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Genotype AA of ACE2 G8790A (rs2285666) Has Protective Potential Against COVID-19 Disease Severity

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Background: SARS-CoV-2 virus uses angiotensin converting enzyme 2 (ACE2), a key enzyme of the renin angiotensin system (RAS) as the functional receptor for cell fusion and induction of infections in the respiratory system. Functional ACE2 gene polymorphisms may lead to RAS imbalance and are associated with COVID-19 susceptibility and severity. ACE2 G8790A (rs2285666), a splice region variant, is well characterized in various populations across the world. In the present study, the role of ACE2 G8790A (rs2285666) variant as risk predictor for severity of COVID-19 infection was investigated.

Materials and methods: One-hundred COVID-19 subjects were included in the study and divided into: subjects with a history of severe infection and ICU-admitted (Group 1) and subjects with mild to moderate COVID-19 infection (Group 2). Genotype analysis for rs2285666 of ACE2 was performed using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method.

Results: The distribution of ACE2 G8790A (rs2285666) genotypes were GG 62%, GA 18%, and AA 20% in Group 1 and GG 34%, GA 14%, and AA 52% in Group 2, respectively. The A allele of rs2285666 ($p \leq 0.001$; OR=3.4; 95% CI=1.89–6.107) were less frequent in Group 1 as compared to Group 2. Also, a statistically significant difference was found between severity of COVID-19 infection with age and comorbidities such as diabetes, hypertension, chronic kidney disease, but not gender.

Conclusion: Our findings suggest the possibility of a protective mechanism of the AA genotype of ACE2 G8790A (rs2285666) variant against COVID-19 disease severity.

Keywords: COVID-19, ACE2 gene, renin-angiotensin system, genetic association, rs2285666, sanger sequencing

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly contagious positive strand RNA virus,

reported first in December 2019.¹ The severity of infection in people affected by COVID-19 is related to male gender, older age, presence of serious comorbidities such as

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hypertension, diabetes, cardiovascular disease (CVD), chronic lung disease, and associated liver and kidney damage.^{2,3} However, susceptibility and risk for progression to the severe form of infection has not been similar across different ethnicities across the world. This remains the most controversial aspect in understanding the pathogenesis and clinical course of COVID-19. The surface of lung alveolar epithelial cells contains angiotensin converting enzyme 2 (ACE2), a key enzyme of the renin angiotensin system (RAS) which facilitates the entry of SARS-CoV-2.⁴ Spike (S) protein of SARS-CoV-2 virus, consists of subunit S1 with a receptor-binding domain (RBD) that recognizes ACE2.⁵⁻⁷

Meta analysis studies indicate a possibility of increased mortality risk in co-existence of cardiovascular diseases and COVID-19 infection.^{2,3} Clinical studies suggest lung fibrosis and acute respiratory distress syndrome (ARDS) seen in COVID-19 patients may be related to an unbalanced RAS.^{8,9} In COVID-19-induced inflammation, membrane fusion between SARS-CoV-2 and S1 lead to decrease in ACE2 levels and disruption in angiotensin II metabolism and elevated levels. This leads to the release of inflammatory cytokines and systemic inflammation in COVID-19 infection.^{10,11}

Recent studies have identified few functional SNPs, such as rs2106809 and rs2285666, which can bring about variations in binding affinity of ACE2 for SARS CoV-2 RBD.^{12,13} G8790A (rs2285666) or also called c.439+4G>A (NM_001371415.1) variant of the *ACE2* gene, located in intron 3 of chromosome Xp22 may affect *ACE2* gene expression by altered mRNA splicing. The wild type of this variant enhances the ACE2 production with a greater affinity for virus.^{14,15} Frequency of variant G8790A was found to be higher among the Indian population (mean allele frequency of ~0.6) in comparison with others.¹⁶ Further, the frequency of this allele is significantly higher (two tailed $p < 0.0001$) in the Indian population as compared to European, American, or African ethnicities. Also, the frequency of this allele is significantly higher (two tailed $p < 0.0001$) among Indian populations in comparison with either European, American, or African. The alternate allele (TT-plus strand or AA-minus strand) has been found to increase the expression of the gene up to low infection rate and low mortality rate.¹⁷ In the present study, we hypothesized a correlation between *ACE2* G8790A (rs2285666) variant and severity of COVID-19, since only a limited number of studies have been conducted on the association between *ACE2* gene and susceptibility

and severity of COVID-19 infection in Indian population and in other ethnicities. The aim of the present study was to investigate the role of *ACE2* G8790A (rs2285666) variant as risk predictor for severity of COVID-19 infection.

Materials and methods

Study Design and Subjects Recruitment

The was a cohort study conducted between March and June 2021. One-hundred subjects aged 18 years and above and diagnosed with COVID-19 based on positive polymerase chain reaction (PCR) test and/or other clinical data were recruited. Subjects were classified into two groups based on the disease severity as defined by WHO guidelines^{18,19,20}; Group 1 consisted of patients who suffered severe COVID-19 and were admitted in the intensive care unit (ICU) of Employees State Insurance Corporation (ESIC) Superspeciality Hospital, Hyderabad, India; and Group 2 consisted of patients with mild and moderate COVID-19 infections admitted to the isolation ward in ESIC Superspeciality Hospital.

Severe COVID-19 subjects in Group 1 were included if they had any one of the following conditions as stated by WHO¹⁹: respiratory distress (respiratory rate ≥ 30 /min), oxygen saturation on room air at rest $\leq 93\%$, partial pressure of oxygen in arterial blood/ $\text{FiO}_2 \leq 300$ mm Hg, respiratory failure occurs and mechanical ventilation is required, another organ dysfunction is present, or requiring intensive care unit monitoring and treatment. Whereas, Group 2 included subjects with mild disease *i.e.*, symptomatic patients meeting the case definition for COVID-19 without evidence of viral pneumonia or hypoxia and subjects with moderate disease *i.e.*, clinical signs of pneumonia (fever, cough, dyspnoea, fast breathing) but no signs of severe pneumonia, including $\text{SpO}_2 \geq 90\%$ on room air.¹⁹ Subjects with insufficient medical records or missing data and/or < 18 years of age were excluded from the study. The study was approved by the institutional ethical committee (No. ESICMC/SNR/IEC-F279/05-2021).

Characteristics Data Collection

The demographic data of study subjects, such as age, gender, and any underlying comorbidities and past medication history were obtained from medical records and patient's charts at the hospital admission. High Resolution Computed Topography (HRCT) was used to evaluate the extent of lung involvement and to categorize the subjects involved into groups.²⁰

DNA Isolation and Genotyping

Whole blood samples were collected in EDTA vacutainer from the study subjects and stored at -80°C until the test was performed. The genotyping of *ACE2* G8790A (rs2285666) polymorphism was performed by restriction fragment length polymorphism PCR (PCR-RFLP) and later confirmed by sanger sequencing (Figure 1).²¹ Genomic DNA was extracted from EDTA peripheral blood using the DNEasy Blood Extraction Kit (Macherey and Nagel, Nordrhein-Westfalen, Germany) and was quantified using nanodrop. The primers used and restriction enzyme site are as follows: Forward primer: 5'-CATGTGGTCAAAGGATATGT-3', Reverse primer: 5'AAAGTAAGGTTGGCAGACAT3' and AluI: AG|CT. PCR was performed with total reaction volume of 25 µL consisting of 1µL genomic DNA template, 2.5 µL 10×PCR buffer, 25 µmol/L MgCl₂ 1.5 µL, 10 mmol/L dNTP 0.5 µL, 0.8 µL forward primer, 0.2 µL Taq DNA polymerase and 18.5 µL double distilled water. PCR program conditions were as follows: an initial denaturation at 95°C for 2 min, followed by 34 cycles at 94°C for 30 s, 50.6°C for 30 s, and 72°C for 45 s. The final extension step was at 72°C for 7 min. Five µL of PCR products were digested at 37°C for 4 h with 2U AluI (Takara Bio, Shiga, Japan), 2 µL of 10× buffer supplied and sterile water to a total volume of 20 µL. After digestion, bands were visualized using ETBr

stained 2% agarose gel electrophoresis. Homozygous GG confirmed by a single band at 466 bp, Homozygous AA genotype confirmed with bands at of 281 and 185 bp and Heterozygous AG genotype confirmed by bands at 466, 281 and 185 bp respectively (Figure 2).

Statistical Analysis

The Hardy-Weinberg equilibrium was tested for *ACE2* gene polymorphism and any deviation between the observed and expected frequencies was statistically evaluated for significance using odds ratio (OR) with 95% confidence interval (CI) and the chi-square test. Association between genotypes and severity of COVID-19 was examined. Hardy-Weinberg calculator was used for calculating allele and genotype frequencies. The quantitative data was compared using independent t-test and qualitative was compared using Chi-square test. A $p < 0.05$ was considered as significant. All of the tests were performed using Statistical Package for the Social Sciences (SPSS) version 16 (IBM Corporation, Armonk, NY, USA).

Results

Among 100 subjects diagnosed with COVID-19 infection, 50 subjects were classified as mild to moderate infection

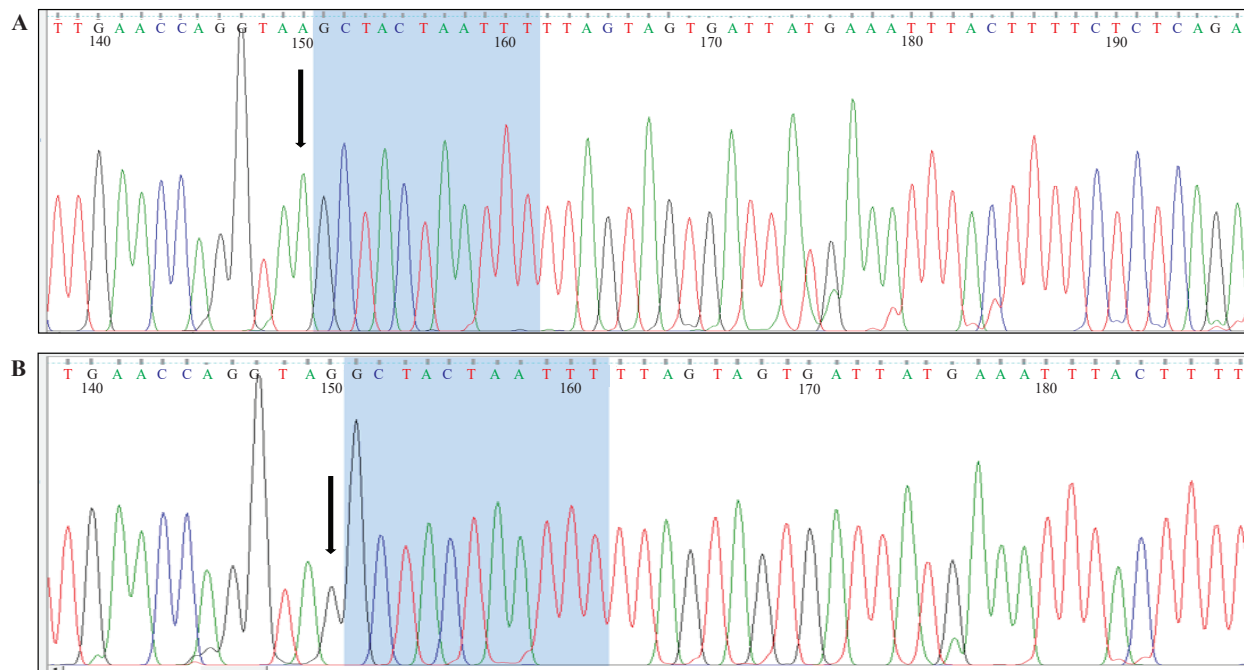


Figure 1. Sanger sequencing results of the homozygous AA and GG genotype. A: Sample 1 represents homozygous (AA) genotype. B: Sample 2 represents a homozygous (GG) genotype.

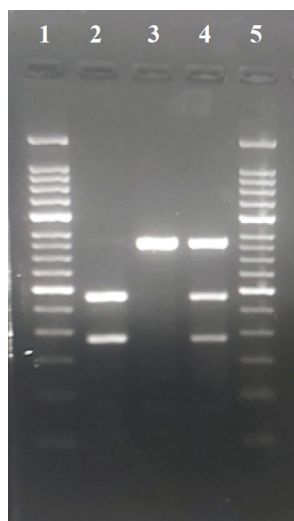


Figure 2. Agarose gel representation of *ACE2* G8790A (rs2285666) genotyping. Lane represents the 100bp ladder. Lanes 1 and 5 represent 50 bp ladder. Lane 2 represents Homozygous (AA) genotype (281 and 185 bp), lane 3 represents homozygous (GG) genotype (466 bp) and lane 4 represents heterozygous (AG) genotype (466, 281 and 185 bp).

and 50 subjects were classified as severe infection. Results of the subjects' demographic and underlying comorbidity analysis showed no significant difference in gender between the two groups ($p=0.22$). However, there were significant differences in age ($p=0.009$), existence of comorbidities: hypertensives ($p=0.023$), diabetes ($p=0.045$), chronic kidney disease ($p=0.013$), chronic lung disease ($p=0.000$) observed between Group 1 and Group 2 (Table 1).

Genotype Distribution and Allele Frequencies of *ACE2* Gene Polymorphism

The frequency of GG 62%, GA 18%, and AA 20 % in Group 1 and GG 34%, GA 14%, and AA 52% in Group 2, respectively (Table 2). TA statistically significant difference was observed in the genotypic distribution and allelic frequency between the Group 1 and Group 2 subjects [for GG vs. AA genotype, OR=0.231; (95% CI=0.095–0.561; $p=0.001$); GG vs. (GA+AA) OR=0.316, (95% CI=0.139–0.715; $p=0.005$); and G allele vs. A allele, OR=3.4 (95% CI=1.89–6.107); $p=0.000$]. An increase in the frequency of A allele was observed in Group 2 as compared to Group 1 subjects, thereby indicating its likely protective effect of A allele in severity of COVID-19 disease.

Discussion

The results of the present study show the association between GG genotype as well as its associated allele, the G allele, of *ACE2* G8790A (rs2285666) variant and the severity of COVID-19 disease. This is similar to other studies results in Indian and Caucasian populations.²¹ However, there were other studies which reported that this variant did not affect the severity of the disease.^{13,22}

ACE2 is the entry receptor of SARS-CoV-2, and is significantly expressed in the airway cells, alveolar epithelial type II cells, and endothelial cells of the respiratory and cardiovascular systems respectively.²³⁻²⁶ Studies have shown that the *ACE2* is a type I transmembrane metallo carboxypeptidase and a key player in RAS and a homology with *ACE*. Previous research has demonstrated that *ACE2* inhibition or knockdown dramatically increases lung damage and inflammatory cytokine release.²⁷ Higher levels of lung damage and RAS imbalance are linked to a disparity between *ACE* and *ACE2* activity in ARDS. This may be because pulmonary Ang-(1-7) levels are decreased and its anti-inflammatory actions in the pulmonary tissue are lost.²⁸⁻³¹ With increased *ACE* activity, decreased *ACE2* availability, and an decrease in Ang-(1-7) generation, enhanced AT1 receptor activation markedly impairs pulmonary function.^{28,31} Ang-(1-7) has vasodilator, anti-inflammatory, anti-proliferative and anti-fibrotic effects when it binds with Mas receptor and thereby regulates multiple intracellular signaling pathways.^{25,32} SARS-CoV-2 induces *ACE2* deficit by suppressing *ACE2* receptor, and thereby leads to an imbalance between *ACE1* and *ACE2*, which are the two components of the RAS pathway.³³ Additionally, this

Table 1. Demography and co-morbidities in the groups and their relation with the COVID-19 severity.

Variables	Group 1 (n=50)	Group 2 (n=50)	<i>p</i> -value ^a
Gender, n (%)			
Female	24 (48)	18 (36)	0.224
Male	26 (52)	32 (64)	
Age (years), (mean±SD)	45.1±8.7	49.5 6±8.03	0.009*
Hypertension, n (%)	26 (52)	37 (74)	0.023*
Diabetes, n (%)	19 (38)	29 (58)	0.045*
Chronic kidney disease, n (%)	8 (16)	19 (38)	0.013*
Chronic lung disease, n (%)	2 (4)	17 (34)	0.000*

* $p<0.05$ is significant. ^aanalyzed with independent t-test.

Table 2. Genotype distribution and allelic frequencies of ACE2 G8790A polymorphism.

ACE2 G8790A G>A	n (%)		OR (95% CI)	p-value ^a
	Group 1 (n=50)	Group 2 (n=50)		
Genotype				
GG	31 (62)	17 (34)	1.00 (ref)	1.00 (ref)
AG	9 (18)	7 (14)	1.348 (0.460–3.956)	0.585
AA	10 (20)	26 (52)	0.231 (0.095–0.561)	0.001*
AG+AA	19 (38)	33 (66)	0.316 (0.139–0.715)	0.005*
Alleles				
G	71 (35.5)	41 (20.5)	3.4 (1.89–6.107)	0.000*
A	29 (14.5)	59 (31.5)		

* $p < 0.05$ is significant. ^aanalyzed with Chi-square test for Group 1 vs. Group 2.

interaction between the virus and receptor prevents the ACE enzyme from conversion of Ang II to Ang-(1-7) with high affinity.²⁵ The RAS imbalance leads to Ang II overproduction and deficit in Ang-(1-7) which facilitates inflammatory and coagulation processes at the level of the lung tissue.^{34,35} A slight or moderate ACE2 deficit cannot protect the host against viral invasion because rs2285666 variant can alter mRNA splicing which affects gene expression and protein level of the enzyme as well.¹⁴ An analysis of the association between circulating ACE2 G8790A (rs2285666) genotypes and Type 2 Diabetes Mellitus patients revealed that the AA genotype exhibits the highest level of expression when compared to other genotypes.³⁶

As a global burden, the severity of disease among COVID-19 affected individuals has been positively associated risk predictors such as advanced age, occurrence of co-morbidities, such as diabetes mellitus, hypertension, chronic lung disease, cardiovascular disease (CVD), and impaired renal and liver function, *etc.*³⁷ The age correlation to severity and comorbid conditions like diabetes, which have been linked to ACE2 deficiency might worsen the COVID-19-induced ACE2 deficit and raise the severity of the disease, which were similar to current study's findings.³³ Clinical course of COVID-19 is extremely heterogeneous both individually and globally. Hence, it has been suggested that an additional factor may have a role in modulating the risk of disease onset and severity. No statistically significant association between gender and severity of COVID-19 disease was observed in the present study was contrary to other studies which was reported that men were more likely to develop severe COVID-19 disease.³⁸

Our study is a preliminary attempt at suggesting the possible role of host susceptibility and genetics in COVID-19 infection and its severity. Further studies on the role of host genomic variant status to various infections are needed regarding the hour in the wake of COVID-19 infection. It could explain the variation in disease susceptibility and severity not only between different ethnicities, but also in subjects of the same population.

Conclusion

In conclusion, variant genotype AA of ACE2 G8790A, might play a significant role in conferring protection against COVID-19 severity. The result of the current study might help to understand the inter-individual variability of the COVID-19 disease severity and importance of host genetics in understanding disease outcome.

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Authors Contribution

SGC and IAS were involved in concepting and planning the research, BKR performed the data acquisition/collection, IAS and SGC performed the interpretation of the results, and conducted data analysis. IAS, SGC and SS drafted the manuscript and performed critical review of the article. All authors took parts in giving critical revision of the manuscript.

References

- Yang W, Sirajuddin A, Zhang X, Liu G, Teng Z, Zhao S, *et al.* The role of imaging in 2019 novel coronavirus pneumonia (COVID-19). *Eur Radiol.* 2020; 30(9): 4874–82.
- Li X, Guan B, Su T, Liu W, Chen M, Bin Waleed K, *et al.* Impact of cardiovascular disease and cardiac injury on in-hospital mortality in patients with COVID-19: a systematic review and meta-analysis. *Heart.* 2020; 106(15): 1142–7.
- Sabatino J, De Rosa S, Di Salvo G, Indolfi C. Impact of cardiovascular risk profile on COVID-19 outcome. A meta-analysis. *PLoS One.* 2020; 15(8): e0237131. doi: 10.1371/journal.pone.0237131.
- Hamming I, Timens W, Bulthuis MLC, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol.* 2004; 203(2): 631–7.
- Shang J, Wan Y, Luo C, Ye G, Geng Q, Auerbach A, *et al.* Cell entry mechanisms of SARS-CoV-2. *Proc Natl Acad Sci USA.* 2020; 117(21): 11727–34.
- Li F. Receptor recognition mechanisms of coronaviruses: A decade of structural studies. *J Virol.* 2015; 89(4): 1954–64.
- Chen Y, Liu Q, Guo D. Emerging coronaviruses: Genome structure, replication, and pathogenesis. *J Med Virol.* 2020; 92(4): 418–23.
- Shenoy V, Ferreira AJ, Qi Y, Fraga-Silva RA, Díez-Freire C, Dooies A, *et al.* The angiotensin-converting enzyme 2/angiogenesis-(1-7)/Mas axis confers cardiopulmonary protection against lung fibrosis and pulmonary hypertension. *Am J Respir Crit Care Med.* 2010; 182(8): 1065–72.
- Wösten-van Asperen RM, Lutter R, Specht PA, Moll GN, van Woensel JB, van der Loos CM, *et al.* acute respiratory distress syndrome leads to reduced ratio of ACE/ACE2 activities and is prevented by angiotensin-(1-7) or an angiotensin II receptor antagonist: ARDS leads to reduced ratio of ACE/ACE2 activities. *J Pathol.* 2011; 225(4): 618–27.
- Liu Z, Xiao X, Wei X, Li J, Yang J, Tan H, *et al.* Composition and divergence of coronavirus spike proteins and host ACE2 receptors predict potential intermediate hosts of SARS-CoV-2. *J Med Virol.* 2020; 92(6): 595–601.
- Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, *et al.* Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci China Life Sci.* 2020; 63(3): 364–74.
- Srivastava A, Bandyopadhyay A, Das D, Pandey RK, Singh V, Khanam N, *et al.* Genetic association of ACE2 rs2285666 polymorphism with COVID-19 spatial distribution in India. *Front Genet.* 2020; 11: 564741. doi: 10.3389/fgene.2020.564741.
- Karakas Çelik S, Çakmak Genç G, Pişkin N, Açikgöz B, Altinsoy B, Kurucu İşsiz B, *et al.* Polymorphisms of ACE (I/D) and ACE2 receptor gene (Rs2106809, Rs2285666) are not related to the clinical course of COVID-19: A case study. *J Med Virol.* 2021; 93(10): 5947–52.
- Patel SK, Velkoska E, Freeman M, Wai B, Lancefield TF, Burrell LM. From gene to protein-experimental and clinical studies of ACE2 in blood pressure control and arterial hypertension. *Front Physiol.* 2014; 5: 227. doi: 10.3389/fphys.2014.00227.
- Pouladi N, Abdolahi S. Investigating the ACE2 polymorphisms in COVID-19 susceptibility: An in-silico analysis. *Mol Genet Genomic Med.* 2021; 9(6): e1672. doi: 10.1002/mgg3.1672.
- 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): 11. doi: 10.1038/s41421-020-0147-1.
- Hou Y, Zhao J, Martin W, Kallianpur A, Chung MK, Jehi L, *et al.* New insights into genetic susceptibility of COVID-19: an ACE2 and TMPRSS2 polymorphism analysis. *BMC Med.* 2020; 18(1): 216. doi: 10.1186/s12916-020-01673-z.
- World Health Organization [Internet]. Weekly Operational Update on COVID-19 - 16 August 2021 [cited 2023 Jul 24]. Available from: <https://www.who.int/publications/m/item/weekly-operational-update-on-covid-19---16-august-2021>.
- World Health Organization [Internet]. Living Guidance for Clinical Management of COVID-19 [cited 2023 Jul 24]. Available from: <https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2021-2>.
- The PLOS ONE Editors. Expression of Concern: Low-dose chest CT for diagnosing and assessing the extent of lung involvement of SARS-CoV-2 pneumonia using a semi quantitative score. *PLoS One.* 2022; 17(12): e0279045. doi: 10.1371/journal.pone.0279045.
- Möhlendick B, Schönfelder K, Breuckmann K, Elsner C, Babel N, Balfanz P, *et al.* ACE2 polymorphism and susceptibility for SARS-CoV-2 infection and severity of COVID-19. *Pharmacogenet Genomics.* 2021; 31(8): 165–71.
- 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. doi: 10.1186/s12985-022-01782-6.
- 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.
- Wang Q, Zhang Y, Wu L, Niu S, Song C, Zhang Z, *et al.* Structural and functional basis of SARS-CoV-2 entry by using human ACE2. *Cell.* 2020; 181(4): 894–904.e9.
- Santos RAS, Sampaio WO, Alzamora AC, Motta-Santos D, Alenina N, Bader M, *et al.* The ACE2/angiotensin-(1-7)/MAS axis of the renin-angiotensin system: Focus on angiotensin-(1-7). *Physiol Rev.* 2018; 98(1): 505–53.
- Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, *et al.* High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *Int J Oral Sci.* 2020; 12(1): 8. doi: 10.1038/s41368-020-0074-x.
- Li Y, Zeng Z, Cao Y, Liu Y, Ping F, Liang M, *et al.* Angiotensin-converting enzyme 2 prevents lipopolysaccharide-induced rat acute lung injury via suppressing the ERK1/2 and NF-κB signaling pathways. *Sci Rep.* 2016; 6: 27911. doi: 10.1038/srep27911.
- Magalhaes GS, Barroso LC, Reis AC, Rodrigues-Machado MG, Gregório JF, Motta-Santos D, *et al.* Angiotensin-(1-7) promotes resolution of eosinophilic inflammation in an experimental model of asthma. *Front Immunol.* 2018; 9: 58. doi: 10.3389/fimmu.2018.00058.
- Wang D, Chai XQ, Magnussen CG, Zosky GR, Shu SH, Wei X, *et al.* Renin-angiotensin-system, a potential pharmacological candidate, in acute respiratory distress syndrome during mechanical ventilation. *Pulm Pharmacol Ther.* 2019; 58(101833): 101833. doi: 10.1016/j.pupt.2019.101833.
- He H, Liu L, Chen Q, Liu A, Cai S, Yang Y, *et al.* Mesenchymal stem cells overexpressing angiotensin-converting enzyme 2 rescue lipopolysaccharide-induced lung injury. *Cell Transplant.* 2015; 24(9): 1699–715.

31. Bastos AC, Magalhães GS, Gregório JF, Matos NA, Motta-Santos D, Bezerra FS, *et al.* Oral formulation angiotensin-(1-7) therapy attenuates pulmonary and systemic damage in mice with emphysema induced by elastase. *Immunobiology*. 2020; 225(2): 151893. doi: 10.1016/j.imbio.2019.12.002.
32. Gironacci MM. Angiotensin-(1-7): beyond its central effects on blood pressure. *Ther Adv Cardiovasc Dis*. 2015; 9(4): 209–16.
33. 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.
34. Costa LB, Perez LG, Palmeira VA, Macedo e Cordeiro T, Ribeiro VT, Lanza K, *et al.* Insights on SARS-CoV-2 molecular interactions with the renin-angiotensin system. *Front Cell Dev Biol*. 2020; 8: 559841. doi: 10.3389/fcell.2020.559841.
35. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet*. 2020; 395(10229): 1033–4.
36. Wu YH, Li JY, Wang C, Zhang LM, Qiao H. The ACE2G8790A polymorphism: Involvement in type 2 diabetes mellitus combined with cerebral stroke. *J Clin Lab Anal*. 2017; 31(2): e22033. doi: 10.1002/jcla.22033.
37. Tandirogang N, Fitriany E, Mardiana N, Jannah M, Dilan BFN, Ratri SR, *et al.* Neutralizing antibody response by inactivated SARS-cov-2 vaccine on healthcare workers. *Mol Cell Biomed Sci*. 2023; 7(1): 18-27.
38. Gómez J, Albaiceta GM, García-Clemente M, López-Larrea C, Amado-Rodríguez L, Lopez-Alonso I, *et al.* Angiotensin-converting enzymes (ACE, ACE2) gene variants and COVID-19 outcome. *Gene*. 2020; 762(145102): 145102. doi: 10.1016/j.gene.2020.145102.