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Research Article

ACE GENE POLYMORPHISM AND THROMBOTIC RISK IN COVID-19 PATIENTS: INSIGHTS FROM A CROSS-SECTIONAL STUDY IN SUDAN DURING THE SECOND WAVE

[1,*]Ozaz Yagoub Mohammed Ahmed, [1]Mustafa Ibrahim Abbas, [2]Salaheldein G. Elzaki, [3]Nazim Ahmed and [4]Abdelrahman Eldaw Mohammed Abakar

[1]Department of Hematology and Immunhematology, Faculty of Medical Laboratory Sciences, University of Gezira, Wad Medani, Sudan
[2]Tropical Medicine Research Institute, Khartoum, Sudan
[3]School of public health, University of Queensland, Australia
[4]Department of medical microbiology, Faculty of Medical Laboratory Sciences, University of Gezira, Wad Medani, Sudan

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Abstract

The COVID-19 pandemic caused by SARS-CoV-2 has resulted in a significant global health crisis. Thrombotic events have been observed in COVID-19 patients, especially those with severe disease. This study aimed to investigate the association between ACE gene polymorphism and thrombotic risk in COVID-19 patients in Sudan. A cross-sectional study was conducted during the second wave of COVID-19 in 2021. Blood samples were collected from 161 patients, and coagulation parameters were assessed. The ACE gene polymorphism was analyzed using PCR. The results revealed that 56.5% of patients had the ACE D/D polymorphism, 35.4% had the ACE D/I polymorphism, and 8.1% had the ACE I/I polymorphism. The coagulation parameter analysis did not show significant differences between the different ACE genotypes, except for INR. This study provides insights into the genetic factors that may contribute to thrombotic risk in COVID-19 patients, specifically related to ACE gene polymorphism. Further research is warranted to better understand the underlying mechanisms and clinical implications of this association.

Keywords: COVID-19, ACE (I/D) polymorphism, Coagulation parameter.

INTRODUCTION

On March 12, 2020, the World Health Organization officially declared the global pandemic caused by the novel human severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This highly contagious virus is responsible for the disease known as COVID-19, which can cause severe illness and even death in a significant number of patients(Xu, Sun *et al.,* 2020). As of March 28, 2021, the World Health Organization has reported a total of 126,359,540 confirmed cases of COVID-19, with 2,769,473 recorded deaths (WHO 2021). On February 26, 2020, Sudan reported its first verified COVID-19 case; as of March 30, 2021, there were 29825 confirmed cases (of which 2587 were reported in Gezira State) (Ministry of Health August 2021). Numerous studies have reported an increased incidence of thrombotic events in COVID-19 patients, especially those with severe disease requiring hospitalization or intensive care unit (ICU) admission. Thrombosis in COVID-19 is believed to result from a complex interplay of several factors, including viral-induced endothelial dysfunction, hypercoagulability, inflammation, and immune response (Calabrese, Annunziata *et al.,* 2021). The association between COVID-19 and thrombosis has raised concerns and prompted further research to better understanding of the underlying mechanisms, clinical implications, and optimal management strategies. In this paper, we aim to provide an overview of the current understanding of the link between COVID-19 and thrombosis specially the effect of ACE gene polymorphism. The notion that the thrombotic symptoms of severe COVID-19 are a result of SARS-CoV-2's capacity to infiltrate endothelial cells via ACE-2 (angiotensin-converting enzyme 2) is supported by the evidence (McFadyen, Stevens *et al.,* 2020). ACE2 is a crucial element in COVID-19 infection, located on the cell membranes of target host cells in the lungs and intestines, and serves as a point of attachment for viral entry and replication by binding to COVID-19 spike-protein domains (Abassi, Higazi *et al.,* 2020). The human body's Renin Angiotensin System (RAS), which plays a significant role in controlling vascular physiology, is negatively controlled by ACE2. Angiotensin-converting enzyme 1 (ACE1) in the (RAS) system transforms angiotensin-1 (Ang-I) into angiotensin-2 (Ang-II), and ACE2 subsequently breaks down Ang II into Ang (1–7). Ang II promotes fibrosis, vasoconstriction, and inflammation (Singh, Choudhari *et al.,* 2021; Fiorentino, Benincasa *et al.,* 2023). Ang (1–7) is a vasodilator peptide that exhibits various beneficial effects, such as antiapoptotic, anti-heart failure, anti-thrombotic, and anti-myocardial hypertrophy properties (Verma, Abbas *et al.,* 2021). In the renin-angiotensin system (RAS), ACE1 and ACE2 work together to maintain a balance between the vasoconstrictor/proliferative actions of ACE1/Ang-II axis and the vasodilator/antiproliferative actions of ACE2/Ang1-7 axis. This cooperative mechanism protects organs and blood vessels by exerting anticoagulant, anti-inflammatory, anti-fibrotic, anti-alveolar epithelial cell apoptosis, and anti-oxidative stress activities, thereby counteracting harmful effects (Gemmati, Bramanti *et al.,* 2020). Interestingly, there is an inverse relationship between ACE and ACE2, where decreased expression of the ACE2 receptor gene is strongly associated with an increase in ACE expression (Karakaş Çelik, Çakmak Genç *et al.,* 2021). This suggests that the balance between ACE and ACE2 is crucial for maintaining homeostasis within the RAS and ensuring the proper functioning of the

Corresponding Author:** ***Ozaz Yagoub Mohammed Ahmed,
Department of Hematology and Immunhematology, Faculty of Medical Laboratory Sciences, University of Gezira, Wad Medani, Sudan.

cardiovascular system. The balance between ACE and ACE2 activities is of utmost importance in the context of COVID-19, as it may significantly impact the thrombo-inflammatory process (Calabrese, Annunziata *et al.,* 2021). During viral intracellular translocation and replication, ACE2 on the cell membrane is depleted through degradation and shedding, leading to its loss (Abassi, Higazi *et al.,* 2020). Consequently, the absence of ACE2 allows unopposed effects of Angiotensin II, resulting in vasoconstriction, endothelial injury, endovascular thrombosis, and increased blood volume (Calabrese, Annunziata *et al.,* 2021). Additionally, Angiotensin II, apart from being a potent vasoconstrictor, acts as a proatherogenic agent by increasing plasminogen activator inhibitor-1 (PAI-1) levels, which inhibits fibrinolysis (Frischmuth, Hindberg *et al.,* 2022).

The levels of ACE1 and ACE2 are tightly regulated by common genetic variants in their respective genes. Significant variations in the frequency of these gene variants have been observed across different sexes and races, with certain subgroups, such as males, individuals of Black ethnicity, and those with cardiovascular disease, being at higher risk of poor prognosis in COVID-19(Gemmati, Bramanti *et al.,* 2020). The ACE1 gene, located on chromosome 17 (locus 17q23.3), consists of 26 exons and plays a key role in converting Ang-I to Ang-II. Various gene variants influence ACE1 expression, resulting in varying levels in different populations (Gemmati, Bramanti *et al.,* 2020). The ACE1 gene exhibits an insertion/deletion polymorphism characterized by the presence of an insertion allele (I) or deletion allele (D) of a 287 base pair marker in intron 16, leading to three genotypes: DD and II homozygotes, and ID heterozygotes. The DD genotype exhibits the highest serum/tissue ACE1 activity, likely due to maintaining both active sites for Ang-I to Ang-II conversion. Conversely, the ID genotype shows intermediate levels, while the II genotype displays the lowest activity, potentially because it only possesses one active site in the ACE1 I-allele (Fiorentino, Benincasa *et al.,* 2023) A global genetic analysis revealed a decline in the frequency of the D-allele from regions with the highest prevalence in Africa and the Arab world, where it serves as an ancestral allele, to the lowest frequency in East Asia, with intermediate frequencies in Europe, Australia, and the Americas. The D-allele may have provided advantages to humans in hot and dry environments by aiding in salt and water retention (Gemmati, Bramanti *et al.,* 2020).

METHODOLOGY

This cross-sectional conducted during the second wave of COVID-19 in 2021 at a Mycetoma isolation center in Gezira state, Sudan. Patients' informed consent was obtained, and the Gezira State, Sudan, Ministry of Health's Scientific and Research Ethics Committee received ethical approval. The study population consisted of 161 patients. Eligible participants were individuals already diagnosed with SARS-CoV-2 infection and admitted to the isolation centers with typical COVID-19 symptoms of fever and cough. A volume of 5 mL of venous blood was drawn from each patient, with 2.5 mL collected in a K3EDTA container for DNA extraction and the remaining 2.5 mL collected in trisodium citrate tubes for coagulation studies. The anticoagulant was thoroughly mixed with the blood sample by repeatedly inverting the container. The EDTA blood samples were transported to the laboratory on crushed ice, while the trisodium citrate tubes were centrifuged to prepare platelet-poor plasma (PPP). Several coagulation parameters were assessed, including platelet count, prothrombin time (PT), activated partial thromboplastin time (APTT), and D-dimer levels. Platelet count and related parameters were measured using the Sysmex XP-300 automated analyzer, following the manufacturer's procedure. PT and APTT were determined using the Coatron M4c coagulometer, according to the manufacturer's instructions. D-dimer levels were measured using the I-Chroma TM method, as per the manufacturer's protocol. Venous blood samples collected in EDTA-containing test tubes were processed within two hours. Plasma was separated, and the pellets were frozen at -20°C. Genomic DNA was extracted from peripheral blood leukocytes using the G-spinTM total DNA Extraction Kit. Molecular Analysis of ACE Gen I/D Polymorphism (rs1799752): The presence of a 287-base pair (bp) Alu repeat sequence in intron 16 was analyzed to determine the D/I polymorphism of the ACE gene. Molecular analysis was conducted using established techniques and protocols.

The polymerase chain reaction was done using specific following primers:

Forward primer	5′-CTGGAGACCACTCCCATCCTTTCT- 3′
Reverse primer	5′-GATGTGGCCATCACATTCGTCAGAT-3′

PCR protocol

Components	Volume
ACEForward primer	0.5 µL
ACE Reverse primer	0.5 µL
Master Mix	4 µL
DNA	5 µL
D.W	10 µL
Total volume	20µL

PCR program

	Temperature	Time	No. of cycles
Initial denaturation	94^0C	1 min	
denaturation	94^0 C	45 sec	30 CYCLE
Annealing	62^0 C	1 min	
Extension	72^0 C	1 min	
Final extension	72^0C	5 min	

- PCR products was checked on 2 % agarose gel and was visualized by gel documentation system.
- Allele (I/D) types and genotype for each sample well be determined based on the PCR product sizes:

Allele	Size
Insertion (I allele)	(481 - 490) bp
Deletion (D allele)	(190 - 194) bp
Heterozygosity	490/190 bp

Genotypes: I/I = Insertion in homozygosis, I/D = Insertion/Deletion, and D/D = Deletion in homozygosis.

SPSS version 25.0 and SNPSTAT were used to conduct the statistical analyses. P value 0.05 indicated significance

RESULTS

The findings of this study involved 161 participants, consisting of both males (110, or 68.3%) and females (51, or 31.7%). Among the participants, the majority belonged to the elder patient group, comprising 83 individuals (51.6%) (Table 1). In Table 2, the results obtained from a conventional PCR test to

determine the frequencies of ACE I/D gene polymorphism in peripheral blood samples of SARS-CoV-2-infected patients are presented. It was observed that 91 patients (56.5%) had ACE D/D polymorphism, 57 patients (35.4%) had ACE D/I polymorphism, and 13 patients (8.1%) had ACE I/I polymorphism. Specifically, out of the total alleles, 148 (91.9%) were mutant alleles, while 13 (8.1%) were wild-type alleles.

The study found that the different allelic variations of the ACE gene in COVID-19 patients did not show variations across age groups and genders. Furthermore, there were no differences in community and household contact among the different allelic variations of ACE1 (Table 3). The coagulation parameter analysis revealed no statistically significant differences between the different ACE 1 genotypes, except for INR (P-value 0.048). The mean and standard deviation are presented in Table 4.

Table 1. Demographic characteristics of study population

Factors	Cases (N = 161)
Age group (years)	
65 years and Less	78 (48.4%)
More than 65 years	83 (51.6%)
Gender	
Male	110 (68.3%)
Female	51 (31.7%)
Gene polymorphism	
D/D	91 (56.5%)
I/D	57 (35.4%)
I/I	13 (8.1%)
Mutant	
Present	148 (91.9%)
Absent	13 (8.1%)
House hold contact	
Yes	57 (35.4%)
No	104 (64.6%)
Community contact	
Yes	63 (39.1%)
No	98 (60.9%)

Table 2. Frequency of ACE (I/D) gene polymorphism among study population

Factors	Cases (N = 161)
Gene polymorphism	
D/D	91 (56.5%)
I/D	57 (35.4%)
I/I	13 (8.1%)
Mutant	
Present	148 (91.9%)
Absent	13 (8.1%)

Table 3. Association between ACE (I/D) genotypes and risk factors

Polymorphism variables		D/D	I/D	I/I	Total	p- Value
Age groups	65 or less years	44 (56.4%)	28 (35.9%)	6 (7.7%)	78 (100%)	0.981
	More than 65 years	47 (56.6%)	29 (34.9%)	7 (8.4%)	83 (100%)	
	Total	91 (56.5%)	57 (35.4%)	13 (8.1%)	161 (100%)	
Gender	Male	63 (57.3%)	37 (33.6%)	109.1%)	110 (100%)	0.675
	Female	28 (54.9%)	20(39.2%)	3 (5.9%)	51 (100%)	
	Total	91 (56.5%)	57 (35.4%)	13 (8.1%)	161 (100%)	
House hold contact	No	56 53.8%)(	38 (36.5%)	10 (9.%)	104 (100%)	0.496
	Yes	35(61.4 %)	19 (33.3%)	3 (5.3%)	57 (100%)	
	Total	91 (56.5%)	57 (35.4%)	13 (8.1%)	161 (100%)	
Community exposure	No	54 (55.1%)	34 (34.7%)	10 (10.2)	98 (100%)	0.441
	Yes	37 58.7%)(	23 (36.5%)	3 (4.8%)	63 (100%)	
	Total	91 (56.5%)	57 (35.4%)	13 (8.1%)	161 (100%)	

Table 4. Association between ACE (I/D) polymorphism and coagulation parameter

		N	Mean	Std. Deviation	95% Confidence Interval for Mean Lower Bound	Upper Bound	P-value
PLT	D/D	91	296.13	97.329	275.86	316.40	0.634
	I/D	57	309.74	110.919	280.31	339.17	
	I/I	13	322.00	195.442	203.90	440.10	
	Total	161	303.04	112.124	285.59	320.49	
PCT	D/D	91	.2914	.14293	.2617	.3212	0.288
	I/D	57	.4721	1.11997	.1749	.7693	
	I/I	13	.3346	.18095	.2253	.4440	
	Total	161	.3589	.67835	.2533	.4645	
MPV	D/D	91	8.714	1.3934	8.424	9.004	0.868
	I/D	57	8.630	1.4410	8.247	9.012	
	I/I	13	8.838	1.0129	8.226	9.451	
	Total	161	8.694	1.3781	8.480	8.909	
PDW	D/D	91	17.008	2.6858	16.448	17.567	0.343
	I/D	57	16.628	2.1014	16.070	17.186	
	I/I	13	17.654	1.4316	16.789	18.519	
	Total	161	16.925	2.4157	16.549	17.301	
D.D	D/D	91	2216.80756	2498.087494	1696.55566	2737.05946	0.617
	I/D	57	1962.86989	2575.912744	1279.38824	2646.35154	
	I/I	13	1564.22692	1718.197002	525.93087	2602.52298	
	Total	161	2074.21132	2468.023598	1690.07814	2458.34450	
PT	D/D	91	19.40	8.605	17.60	21.19	0.092
	I/D	57	17.16	5.175	15.78	18.53	
	I/I	13	15.92	4.132	13.43	18.42	
	Total	161	18.32	7.342	17.18	19.47	
PTT	D/D	91	42.47	10.963	40.19	44.76	0.288
	I/D	57	40.37	8.243	38.18	42.56	
	I/I	13	39.00	6.416	35.12	42.88	
	Total	161	41.45	9.797	39.92	42.97	
INR	D/D	91	1.358	.6020	1.233	1.484	0.048
	I/D	57	1.177	.3674	1.080	1.275	
	I/I	13	1.092	.2871	.919	1.266	
	Total	161	1.273	.5171	1.192	1.353	
Fibrogen	D/D	57	223.449	111.7900	193.787	253.111	0.774
	I/D	31	240.484	103.0030	202.702	278.266	
	I/I	5	222.520	105.8614	91.076	353.964	
	Total	93	229.077	107.7942	206.877	251.277	

DISCUSSION

This study aimed to investigate the frequencies of ACE I/D gene polymorphism in SARS-CoV-2-infected patients and its association with demographic characteristics and coagulation parameters. The results indicated that the majority of the participants were males (68.3%) and the elder patients represented the predominant group (51.6%). These findings are consistent with previous studies that have reported a higher susceptibility of males and older individuals to severe COVID-19 outcomes (Lakbar, Luque-Paz *et al.,* 2020) (Farshbafnadi, Zonouzi *et al.,* 2021). The community exposure was main source of infection followed by household contact (39.1%) and (35.4%) respectively, notably the unknown source of infection represented (25.4%) of cases. Hypertension and D.M appear as the most common comorbid diseases associated with COVID-19 under cases of study (52.8%), followed by asthma, renal disorders and cardiac diseases (25%), (9.9%) and (506%) respectively this finding deal with (Zhu *et al.,* 2020). In Table 2, the distribution of ACE I/D gene polymorphism in the patient population was analyzed using a conventional PCR test, the majority of SARS-CoV-2-infected patients had ACE gene polymorphisms.

The results revealed that the most common genotype was ACE D/D, present in 56.5% of the SARS-CoV-2-infected patients. This was followed by ACE D/I (35.4%) and ACE I/I (8.1%) genotypes. These findings suggest that the D allele of the ACE gene may be associated with an increased risk of SARS-CoV-2 infection, while the I allele may confer some level of protection. This is in line with previous studies that have suggested an association between ACE I/D polymorphism and susceptibility to viral respiratory infections, including SARS-CoV-1 and MERS-CoV (Rezaiezadeh, Lord *et al.,* 2022) (Mir, Mir *et al.,* 2021). Moreover, the study found that the majority of the alleles were mutant alleles (91.9%), indicating a higher prevalence of the D allele in the study population. This observation is consistent with the known higher frequency of the D allele in various populations (Sarangarajan, Winn *et al.,* 2021). However, it is important to note that the distribution of ACE D genotypes /alleles and I can vary among different ethnic groups, and further studies with larger sample sizes and diverse populations are needed to confirm these findings. The study also explored the association between ACE I/D gene polymorphism and demographic characteristics. Interestingly, no significant differences were observed in the distribution of genotypes across different age groups and genders. This

suggests that ACE I/D polymorphism may not contribute to the variation in susceptibility to SARS-CoV-2 infection based on age or gender. These findings align with some previous studies that reported similar results (Sabater Molina, Nicolás Rocamora *et al.,* 2022; Fiorentino, Benincasa *et al.,* 2023). Additionally, there were no differences in community and home contact across all ACE1 genetic variations, suggesting that ACE gene polymorphisms may not be associated with differences in disease transmission. This study did not find significant differences between PLTs count and their parameters PCT, MPV and PDW (p-value: 0.634, 0.288, 0.868 and 0.343) respectively when compared among different ACE (I/D) genotypes, this finding was disagree with (Lapić, Radić Antolic *et al.,* 2022) the course duration dose and medication types may contributed with this finding and furtrue more the ACE (D/D) genotype associated with lower mean of plts count followed by (I/D) then (I/I) (296.13, 309.74 and 322) this finding suggest that the presence of the mutant (D) allele was indirectly proportional with plts count, so the (D) allele one of causes of thrombotic tendency among COVID-19 patients. The result of D-dimer in this study as expected more than upper limit of normal rang in all cases , this result was agree with (Fiorentino, Benincasa *et al.,* 2023) (Debi, Itu *et al.,* 2022). Interestingly, the ACE (I/D) polymorphism was not associated with significant difference between different genotypes (p-value 0.617) this may due to different cuases of thrombosis associated with COVID-19, over that the ACE (D/D) genotype was associated with higher mean of D-dimer followed by (I/D) then (I/I) (2216, 1962 and 1564) respectively, this support the hypothesis of which the mutant (D) allele associated with increased risk of thrombosis this finding agree with (Calabrese, Annunziata *et al.,* 2021)

The high D-dimer with (I/I) genotype explained by the fact of association between ACE 2 as receptor for Covid-19 infection and their indirectly proportional with plasma level of Ang II catalyzed by ACE 1 then lead to accumulation of their products which act as enhancer for pro-inflammation, pro-fibrosis and vasoconstriction and then thrombosis Furfure more COVID19 associated thrombosis by different acquired and hereditary causes. The study aimed to investigate the relationship between different genetic variations of ACE (I/D) polymorphism and various clotting parameters, such as PT (Prothrombin Time), APTT (Activated Partial Thromboplastin Time), INR (International Normalized Ratio), and plasma fibrinogen level. The findings indicated that there were no significant differences in PT and APTT among the different genetic variations of ACE (I/D) polymorphism. However, it was unexpectedly observed that individuals carrying the mutant D allele had higher PT and APTT compared to those with the wild-type allele. This unexpected association may be attributed to various factors that can influence the calculation of therapeutic dose for thrombosis. These factors include ethnicity, age, weight, and height, which might affect the individual response to anticoagulant therapy. Additionally, the study revealed a significant difference in the INR values among individuals with different (I/D) genotypes, with a p-value of 0.048. This suggests that the ACE (I/D) polymorphism may have an impact on the effectiveness or response to anticoagulant treatment, as reflected by the INR values. On the other hand, when examining the plasma fibrinogen levels, the study did not find any significant association or impact related to ACE (I/D) polymorphism in the Sudanese population. This implies that genetic variations in ACE (I/D) polymorphism may not play a significant role in determining plasma fibrinogen levels among this specific population. The study highlights the potential influence of ACE (I/D) polymorphism on clotting parameters, such as PT, APTT, and INR, indicating the need for further investigation into the underlying mechanisms and the implications for personalized thrombosis treatment. However, it suggests that ACE (I/D) polymorphism may not have a direct impact on plasma fibrinogen levels in the Sudanese population.

Conclusion

The study indicated no statistically significant differences in most coagulation parameters among the different ACE 1 genotypes, except for the international normalized ratio (INR) which showed a statistically significant difference. This finding suggests that the ACE I/D polymorphism may have a modest influence on the coagulation status of SARS-CoV-2-infected patients, specifically affecting INR values. However, further research is required to elucidate the underlying mechanisms and clinical implications of this association.

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REFERENCES

Abassi, Z., A. A. R. Higazi, *et al.,* (2020). "ACE2, COVID-19 infection, inflammation, and coagulopathy: missing pieces in the puzzle." *Frontiers in physiology* 11: 574753.

Calabrese, C., A. Annunziata, *et al.,* (2021). "ACE gene I/D polymorphism and acute pulmonary embolism in COVID19 pneumonia: A potential predisposing role." *Frontiers in medicine* 7: 631148.

Debi, H., Z. T. Itu, *et al.,* (2022). "Association of serum C-reactive protein (CRP) and D-dimer concentration on the severity of COVID-19 cases with or without diabetes: a systematic review and meta-analysis." *Expert Review of Endocrinology & Metabolism* 17(1): 83-93.

Farshbafnadi, M., S. K. Zonouzi, *et al.,* (2021). "Aging & COVID-19 susceptibility, disease severity, and clinical outcomes: The role of entangled risk factors." *Experimental gerontology* 154: 111507.

Fiorentino, G., G. Benincasa, *et al.,* (2023). "Targeted genetic analysis unveils novel associations between ACE I/D and APO T158C polymorphisms with D-dimer levels in severe COVID-19 patients with pulmonary embolism." *Journal of Thrombosis and Thrombolysis* 55(1): 51-59.

Frischmuth, T., K. Hindberg, *et al.,* (2022). "Elevated plasma levels of plasminogen activator inhibitor-1 are associated with risk of future incident venous thromboembolism." *Journal of Thrombosis and Haemostasis* 20(7): 1618-1626.

Gemmati, D., B. Bramanti, *et al.,* (2020). "COVID-19 and individual genetic susceptibility/receptivity: role of ACE1/ACE2 genes, immunity, inflammation and coagulation. Might the double X-chromosome in females be protective against SARS-CoV-2 compared to the single X-chromosome in males?" *International journal of molecular sciences* 21(10): 3474.

Karakaş Çelik, S., G. Çakmak Genç, *et al.,* (2021). "Polymorphisms of ACE (I/D) and ACE2 receptor gene

(Rs2106809, Rs2285666) are not related to the clinical course of COVID-19: a case study." *Journal of Medical Virology* 93(10): 5947-5952.

Lakbar, I., D. Luque-Paz, *et al.,* (2020). "COVID-19 gender susceptibility and outcomes: A systematic review." *PLoS One* 15(11): e0241827.

Lapić, I., M. Radić Antolic, *et al.,* (2022). "Association of polymorphisms in genes encoding prothrombotic and cardiovascular risk factors with disease severity in COVID-19 patients: A pilot study." *Journal of Medical Virology* 94(8): 3669-3675.

McFadyen, J. D., H. Stevens, *et al.,* (2020). "The emerging threat of (micro) thrombosis in COVID-19 and its therapeutic implications." *Circulation research* 127(4): 571-587.

Ministry of Health, S. (August 2021). "COVID-19 monthly epidemiological update."

Mir, M. M., R. Mir, *et al.,* (2021). "Strong association of angiotensin converting enzyme-2 gene insertion/deletion polymorphism with susceptibility to SARS-CoV-2, hypertension, coronary artery disease and COVID-19 disease mortality." *Journal of personalized medicine* 11(11): 1098.

Rezaiezadeh, J. S., J. S. Lord, *et al.,* (2022). "The association of ACE I/D polymorphism with the severity of COVID-19 in Iranian patients: A case-control study." *Human Gene* 34: 201099.

Sabater Molina, M., E. Nicolás Rocamora, *et al.,* (2022). "Polymorphisms in ACE, ACE2, AGTR1 genes and severity of COVID-19 disease." *PLoS One* 17(2): e0263140.

Sarangarajan, R., R. Winn, *et al.,* (2021). "Ethnic prevalence of angiotensin-converting enzyme deletion (D) polymorphism and COVID-19 risk: rationale for use of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers." *Journal of Racial and Ethnic Health Disparities* 8: 973-980.

Singh, H., R. Choudhari, *et al.,* (2021). "ACE2 and TMPRSS2 polymorphisms in various diseases with special reference to its impact on COVID-19 disease." *Microbial pathogenesis* 150: 104621.

Verma, S., M. Abbas, *et al.,* (2021). "Impact of I/D polymorphism of angiotensin-converting enzyme 1 (ACE1) gene on the severity of COVID-19 patients." *Infection, Genetics and Evolution* 91: 104801.

WHO (2021). "COVID-19 weekly epidemiological update, 22 December 2021."

Xu, X., J. Sun, *et al.,* (2020). "Seroprevalence of immunoglobulin M and G antibodies against SARS-CoV-2 in China." *Nature medicine* 26(8): 1193-1195.

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