicted to elevate the force of the splicing site to 9.2%, causing increased ACE2 expression (13). Additionally, the patients detected with ACE1 with more significant activity and reduced ACE2 activity would be more susceptible to hypertension, principally companies with traditional cardiovascular risks such as dyslipidaemia and diabetes related to ageing (20). Inappropriately, the entry of SARS-CoV-2 inside cells through membrane receptor fusion down-regulates ACE2 receptors and causes loss of ACE2 catalytic activity (21). It has been reported that increased lung inflammation and coagulation as undesirable effects and boosted angiotensin II outcomes via the RAS system (22). The clinical reports of SARS-CoV-2 patients reveal numerous characteristics associated with the degree of infection and severity of the disease, distributing a variable mark of ACE2 deficiency (5). Therefore, ACE2 down-regulation induced by the viral attack may harm people with boundary ACE2 deficiency related to the above conditions (23).

In conclusion, there is an association between increased severity of SARS-Cov-2 infection and rs2285666 polymorphism of the codominant genotype model of the Iraqi population. However, several other factors may affect disease severity, such as ethnic group differences, sex, comorbidity, virus strain, and others.

Table 1. Table of genotypes difference between groups involved in the study

ACE-2 Polymorphisms	Sever SARS-CoV-2	Healthy Control group	Unadjusted Model		Adjusted Model *	
			OR (95% CI)	*p*	OR (95% CI)	*p*
rs6777038	n=50	n=50				
Allele (%)						
C	70(0.7)	91(0.91)				
T	30(0.3)	9(0.09)	4.33(1.93-9.72)	0.0004	4.51(1.99-10.18)	0.0003
Genotype (%)						
CC	28(0.28)	43(0.43)				
CT	14(0.14)	5(0.05)	4.3(1.39-13.27)	0.011	4.26(1.37-13.23)	0.012
TT	8(0.08)	2(0.02)	6.14(1.21-31.07)	0.028	6.7(1.3-34.57)	0.023
Dominant Model (%)						
CC	36(0.36)	43(0.43)				
CT+TT	14(0.14)	7(0.07)	2.39(0.87-6.56)	0.091	2.43(0.88-6.71)	0.087
Recessive Model (%)						
CC+CT	45(0.45)	48(0.48)				
TT	5(0.05)	2(0.02)	0.49(14.44-0.21)	0.26	3.03(0.54-16.87)	0.21

*Adjusted for Gender & Age

Authors' Contribution

Study concept and design: M. A. A.
Acquisition of data: M. N. M.
Analysis and interpretation of data: M. A. A.
Drafting of the manuscript: I. M. N.
Critical revision of the manuscript for important intellectual content: M. N. M.
Statistical analysis: I. M. N.
Administrative, technical, and material support: M. A. A.

Ethics

Ethical issues were approved according to the Iraqi Ministry of Health, and all sampling processes were performed at the Public Health Laboratory in Al-Najaf Province.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Gorbalenya A, Baker S, Baric R, De Groot R, Drosten C, Gulyaeva A, et al. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol. 2020;5(4):536-44.
2. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-3.
3. Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci China Life Sci. 2020;63(3):457-60.
4. Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature. 2020;581(7807):215-20.
5. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020;395(10224):565-74.
6. Shi C-S, Qi H-Y, Boularan C, Huang N-N, Abu-Asab M, Shelhamer JH, et al. SARS-coronavirus open reading frame-9b suppresses innate immunity by targeting mitochondria and the MAVS/TRAF3/TRAF6 signalosome. J Immun. 2014;193(6):3080-9.
7. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. J Virol. 2020;94(7):e00127-20.
8. Yang X-h, Deng W, Tong Z, Liu Y-x, Zhang L-f, Zhu H, et al. Mice transgenic for human angiotensin-converting enzyme 2 provide a model for SARS coronavirus infection. Comp Med. 2007;57(5):450-9.
9. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature. 2005;436(7047):112-6.
10. Zhao Y, Zhao Z, Wang Y, Zhou Y, Ma Y, Zuo W. Single-cell RNA expression profiling of ACE2, the receptor of SARS-CoV-2. Am J Respir Crit Care Med. 2020;202(5):756-9.
11. Cao Y, Li L, Feng Z, Wan S, Huang P, Sun X, et al. Comparative genetic analysis of the novel coronavirus (2019-nCoV/SARS-CoV-2) receptor ACE2 in different populations. Cell Discov. 2020;6(1):1-4.
12. Stawiski EW, Diwanji D, Suryamohan K, Gupta R, Fellouse FA, Sathirapongsasuti JF, et al. Human ACE2 receptor polymorphisms predict SARS-CoV-2 susceptibility. BioRxiv. 2020.
13. Asselta R, Paraboschi EM, Mantovani A, Duga S. ACE2 and TMPRSS2 variants and expression as candidates to sex and country differences in COVID-19 severity in Italy. Aging (Albany NY). 2020;12(11):10087.
14. Li Y-y. Lack of association of ACE2 G8790A gene mutation with essential hypertension in the Chinese population: a meta-analysis involving 5260 subjects. Front Physiol. 2012;3:364.
15. Coronavirus N. Situation Report–221 February 2020. 2019.
16. Fu J, Zhou B, Zhang L, Balaji KS, Wei C, Liu X, et al. Expressions and significances of the angiotensin-converting enzyme 2 gene, the receptor of SARS-CoV-2 for COVID-19. Mol Biol Rep. 2020;47(6):4383-92.
17. Monteil V, Kwon H, Prado P, Hagelkrüys A, Wimmer RA, Stahl M, et al. Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. Cell. 2020;181(4):905-13.
18. Strafella C, Caputo V, Termine A, Barati S, Gambardella S, Borgiani P, et al. Analysis of ACE2 genetic

variability among populations highlights a possible link with COVID-19-related neurological complications. Genes. 2020;11(7):741.

19. Chaoxin J, Daili S, Yanxin H, Ruwei G, Chenlong W, Yaobin T. The influence of angiotensin-converting enzyme 2 gene polymorphisms on type 2 diabetes mellitus and coronary heart disease. Eur Rev Med Pharmacol Sci. 2013;17(19):2654-9.
20. Ghafouri-Fard S, Noroozi R, Omrani MD, Branicki W, Pośpiech E, Sayad A, et al. Angiotensin converting enzyme: a review on expression profile and its association with human disorders with special focus on SARS-CoV-2 infection. Vasc Pharmacol. 2020;130:106680.
21. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive Care Med. 2020;46(4):586-90.
22. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus–induced lung injury. Nat Med. 2005;11(8):875-9.
23. Verdecchia P, Cavallini C, Spanevello A, Angeli F. The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. Eur J Intern Med. 2020;76:14-20.