ORIGINAL RESEARCH

DOI: 10.5336/jcog.2023-96185

Host Genetic Polymorphisms and Disease Severity in Pregnant Women with COVID-19 in Türkiye

Seher POLAT[a], Sevil KİREMİTLİ[b], Tunay KİREMİTLİ[b], Ahmet KIRKINCI[b], Fatma Zehra KURNUÇ[b], Yusuf Kemal ARSLAN[c]

[a]Erzincan Binali Yıldırım University Faculty of Medicine, Department of Medical Genetics, Erzincan, Türkiye
[b]Erzincan Binali Yıldırım University Faculty of Medicine, Mengücek Gazi Training and Research Hospital, Department of Obstetrics and Gynecology, Erzincan, Türkiye
[c]Çukurova University Faculty of Medicine, Department of Biostatistics, Adana, Türkiye

ABSTRACT Objective: The study aimed to analyze the association between coronavirus disease-2019 (COVID-19) disease severity and genetic susceptibility in pregnant women. **Material and Methods:** The research included 54 pregnant women with confirmed COVID-19 diagnosis. All volunteers were evaluated physically and biochemically. Angiotensin-converting enzyme (ACE)2 (p.T27A A>G, p.G326E G>A, p.K419T A>C, ACE (p.T776T A>G, and g.16471_16472delinsALU (I/D), AGTR1 c.*86A>C, methylenetetrahydrofolate-reductase (*MTHFR*) p.A222V C>T and *PAI-1*-844 G>A were analyzed. **Results:** The allele frequency was also compared with control groups of the different studies made on Turkish women. *MTHFR* "CT" genotype compared to "CC" had lower platelet counts (p=0.015). In ACE "ID" genotype, there was a lower D-dimer level compared to "DD" genotype (p=0.02). In *PAI-1*–844G>A, the AA vs. AG+GG genotype and AA vs. GG genotype elevate the risk of hospitalization 6.4-fold (OR: 6.4 95% (Cl): 1.6-25.8 p=0.009), and 4.6-fold (OR: 4.6 95% CI:1.0-21.6 p=0.049), respectively. In *MTHFR* p.A222V, to have CC vs. CT genotype increased the risk of enoxaparin and antibiotic use 4.1-fold and 3.2-fold at the borderline significance (OR: 4.1 95% Cl: 0.99-16.9 p=0.052 and OR: 3.2 95% Cl: 0.98-10.5 p=0.053), respectively. An allele frequency difference wasn't found between the patient and the healthy women related to the investigated polymorphisms. **Conclusion:** *PAI-1*-844G>A, *MTHFR* p.A222V, and ACE (I/D) associated with a poor COVID-19 outcome, the risk of enoxaparin and antibiotic use, and also increased risk of hospitalization. Allele frequencies of the genes were not different between healthy control women and women with COVID-19; genetic variation may not influence the risk of infection but disease severity.

Keywords: COVID-19, pregnant women, genetic susceptibility, gene polymorphism

The coronavirus disease-2019 (COVID-19) is a pandemic caused by a single-stranded RNA virus belonging to the family of coronavirus. Over 192 million people were infected with COVID-19 and almost 2% of them died according to the World Health Organization COVID-19 dashboard and the virus continues to spread. COVID-19 primarily appears as lung disease and shows flu-like symptoms including cough, fever, fatigue, shortness of breath, and headache. Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection can be asymptomatic or symptoms may range from pneumonia and respiratory failure. Various factors have been identified to elevate COVID-19 disease severity, such as age, male gender, and comorbidities including hypertension, cardiovascular disease, diabetes mellitus, and genetic susceptibility.[1]

Pregnancy increases the risk of thromboembolic complications due to boost of clotting factors in the blood. COVID-19 can advance hypercoagulability risk in pregnant women further and create a higher risk for thromboembolism.[2] Variants on methylenete-

TO CITE THIS ARTICLE:
Polat S, Kiremitli S, Kiremitli T, Kırkıncı A, Kurnuç FZ, Arslan YK. Host genetic polymorphisms and disease severity in pregnant women with COVID-19 in Türkiye. JCOG. 2024;34(1):1-9.

Correspondence: Seher POLAT
Erzincan Binali Yıldırım University Faculty of Medicine, Department of Medical Genetics, Erzincan, Türkiye
E-mail: spolat@erzincan.edu.tr

Peer review under responsibility of Journal of Clinical Obstetrics & Gynecology.

Received: 17 Feb 2023 ***Accepted:*** 26 Mar 2024 ***Available online:*** 28 Mar 2024

trahydrofolate reductase (*MTHFR*) gene dysregulate homocysteine (Hcy) metabolism and stimulates hyperhomocysteinemia (HHcy). The increase in Hcy level in *MTHFR* mutant patients causes an increase in cytokine activity, vascular endothelial damage accompanied by lipid peroxidation, prothrombotic process, atherothrombogenesis, thromboembolism, hypercoagulation, systemic vascular occlusive diseases and plays a role as a risk factor for vascular stiffness.[3] Plasminogen activator inhibitor-1 (*PAI-1*) is the main regulator of the fibrinolytic system involved in normal blood coagulation and its elevation is associated with impaired fibrinolytic system.[4] Angiotensin-converting enzyme II (*ACE2*), *ACE* I, and angiotensin II receptor Type 1 (*AGTR1* or *AT1R*) are recently conspicuous candidate genes. Single-nucleotide polymorphisms located on them may alter binding or entry of SARS-CoV-2.[5] The expression patterns of the *ACE2* receptor may modulate the cellular entrance of SARS-CoV-2 in most of the host tissues as well.[6] p.T27A and p.K419T predicted to increase susceptibility while p.K419T and p.G326E putative protective variant predicted to show decreased binding to SARS-CoV-2 S-protein.[5,7.]

There is insufficient data on its impact on vulnerable populations to COVID-19, such as pregnant women. The study aims to analyze an association of COVID-19 disease severity and genetic susceptibility by examining DNA polymorphisms of *ACE2* (p.T27A A>G (rs781255386), p.G326E G>A (rs759579097), p.K419T A>C (rs1209307377), *ACE* (p.T776T A>G (rs4343), and g.16471_16472delinsALU (also referred to as I/D polymorphism; rs1799752) *AGTR1* c.*86A>C (also referred to as A1166C; rs5186), *MTHFR* p.A222V C>T (also referred to as C677T; rs1801133) and *PAI-1* -844 G>A (rs2227631) and to analyze the relationship of the variants with biochemical and clinical outcomes.

MATERIAL AND METHODS

PATIENTS COHORT AND DIAGNOSTIC CRITERIA

Fifty-four pregnant women with COVID-19 aged between 19 and 38 years were enrolled in the study. All the volunteers had COVID-19 diagnosis confirmed by qRT-PCR using nasopharyngeal or oropharyngeal swabs. The volunteers applied to Erzincan Binali Yıldırım University Mengücek Gazi Research and Training Hospital between July 2020 and June 2021 with complaints of fever and/or weakness and/or joint pain and/or headache and/or shortness of breath and/or cough and/or nausea and/or vomiting. The study group was formed from women who were between five and forty weeks of pregnancy. Physical examination and biochemical evaluation were done to all volunteers. Clinical and laboratory information was gathered from the patients on the first day of consultation at the hospital. When genetic variants were examined, three different genotypes were obtained as wild type homozygous, heterozygous, and mutant homozygous. Within the scope of the study, the differences in clinical findings in the three genotypes were examined. Therefore, in the sample size calculation, large effect size was selected from the effect size conventions recommended by Cohen (d=0.5), and the sample size calculated in the three genotypes with 80% power was found to be 42 people. The number of people to be included in the study was determined as 54 with an increase of approximately 25% due to withdrawal from the study or difficulty in patient follow-up. The research was applied according to the Declaration of Helsinki and approval was obtained from the Ethics Committee of the Medical School at Erzincan Binali Yıldırım University (date: August 3, 2021, no: 94971). All volunteers gave their informed consents.

BIOCHEMICAL ANALYSES

Lymphocyte, platelet, hemoglobin and white blood cell (WBC) count were performed with Sysmex XN-1000 Hematology System (Sysmex Corporation, Kobe, Japan). Aspartate transaminase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), glucose, creatinine measurements were done from blood serum by using the spectrophotometric method using the Beckman Coulter Olympus AU2700 Plus Chemistry Analyzer (Beckman Coulter, Tokyo, Japan). An immunoassay of chemiluminescence (Centaur XP, Siemens Healthcare, Germany) was used for ferritin assessment. D-dimer and C-reactive protein (CRP) were assessed by using the AQT90 flex Radiometer® (Bronshoj, Denmark) and the nephelomet-

ric method on BN™ II System (Siemens, Munich, Germany), respectively. Peripheral capillary oxygen saturation (SpO_2) was measured using the ABL800 FLEX blood gas analyzer (Bronshoj, Denmark). Reference ranges of the lymphocyte, platelet, hemoglobin, D-dimer, glucose, BUN, creatinine, SpO_2, CRP, AST, ALT, and WBC were 1-5×10^9/L, 150-450×10^9/L, 13.5-17.5 g/L, 0-500 ng/dL, 60-100 ng/dL 17-43 ng/dL, 0.84-1.25 ng/dL, 75-99 mm/Hg, 0-5 mg/L, 0-50 U/L, 0-50 U/L and 4.49-12.68×10^9/L, respectively.

GENETIC ANALYSES

Genotype analyses were performed to all patients. Genomic DNA isolation kit (Roche, Germany) was used for DNA extraction from peripheral blood leukocytes. The variants (*ACE* p.T776T A>G (rs4343), *AGTR1* c.*86A>C (rs5186), *MTHFR* p.A222V C>T (rs1801133), and *PAI-1* -844 G>A (rs2227631) were analyzed by using polymerase chain reaction (PCR) and restriction fragment length polymorphism method, and *ACE* g.16471_16472delinsALU (rs1799752) polymorphism was analysed by PCR as previously described.[8-12] *ACE2* p.K419T A>C (rs1209307377), *ACE2* p.T27A A>G (rs781255386), and *ACE2* p.G326E G>A (rs759579097) were amplified using a forward and reverse primer GAGGTGGGTACTCAAGATT CACTG-CCAGTTACCCATAAATACCTCAT-ACC, GGCCATAAAGTGACAGGAGAG-GACT CCAAAATCAGGGATATGG, and CCAACTTA TTCATCCTGTTTGAG-GTTAAACTTCAGCCT-GCCTCTG. PCR products were digested with DraI, StyI, and EcoRI, respectively.

Dream Taq DNA polymerase (Thermo Fisher Scientific Inc., USA) was used to amplify the genomic regions of rs781255386, rs759579097, rs1209307377, rs4343, rs5186, rs1799752, and rs2227631 polymorphisms. The genomic region of rs1801133 variant was amplified by Takara LA Taq DNA polymerase (Takara, Japan). All the reactions were performed based on manufacturer guidelines. 2.5% agarose gels were used to separate digested PCR products and those were visualized by staining a red-safe stain (Intronbio, Korea). PCR product of the rs1801133 variant was loaded the agarose gel directly for visualization. PCR products were digested based on manufacturer guidelines (New England BioLabs, Inc., UK).

STATISTICAL ANALYSES

Results were shown as mean (standard deviation; SD), and median (minimum-maximum) for the variables which were continuous, and as n (%) for categorical variables. The normality of variables was analyzed by using the Kolmogorov-Smirnov test. The Mann-Whitney U test was used for the variables not normally distributed. For three or more groups. One-way ANOVA or Kruskal-Wallis test was used according to normality. As post-hoc, Bonferroni or Dunn test was used for pairwise comparisons. Genotype numbers were used to calculate genotypes and allele frequencies of the study groups. The frequency of allele was compared with other studies using chi-square test. Logistic regression analysis was used while calculating odds ratios (OR) and confidence intervals (95% CI) for testing the relative risk associated with risk allele for PCOS directly from the model. The *p* value lower than 0.05 was applied as significant for all analyses. The statistical analyses were applied using the IBM SPSS 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp).

RESULTS

CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF THE STUDY GROUP

The study group was in three different pregnancy trimesters, 16 of them were in 1st trimester, 23 in 2nd trimesters, and 15 in 3rd trimester. The pregnancies were resulted in 9.3% (n=5) abortions, 27.8% (n=15) vaginal, and 29.6% (n=16) caesarean deliveries. Of the women, 33.3% (n=18) were pregnant at the time of data collection. All the vaginal and caesarean deliveries were resulted in live births in the study group. Three patients 5.5% (n=3) were treated in intensive care unit, one out of them had asthma as a chronic disease. All three pregnancies resulted in live birth.

Biochemical data of the study group was given in Table 1. While the CRP and D-dimer levels of the patients were found to be high in the study group, lymphocyte, platelet, hemoglobin, WBC, glucose,

TABLE 1: Biochemical data and its distribution according to genotype in women with COVID-19.

		PAI-1 -844A>G (rs2227631)			MTHFR C677T (rs1801133)			ACE I/D (rs1799752)			AGTR1 A1160C (rs5186)			ACE p.T776T A>G (rs4343)		
	COVID-19 patients (n=54)	AA	AG	GG	CC	CT	TT	DD	ID	II	AA	AC	CC+	AA+	AG	GG
Age	26.0 (4.1)	25.1 (4.2)	26.4 (4.3)	26.8 (3.6)	26.2 (4.6)	25.5 (3.7)	-	27.1 (4.4)	25.2 (3.9)	25.6 (3.9)	25.6 (3.89)	26.7 (4.8)	25.0	24.0	25.6 (3.2)	26.2 (4.4)
Height	163.9 (4.6)	164.7 (5.5)	163.2 (3.8)	163.8 (4.3)	163.8 (4.6)	164.0 (4.9)	-	164.1 (4.5)	163.8 (4.7)	163.7 (4.8)	164.1 (3.9)	163.8 (5.6)	160.0	165.0	164.1 (6.5)	163.8 (4.1)
Weight	71.3 (10.9)	67.8 (17.1)	70.9 (13.7)	72.3 (9.6)	68.7 (16.3)	72.8 (10.8)	-	72.8 (10.8)	67.2 (16.3)	71.3 (15)	71.4 (10.9)	68.4 (18.8)	61.0	74.0	67.4 (24.7)	70.7 (10.8)
Lymphocyte	1.7 (1.6)	2.1 (2.5)	1.6 (0.5)	1.3 (0.5)	1.7 (0.8)	1.8 (2.4)	-	1.5 (0.9)	1.9 (2.2)	1.5 (0.6)	1.8 (1.8)	1.5 (0.9)	1.02	0.85	1.4 (0.6)	1.8 (1.7)
Platelet	191.6 (75.5)	178.1 (61.6)	200.1 (59)	198.3 (117.3)	214.8 (83.7)	162.0 (52.4)*	-	202.9 (97.9)	192.2 (52)	166.3 (68.1)	191.6 (88.4)	188.1 (47.2)	256.0	121.0	164.9 (63.9)	199.4 (77.3)
Haemoglobin	12.2 (1.5)	12.3 (1.9)	12.4 (1)	11.7 (1.4)	12.3 (1.7)	11.9 (1.1)	-	12.0 (1.3)	12.1 (1)	12.8 (2.5)	12.4 (1.7)	11.8 (1)	11.7	13.6	12.9 (2.5)	12.0 (1.1)
D-dimer	815 (179-4317)	891.0 (183-3258)	640.0 (179-2560)	989.0 (356-4317)	812.0 (179-4317)	708.5 (183-3258)	-	1072.0 (183-4317)	529.0 (179-2560)*	707.0 (406-3258)	813.5 (179-4317)	1019.3 (421-2360)	213.0 (328-2156)	2560.0	602.0	852.0 (179-4317)
Glucose	100.7 (14.3)	98.3 (12.7)	100.1 (16.4)	105.8 (13)	98.6 (14.6)	104.5 (14.1)	-	100.6 (14.6)	101.3 (15.5)	99.9 (12.7)	103.0 (14.8)	96.2 (13)	101.0	103.0	97.3 (14.4)	101.5 (14.6)
BUN	22.4 (9.2)	23.6 (11.2)	21.6 (6.8)	21.9 (9.7)	21.4 (8.9)	24.7 (9.7)	-	24.8 (9.9)	20.3 (7.4)	22.7 (11)	21.0 (8.7)	25.5 (10.2)	21.0	12.0	23.3 (11.4)	22.5 (8.7)
Creatinine	0.7 (0.23-1.60)	0.6 (0.23-1.3)	0.7 (0.38-1.6)	0.8 (0.36-1.2)	0.6 (0.23-1.6)	0.9 (0.5-1.2)	-	0.7 (0.43-1.6)	0.7 (0.23-1.3)	0.77 (0.4-1.3)	0.7 (0.38-1.6)	0.63 (0.23-1.4)	1.1	0.7	0.6 (0.4-1.3)	0.7 (0.23-1.6)
SpO_2	94.6 (3.4)	94.5 (5.1)	95.6 (1.7)	93.2 (2.4)	94.6 (4)	94.5 (2.5)	-	95.1 (2.4)	94.9 (2.1)	92.9 (6.2)	93.8 (3.9)	96.0 (1.5)	97.0	93.0	91.1 (6.3)	95.3 (2.2)
CRP	12.7 (3-123)	12.3 (5-123)	12.0 (3-102)	18.7 (3-115)	12.3 (3-123)	13.6 (5-102)	-	15.5 (4-115)	12.0 (3-102)	15.9 (3-123)	13.2 (3-123)	13.0 (3-62)	11.0	12.0	15.1 (5-123)	12.0 (3-115)
AST	30.5 (15.4)	30.0 (16.7)	29.5 (13.5)	32.9 (17.2)	28.4 (15.7)	35.1 (14)	-	29.8 (13.7)	29.2 (16.6)	34.6 (16.7)	30.7 (16.5)	30.6 (13.9)	21.0	41.0	35.4 (16.6)	29.0 (15.1)
ALT	28.4 (14.3)	28.0 (17.7)	26.6 (11.4)	32.2 (13.2)	28.5 (15.7)	29.8 (12.5)	-	27.6 (13.6)	27.6 (10.7)	31.6 (22)	30.0 (15.3)	25.8 (12.8)	26.0	43.0	34.8 (20.4)	26.5 (12.1)
WBC	9.8 (4.3)	9.3 (4.8)	10.3 (3.6)	9.8 (4.9)	9.8 (4.4)	10.1 (4.4)	-	11.6 (4.6)	8.9 (4.1)	8.2 (2.8)	9.9 (4.2)	9.6 (4.7)	8.5	11.2	8.2 (2.9)	10.2(4.6)

+ determined only in one individual, therefore could not include in the statistical analysis. *MTHFR* "TT" genotype was not determined in the complete study group. *Shows significant differences; BUN: Blood urea nitrogen; CRP: C-reactive protein; ALT: Alanine transaminase; AST: Aspartate transaminase; SpO_2: Peripheral capillary oxygen saturation; WBC: White blood cell; ACE: Angiotensin-converting enzyme; AGTR1: Angiotensin II receptor type 1; MTHFR: Methylenetetrahydrofolate reductase; *PAI-1*: Plasminogen activator inhibitor 1.

creatinine, BUN, SpO_2 AST, and ALT values were determined within the reference range (Table 1). CRP levels were found to be high in hospitalized patients (30.8 mg/L vs. 11.5 mg/L, p<0.001). The D-dimer rate was found to be high in those admitted to the intensive care unit (3236.5 ng/dL vs. 812.0 ng/dL p=0.015).

DISTRIBUTION OF THE GENOTYPE AND ALLELE FREQUENCY

The genotype and allele frequency distribution of the genes were calculated from genotype numbers and were given in Table 2. All the volunteers had homozygous wild-type genotypes AA, AA, and GG for rs1209307377, rs781255386, and rs759579097 polymorphisms, respectively.

AA, AG, and GG genotype frequencies of *PAI-1* (-844 G>A) were 37.04% (20), 40.74% (22), 22.22% (12); CC, CT, and TT genotype frequencies of *MTHFR* (C677T) were 60.8% (31), 39.2% (20), and 0% (0); DD, ID, and II genotype frequencies of *ACE* (I/D) were 38.9% (21), 42.6% (23) and 18.5% (10); AA, AC and CC genotype frequencies of *AGTR1* (A1166C) were 63% (34), 35.2% (19), and 1.8% (1); GG, AG, and AA genotype frequencies of *ACE* (p.T776T A>G) were 79.6% (43), 18.5% (10), and 1.9% (1), respectively.

The frequencies A and G alleles of *PAI-1* (-844 G>A) were 57.4% (A: 0.57/62) and 43.6% (G: 0.43/46); C and T allele frequencies of *MTHFR* (C677T) were 80.4% (C: 0.8/82) and 19.6% (T: 0.2/20); D and I allele frequencies of *ACE* (I/D) were 60.2% (D: 0.6/65) and 39.8% (I: 0.4/43); A and C allele frequencies of *AGTR1* (A1166C) were 80.6 % (A: 0.81/87) and 19.4% (C: 0.19/21); G and A alleles frequencies of *ACE* (p. T776T A>G) were 89.9% (G: 0.9/96) and 11.1% (A: 0.1/12). *MTHFR* C677T polymorphism was not analyzed in three patients.

ALLELE FREQUENCY COMPARISON WITH TURKISH CONTROL WOMEN OF DIFFERENT STUDIES

Data on the frequency of alleles regarding the different studies are presented in Table 2. *PAI-1*-844 G>A, *MTHFR* C677T, *AGTR1* A1160C, and *ACE* I/D poly-

TABLE 2: Genotypes and alleles frequencies of the genes in the study and allele frequency comparison between different studies in Turkish women.

	Genotype	The genotype frequency %(n)	The allele frequency %(n)	References	Clinical phenotype	*PAI-1* (-844 G>A)	*MTHFR* (C677T)	*ACE* (I/D)	*AGTR1* (A1166C)
PAI-1 (-844 G>A)	AA	37 (20)	A: 0.574 (62)	Current study	COVID-19 (n=54)	A 0.57 (62)	C: 0.80 (82)	D: 0.60 (65)	A: 0.81 (87)
(rs2227631)						G: 0.43 (46)	T: 0.20 (20)	I: 0.40 (43)	C: 0.19 (21)
	AG	40.7 (22)	G: 0.426 (46)	Polat and Şimşek,[16]	HC (n=104)	A: 0.52(110)	-	-	-
						G: 0.48(98)			
	GG	22.2 (12)		p value		0.44	-	-	-
MTHFR (C677T)	CC	60.8 (31)	C: 0.804 (82)	Polat and Şimşek,[12]	HC (n=110)	-	C: 0.74(164)	-	-
(rs1801133)							T: 0.26(56)		
	CT	39.2 (20)	T: 0.196 (20)	p value		-	0.25	-	-
	TT	0		Karadeniz et al.[24]	HC (n=70)	-	C: 0.70(98)	-	-
							T: 0.30(42)		
ACE (I/D)	DD	38.9 (21)	D: 0.602 (65)	p value		-	0.067	-	-
(rs1799752)	ID	42.6 (23)	I: 0.398 (43)	İnanir et al,[28]	HC (n=210)	-		D: 0.60 (251)	-
								I: 0.40 (169)	
	II	18.5 (10)		p value		-		0.93	-
AGTR1 (A1166C)	AA	63 (34)	A: 0.806 (87)	Bayram et al.[29]	HC (n=100)	-	-	D: 0.52 (104)	-
(rs5186)								I: 0.49 (96)	
	AC	35.2 (19)	C: 0.194 (21)	p value		-	-	0.16	-
	CC	1.9 (1)		Alkanli et al,[30]	HC (n=75)	-	-	D: 0.64(96)	A: 0.83(124)
								I: 0.36 (54)	C: 0.17 (26)
ACE (p.T776T A>G)	GG	79.6 (43)	G: 0.899 (96)	p value				0.53	0.66
(rs4343)	AG	18.5 (10)	A: 0.111 (12)						
	AA	1.9 (1)							
ACE2 (p.K419T A>C)	AA	100 (54)	A: 1 (108)						
(rs1209307377)	AC	-	C: 0 (0)						
	CC	-							
ACE2 (p.T27A A>G)	AA	100 (54)	A: 1 (108)						
(rs781255386)	AG	-	G: 0(0)						
	GG	-							
ACE2 (p.G326E G>A)	GG	100 (54)	G: 1 (108)						
(rs759579097)	GA	-	A: 0 (0)						
	AA	-							

Results are presented as % (n). All the p value was calculated compared to the current study using the chi-square test. The other polymorphisms could not include the analyses since there is no valid data. ACE: Angiotensin-converting enzyme; ACE2: Angiotensin-converting enzyme II; AGTR1: Angiotensin II receptor type 1; *MTHFR*: Methylenetetrahydrofolate reductase; *PAI-1*:Plasminogen activator inhibitor 1; HC: Healthy control. There was no related data to compare for ACE2 polymorphisms.

morphisms were included in the analysis since there is no valid data in the literature related to the other polymorphisms. Any significant difference was not found between the control women and women with COVID-19 regarding allele frequencies of the genes (p>0.05).

CLINICAL AND BIOCHEMICAL OUTCOMES IN DIFFERENT rs2227631, rs1801133, rs1799752, rs5186, AND rs4343 GENOTYPES AND RISK ANALYSES

Biochemical and clinical outcomes in different rs2227631, rs1801133, rs1799752, rs5186, and rs4343 genotypes were given in Table 1, and Table 3. In our study group, the percentage of *PAI-1* "GG" genotype frequency in hospitalized patients was higher than in non-hospitalized patients (66.7% vs. 33.3% p=0.015), the percentage of *PAI-1* "GG" genotype frequency was found to be higher in enoxaparin users compared to non-users (91.7% vs. 8.3% p=0.044), and the percentage of *PAI-1* "GG" genotype frequency was higher in antibiotic users than non-users (75% vs. 25% p=0.001) (Table 3). It was determined that AA+AG vs. GG genotype increased the risk of antibiotic use 4.7-fold (OR: 4.68 95% CI:

TABLE 3: Clinical outcomes in different rs2227631, rs1801133, rs1799752, rs5186, and rs4343 genotypes of the women with COVID-19.

		Intensive care			Hospitalisation			Enoxaparin			Antibiotic		
	Genotype	No	Yes	*p value*	No	Yes	*p value*	No	Yes	*p value*	No	Yes	*p value*
PAI-1	AA	19	1	0.893	14	6	0.015*	6	14	0.044*	7	12	0.001*
(-844 G>A)	AG	21	1		18	4		11	11		18	4	
(rs2227631)	GG	11	1		4	8		1	11		3	9	
MTHFR (C677T)	CC	28	3	0.152	22	9	0.244	13	18	0.043*	19	11	0.049*
(rs1801133)	CT	20	0		11	9		3	17		7	13	
	TT	0	0		0	0		0	0		-	-	
ACE (I/D)	DD	20	1	0.792	14	7	0.866	7	14	0.408	11	9	0.662
(rs1799752)	ID	22	1		16	7		6	17		13	10	
	II	9	1		6	4		5	5		4	6	
AGTR1 (A1166C)	AA	32	2	0.966	21	13	0.525	11	23	0.360	21	13	0.094
(rs5186)	AC	18	1		14	5		6	13		6	12	
	CC	1	0		1	0		1	0		1	1	
ACE	AA	1	0	0.087	0	1	0.303	0	1	0.742	1	0	0.561
(p.T776T A>G)	AG	8	2		6	4		3	7		4	5	
(rs4343)	GG	42	1		30	13		15	28		23	20	

* Shows significant differences. Results were presented as "n". ACE: Angiotensin-converting enzyme; AGTR1: Angiotensin II receptor type 1; *MTHFR*: Methylenetetrahydrofolate reductase; *PAI-1*: Plasminogen activator inhibitor 1.

1.1-19.9 p=0.037). The AA vs. AG+GG genotype and AA vs. GG genotype increased the risk of hospitalization 6.4-fold (OR: 6.4 95% Cl: 1.6-25.8 p=0.009) and 4.6-fold (OR: 4.6 95% CI: 1.0-21.6 p=0.049), respectively.

MTHFR "CT" genotype frequency was higher in enoxaparin (85% vs. 15% p=0.049) and antibiotic users (65% vs. 35% p=0.043) (Table 3). CC vs. CT genotype increased the risk of enoxaparin and antibiotic use 4.1-fold and 3.2-fold at borderline significance (OR: 4.1 95% Cl: 0.99-16.9 p=0.052 and OR: 3.2 95% Cl: 0.98-10.5 p=0.053), respectively. The "CT" genotype compared to "CC" showed significantly lower platelet counts [162.0×10^9/L (±52.4) vs. 214.8×10^9/L (±83.7) p=0.015]. *ACE* "ID" genotype showed lower D-dimer compare to "DD" genotype [529.0 ng/dL (179.0-2560.0) vs. 1072.0 ng/dL (183.0-4317.0) p=0.02] (Table 1).

DISCUSSION

Even though genetic polymorphism is considered associated with COVID-19, there is no case cohort study in pregnant women with COVID-19 disease at genetic level up to our knowledge. Therefore, in this study, the association between *PAI-1, ACE2, ACE (I/D), MTHFR* polymorphisms with clinical and biochemical parameters were examined in pregnant COVID-19 female patients in Turkish cohort.

In the meta-analyses, Dubey et al. found that 27% of pregnant women with COVID-19 showed pregnancy complications like fetal vascular malperfusion, preterm birth, and premature fetal membrane rupture.[13] Zambrano et al. suggested that pregnant women with COVID-19 might be more susceptible to suffer from severe illness than non-pregnant women with an increase in the admission rate to the intensive care unit, ventilation, need for oxygen supplementation and mortality.[14] In our study group, only 3 (5.5%) patients received intensive care unit treatment, and only one out of them had asthma as a chronic disease.

SERPINE1 (PAI-1) gene variants lead to complete PAI-1 deficiency. The *SERPINE1* encodes a protein known *PAI-1* that functions in normal blood clotting and a potent regulator of fibrinolysis.[4] The *PAI-1* gene consists of 9 exons and locates on chromosome 7 (7q21.3-22). *PAI-1* may play significant roles in pathological vascular remodeling and vascular homeostasis. Although there was no statistical relation between *PAI-1* genotypes and biochemical data, the rates of hospitalization, enoxaparin, and an-

tibiotic use were found higher than women with *PAI-1* "GG" genotypes in the current study. Zuo et al. found elevated *PAI-1* levels in hospitalized patients with COVID-19.[15] In our study, "GG" genotype was found related to an increasing risk of hospitalization; it is likely that increased PAI-I level may be associated with "GG" genotype. There was no significant allele frequency difference between Turkish healthy women and women with COVID-19 for the *PAI-1* -844 G>A.[16]

MTHFR gene variant, C677T polymorphism causes the substitution of alanine (A) to valine (V) at codon 222, results in 30% and 70% decline in the enzyme activity in heterozygous and homozygous genotypes, respectively. Since its discovery in 1932, Hcy has gained attention. Significantly increased occurrence of vascular damage in both large and small vessels was found in case of high plasma level of Hcy.[17] Neurotoxic, neurodegenerative, neuroinflammatory, prooxidative prothrombotic, and proatherogenic effects have been caused by HHcy.[18] *MTHFR* C677T polymorphism has capacity to modulate the severity and incidence of COVID-19 infection. In our study groups, "CT" genotype was determined at a high rate in enoxaparin users. Similar to our study, Ponti et al. found that defective Hcy metabolism caused by the *MTHFR* gene polymorphisms were implicated in the COVID-19 disease and strong correlation between C677T and death from coronavirus.[19,20] Hcy is an intermediate amino acid as a metabolite of methionine degradation. Its elevation in plasma is quite common and mostly being dependent on diet, used drugs, and genetic polymorphism.[21] HHcy is an accepted marker of abnormalities in platelet function.[22] Rongioletti et al. found an inverse correlation between plasma Hcy levels and blood platelet counts in the women homozygous for *MTHFR* C677T.[23] In our study, *MTHFR* "CT" genotype compared to "CC" showed significantly lower platelet counts. On the other hand, C677T allele frequencies were not significantly different compared to control groups of the different studies.[24]

Intronic variant I/D polymorphism of *ACE* gene is located in intron 16. D-dimer levels are frequently elevated during sepsis, severe infections, thrombosis, deep vein and pulmonary embolism as a marker hypercoagulable state and intravascular thrombosis. Expression of the *ACE* gene directly under influence of an intronic I/D polymorphism; individuals having a homozygous "DD" genotype exhibit the highest expression of *ACE*, and those with homozygous II genotype exhibit the lowest.[25] It was suggested that *ACE* I/D polymorphisms could be related to differences in the regulation of sodium, fibrinolytic system, and possibly, inflammation.[26] In our study group, D-dimer level was lower than those with "ID" genotype compared to "DD" genotype. In a recent study, poor clinical outcomes were reported in COVID-19 patients with elevated D-dimer levels, and its 4-fold increase (>2 µg/mL) was predicted to be related to in hospital mortality.[27] In our study group, the D-dimer level of three patients treated in the intensive care unit was found higher than the others. There were no allele frequency differences between Turkish control women and women with COVID-19 for the *ACE I/D*.[28-30]

The *ACE2* gene is located on the X chromosome at the position Xp22. It contains 18 exons and shows close homology to *ACE* gene, since ACE2 discovered as a homolog of *ACE*.[31] In principle, it is possible that genetic variability of the ACE2 receptor is one of the factors that modulate virion uptake and thus disease severity. Human *ACE2* variant p.T27A is predicted to increase disease susceptibility. While the other *ACE2* variants p.G326E is predicted as protective variant to show decreased binding to SARS-CoV-2 S-protein.[5] p.G326E and p.T27A variants were not found in our study group. The S1 protein/receptor binding is the key regulator of SARS-CoV-2 to infection of host. The S1 contains the receptor binding domain able to bind the peptidase domain (PD) of ACE2 to enter host cells directly.[32] p.K419T located in PD of the ACE2 protein and the variant was not found in our study group.[5]

The *AT1R* gene is located on the 3rd chromosome and A1166C is one of the most studied polymorphism. "C" allele found to be associated with an increased response to angiotensin II (Ang II).[33] Peng et al. described the Ang II and CRP interaction in the vascular wall. The study provided evidence that CRP generation is induced by Ang II in vascular smooth muscle cells (VSMC) both *in vitro* and *in vivo*, which is mediated predominantly through *AT1R* in the VSMCs.[34]

On the other hand, CRP itself also has the ability to up-regulate *AT1R* in vascular smooth muscles.[35] In the study, CRP concentration in blood was not found related to *ACE Alu* I/D, *ACE* p.T776T, and *AGT1R* A1166C polymorphisms in COVID-19 patients.[36] However, in our study group, the patients hospitalized due to COVID-19 had higher CRP levels. Similarly, in another study, it was found that CRP had a modest effect on clinical outcomes in COVID-19 patients based on statistical models.[37] There were no allele frequency differences between Turkish healthy women and women with COVID-19 for the A1166C.[30]

The study has several limitations, firstly small sample size. Secondly, the study group was formed with volunteers from the North-East Anatolia region of Türkiye. For the first time, the results showed that the genetic polymorphisms did not change the risk of developing the disease but, *PAI-1*-844G>A, *MTHFR* 677C>T, and *ACE* (I/D) were associated with its severity. Moreover, this is the first study provides data of genetics and COVID-19 disease severity in pregnant women. Further studies with a larger study group might be more informative and extend our understanding between the disease severity and host DNA variation. Additionally, the identification of host DNA polymorphisms that modulate the risk of infection and disease severity may lead development of new preventive and/or therapeutic strategies for COVID-19.

CONCLUSION

For the first time, *PAI-1*–844G>A, *MTHFR p.*A222V, and *ACE* (I/D) were associated with a poor COVID-19 outcome; the risk of enoxaparin and antibiotic use and increased risk of hospitalization. Allele frequencies of the genes were not different between healthy control women and women with COVID-19; genetic variation may not influence the risk of infection but disease severity. The study may contribute to finding markers for determining the COVID-19 prognosis or possible severe cases.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Seher Polat;* ***Design:*** *Seher Polat;* ***Control/Supervision:*** *Seher Polat, Sevil Kiremitli;* ***Data Collection and/or Processing:*** *Seher Polat, Sevil Kiremtli, Tunay Kiremitli, Ahmet Kırkıncı, Fatma Zehra Kurnuç, Yusuf Kemal Arslan;* ***Analysis and/or Interpretation:*** *Seher Polat, Yusuf Kemal Arslan;* ***Literature Review:*** *Seher Polat, Sevil Kiremtli, Tunay Kiremitli, Ahmet Kırkıncı, Fatma Zehra Kurnuç, Yusuf Kemal Arslan;* ***Critical Review:*** *Seher Polat, Sevil Kiremtli, Tunay Kiremitli, Ahmet Kırkıncı, Fatma Zehra Kurnuç, Yusuf Kemal Arslan;* ***Materials:*** *Seher Polat, Sevil Kiremtli, Tunay Kiremitli, Ahmet Kırkıncı, Fatma Zehra Kurnuç.*

REFERENCES

1. Figliozzi S, Masci PG, Ahmadi N, Tondi L, Koutli E, Aimo A, et al. Predictors of adverse prognosis in COVID-19: a systematic review and meta-analysis. Eur J Clin Invest. 2020;50(10):e13362. [Crossref] [PubMed]
2. Benhamou D, Keita H, Ducloy-Bouthors AS; Obstetric Anaesthesia and Critical Care Club Working Group. Coagulation changes and thromboembolic risk in COVID-19 obstetric patients. Anaesth Crit Care Pain Med. 2020;39(3):351-3. [Crossref] [PubMed] [PMC]
3. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet. 1995;10(1):111-3. [Crossref] [PubMed]
4. Torres-Carrillo NM, Torres-Carrillo N, Vázquez-Del Mercado M, Delgado-Rizo V, Oregón-Romero E, Parra-Rojas I, et al. The -844 G/A PAI-1 polymorphism is associated with mRNA expression in rheumatoid arthritis. Rheumatol Int. 2008;28(4):355-60. [Crossref] [PubMed]
5. Suryamohan K, Diwanji D, Stawiski EW, Gupta R, Miersch S, Liu J, et al. Human ACE2 receptor polymorphisms and altered susceptibility to SARS-CoV-2. Commun Biol. 2021;4(1):475. [Crossref] [PubMed] [PMC]
6. Qi F, Qian S, Zhang S, Zhang Z. Single cell RNA sequencing of 13 human tissues identify cell types and receptors of human coronaviruses. Biochem Biophys Res Commun. 2020;526(1):135-40. [Crossref] [PubMed] [PMC]

7. Raghav PK, Raghav A, Lathwal A, Saxena A, Mann Z, Sengar M, et al. Experimental and clinical data analysis for identification of COVID-19 resistant ACE2 mutations. Sci Rep. 2023;13(1):2351. [Crossref] [PubMed] [PMC]
8. Abboud N, Ghazouani L, Saidi S, Ben-Hadj-Khalifa S, Addad F, Almawi WY, et al. Association of PAI-1 4G/5G and -844G/A gene polymorphisms and changes in PAI-1/tissue plasminogen activator levels in myocardial infarction: a case-control study. Genet Test Mol Biomarkers. 2010;14(1):23-7. [Crossref] [PubMed]
9. Rigat B, Hubert C, Corvol P, Soubrier F. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). Nucleic Acids Res. 1992;20(6):1433. [Crossref] [PubMed] [PMC]
10. Herrera CL, Castillo W, Estrada P, Mancilla B, Reyes G, Saavedra N, et al. Association of polymorphisms within the Renin-Angiotensin System with metabolic syndrome in a cohort of Chilean subjects. Arch Endocrinol Metab. 2016;60(3):190-8. [Crossref] [PubMed] [PMC]
11. Ghafil FA, Mohammad BI, Al-Janabi HS, Hadi NR, Al-Aubaidy HA. Genetic polymorphism of angiotensin converting enzyme and angiotensin II type 1 receptors and their impact on the outcome of acute coronary syndrome. Genomics. 2020;112(1):867-72. [Crossref] [PubMed]
12. Polat S, Şimşek Y. MTHFR C677T polymorphism in Turkish women with polycystic ovary syndrome. Turkish Journal of Endocrinology and Metabolism. 2021;25(1):102-12. [Crossref]
13. Dubey P, Reddy SY, Manuel S, Dwivedi AK. Maternal and neonatal characteristics and outcomes among COVID-19 infected women: an updated systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2020;252:490-501. [Crossref] [PubMed] [PMC]
14. Zambrano LD, Ellington S, Strid P, Galang RR, Oduyebo T, Tong VT, et al; CDC COVID-19 Response Pregnancy and Infant Linked Outcomes Team. Update: characteristics of symptomatic women of reproductive age with laboratory-confirmed SARS-CoV-2 infection by pregnancy status - United States, January 22-October 3, 2020. MMWR Morb Mortal Wkly Rep. 2020;69(44):1641-7. [Crossref] [PubMed] [PMC]
15. Zuo Y, Warnock M, Harbaugh A, Yalavarthi S, Gockman K, Zuo M, et al. Plasma tissue plasminogen activator and plasminogen activator inhibitor-1 in hospitalized COVID-19 patients. Sci Rep. 2021;11(1):1580. [Crossref] [PubMed] [PMC]
16. Polat S, Şimşek Y. Plasminogen activator inhibitor-1 polymorphism and risk of polycystic ovary syndrome in Turkish women. Meta Gene. 2021;30:100959. [Crossref]
17. Balint B, Jepchumba VK, Guéant JL, Guéant-Rodriguez RM. Mechanisms of homocysteine-induced damage to the endothelial, medial and adventitial layers of the arterial wall. Biochimie. 2020;173:100-6. [Crossref] [PubMed]
18. Zoccolella S, Martino D, Defazio G, Lamberti P, Livrea P. Hyperhomocysteinemia in movement disorders: current evidence and hypotheses. Curr Vasc Pharmacol. 2006;4(3):237-43. [Crossref] [PubMed]
19. Ponti G, Pastorino L, Manfredini M, Ozben T, Oliva G, Kaleci S, et al. COVID-19 spreading across world correlates with C677T allele of the methylenetetrahydrofolate reductase (MTHFR) gene prevalence. J Clin Lab Anal. 2021;35(7):e23798. [Crossref] [PubMed] [PMC]
20. Tekcan A, Cihangiroglu M, Capraz M, Capraz A, Yigit S, Nursal AF, et al. Association of ACE ID, MTHFR C677T, and MIF-173GC variants with the clinical course of COVID-19 patients. Nucleosides Nucleotides Nucleic Acids. 2023;42(10):782-96. [Crossref] [PubMed]
21. McCully KS. Homocysteine and the pathogenesis of atherosclerosis. Expert Rev Clin Pharmacol. 2015;8(2):211-9. [Crossref] [PubMed]
22. Naghshtabrizi B, Shakerian F, Hajilooi M, Emami F. Plasma homocysteine level and its genotypes as a risk factor for coronary artery disease in patients undergoing coronary angiography. J Cardiovasc Dis Res. 2012;3(4):276-9. [Crossref] [PubMed] [PMC]
23. Rongioletti M, Baldassini M, Papa F, Capoluongo E, Rocca B, Cristofaro RD, et al. Homocysteinemia is inversely correlated with platelet count and directly correlated with sE- and sP-selectin levels in females homozygous for C677T methylenetetrahydrofolate reductase. Platelets. 2005;16(3-4):185-90. [Crossref] [PubMed]
24. Karadeniz M, Erdogan M, Zengi A, Eroglu Z, Tamsel S, Olukman M, et al. Methylenetetrahydrofolate reductase C677T gene polymorphism in Turkish patients with polycystic ovary syndrome. Endocrine. 2010;38(1):127-33. [Crossref] [PubMed]
25. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest. 1990;86(4):1343-6. [Crossref] [PubMed] [PMC]
26. de Carvalho SS, Simões e Silva AC, Sabino Ade P, Evangelista FC, Gomes KB, Dusse LM, et al. Influence of ACE I/D Polymorphism on circulating levels of plasminogen activator inhibitor 1, D-dimer, ultrasensitive C-reactive protein and transforming growth factor β1 in patients undergoing hemodialysis. PLoS One. 2016;11(3):e0150613. [Crossref] [PubMed] [PMC]
27. Zhang L, Yan X, Fan Q, Liu H, Liu X, Liu Z, et al. D-dimer levels on admission to predict in-hospital mortality in patients with Covid-19. J Thromb Haemost. 2020;18(6):1324-9. [Crossref] [PubMed] [PMC]
28. İnanir S, Yiğit S, Çam Çelikel F, Ates O, Taycan SE, Nursal AF, et al. Relationship between major depressive disorder and ACE gene I/D polymorphism in a Turkish population. Archives of Clinical Psychiatry. 2016;43(2):27-30. [Crossref]
29. Bayram B, Kılıççı C, Onlü H, Ozkurt M, Erkasap N, Yıldırım E, et al. Association of angiotensin converting enzyme (ACE) gene I/D polymorphism and polycystic ovary syndrome (PCOS). Gene. 2011;489(2):86-8. [Crossref] [PubMed]
30. Alkanli N, Sipahi T, Okman Kilic T, Sener S. Lack of association between ACE I/D and AGTR1 A1166C Gene polymorphisms and preeclampsia in Turkish pregnant women of Trakya region. Journal of Gynecology and Obstetrics. 2014;2(4):49-53. [Crossref]
31. Cao Y, Sun Y, Tian X, Bai Z, Gong Y, Qi J, et al. Analysis of ACE2 gene-encoded proteins across mammalian species. Front Vet Sci. 2020;7:457. [Crossref] [PubMed] [PMC]
32. Turner AJ, Tipnis SR, Guy JL, Rice G, Hooper NM. ACEH/ACE2 is a novel mammalian metallocarboxypeptidase and a homologue of angiotensin-converting enzyme insensitive to ACE inhibitors. Can J Physiol Pharmacol. 2002;80(4):346-53. [Crossref] [PubMed]
33. van Geel PP, Pinto YM, Voors AA, Buikema H, Oosterga M, Crijns HJ, et al. Angiotensin II type 1 receptor A1166C gene polymorphism is associated with an increased response to angiotensin II in human arteries. Hypertension. 2000;35(3):717-21. [Crossref] [PubMed]
34. Peng N, Liu JT, Gao DF, Lin R, Li R. Angiotensin II-induced C-reactive protein generation: inflammatory role of vascular smooth muscle cells in atherosclerosis. Atherosclerosis. 2007;193(2):292-8. [Crossref] [PubMed]
35. Wang CH, Li SH, Weisel RD, Fedak PW, Dumont AS, Szmitko P, et al. C-reactive protein upregulates angiotensin type 1 receptors in vascular smooth muscle. Circulation. 2003;107(13):1783-90. [Crossref] [PubMed]
36. Bahramali E, Firouzabadi N, Jonaidi-Jafari N, Shafiei M. Renin-angiotensin system genetic polymorphisms: lack of association with CRP levels in patients with coronary artery disease. J Renin Angiotensin Aldosterone Syst. 2014;15(4):559-65. [Crossref] [PubMed]
37. Gormez S, Ekicibasi E, Degirmencioglu A, Paudel A, Erdim R, Gumusel HK, et al. Association between renin-angiotensin-aldosterone system inhibitor treatment, neutrophil-lymphocyte ratio, D-Dimer and clinical severity of COVID-19 in hospitalized patients: a multicenter, observational study. J Hum Hypertens. 2021;35(7):588-97. [Crossref] [PubMed] [PMC]