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ORIGINAL ARTICLE

Possible effect of genetic background in thrombophilia genes on clinical severity of patients with coronavirus disease-2019: A prospective cohort study

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ABSTRACT

Background and objective: Thrombotic and microangiopathic effects have been reported in Coronavirus Disease-2019 (COVID-19) patients. In the present study, we aimed to examine the relationship between hereditary thrombophilia factors and the clinical picture severity of COVID-19 patients.
Methods: Ninety COVID-19 patients were included and grouped according to the severity to three groups: severe/critical (n=30), mild/moderate (n=30) and asymptomatic (n=30). Hereditary thrombophilia genetic markers [prothrombin (FII) G20210A, factor V Leiden (FVL) G1691A, factor XIII (FXIII) V34L, methylene tetrahydrofolate reductase (MTHFR) A1298C and C677T, and plasminogen activator inhibitor-1 (PAI-1) 4G & 5G] were genotyped for all patients.
Results: Seventeen (18.9%) patients had the polymorphism 4G/4G PAI-1 and 48 (53.3%) had 4G/5G. In addition, the heterozygous GA FVL, MTHRF677CT, and MTHRF1298AC polymorphisms were detected in 11 (12.2%), 26 (28.9%), and 38 (42.2%) patients, respectively. The rate of severe/critical patients with PAI 4G/5G gene polymorphism was higher than the asymptomatic+moderate/mild patients, and the rate of severe/critically ill patients with PAI 4G/4G polymorphism was found to be lower than the asymptomatic+moderate/mild patients. No difference was evidenced between the distribution of deceased and survivors of the genotype groups.
Conclusions: In the present study, we found that heterozygous 4G/5G PAI-1 polymorphism is associated with critical or severe COVID-19 picture, and that FVL, MTHFR, FXIII, and prothrombin polymorphisms were not directly related to COVID-19 severity.

Keywords COVID-19, FVL, MTHFR, PAI-1, polymorphism, thrombophilia

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INTRODUCTION

In December 2019, pneumonia caused by the newly described Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), which spread to the world from the city of Wuhan, China and caused the pandemic, was identified as Coronavirus Disease-2019 (COVID-19).[1] Clinical findings caused by SARS-CoV-2 infection is observed as asymptomatic disease and mild upper respiratory tract infection, as well as respiratory failure that may result in death.[2]

It has been shown that venous thromboembolism (VTE) due to coagulation dysfunction is associated with a poor prognosis in COVID-19 patients.[1,2] The risk of immune dysfunction and VTE is increased in this patients followed up in the intensive care unit. It has been stated that in the sepsis picture that develops in severe infections, it may predispose to coagulation with the release of inflammatory cytokines, and may lead to disseminated intravascular coagulation (DIC). DIC has been associated with abnormal coagulation, thrombus, and poor prognosis.[1,3]

Studies on factors that cause different clinical pictures among infected patients are very limited[4]. Microvascular embolisms and coagulation disorders worsen the clinical picture, especially in patients who need intensive care, and death is observed more frequently in patients who develop coagulopathy.[2,3]

Attention has been drawn to the importance of coagulopathy management in the course of COVID-19. Microangiopathic and thrombotic effects of the SARS-CoV-2 have been reported in patients with COVID-19. Thromboembolic events are thought to develop in the course of COVID-19 by various mechanisms; (i) development of vascular microthrombotic disease in sepsis, (ii) development of endothelial damage by complement activation, (iii) activation of the inflammatory and microthrombotic pathway, and (iv) stasis resulting from inactivity during the hospitalization period of the patients.[4,5] Also, in the advanced stages of the disease, diffuse intravascular coagulation, whose pathophysiology is complex and multifactorial, was observed. Anticoagulants and antiplatelet drugs are used in the treatment of some of the COVID-19 patients.[3,5,6]

A transient increased risk of venous thromboembolic events is associated with acute infections. This is also observed in COVID-19. Thrombophilia can be identified as an increased tendency to clot in the blood. The clinical manifestation of thrombophilia is pulmonary embolism and/or deep vein thrombosis.[3,5] It is an important cause of mortality and morbidity in current clinical practice. Many hereditary (primary) and acquired (secondary) factors have been identified that cause thrombophilia. Inherited and acquired factors may be the sole factor of thrombophilia, in most cases both hereditary and acquired causes coexist and cause the clinical picture to become aggravated. The fact that COVID-19 is more severe in elderly patients and the factors such as some sudden deaths in young aged-patients, as similar clinical findings in patients with relationship, and as VTE, microangiopathic thrombus, and distal extremity circulatory disorders reveal the necessity of evaluating the relationship between hereditary thrombophilia factors and COVID-19.[3,5,6]

The most common genetic causes of hereditary thrombophilia are; prothrombin (FII) G20210A, factor V Leiden (FVL) G1691A, methylene tetrahydrofolate reductase (MTHFR) A1298C and C677T, factor XIII and plasminogen activator inhibitor-1 (PAI-1).[6]

There have been some studies showing a relationship between thrombophilia and disease severity in COVID-19 patients.[3–5] In this study, it was aimed to investigate the relationship between the genes predisposing to thrombophilia in patients with COVID-19 with the clinic of the disease and the effect on mortality and morbidity in these patients.

MATERIALS AND METHODS

The present study was approved by the Republic of Turkey Ministry of Health (2020-05-05T16_10_59) and the local medical ethics committee (KAEK 2020/4/6). In addition, the study was conducted in accordance to complied with the principles of the Helsinki Declaration, and animal and human rights. Informed consent was obtained before collecting specimens from all patients.

Definition of RT-PCR positive COVID-19

Laboratory confirmation was defined as a positive result on real-time polymerase chain reaction (RT-PCR) assay (Bioeksen, Istanbul, Turkey) (Bio-speedy, SARS-Cov-2 (2019-nCoV) RT-qPCR detection kit) of pharyngeal and nasal swab samples.

Study design

Examinations of 90 RT-PCR-positive patients with COVID-19 who were followed up at Samsun Training and Research Hospital from April 1, 2020 to July 1, 2020 were included in the present study.

Whole blood samples and clinical data of 90 RT-PCR positive patients with COVID-19 were collected. The clinical conditions of the patients are asymptomatic in line with the recommendations of Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia and the Ministry of Health COVID-19 Guide were divided into three groups as moderate/mild and critical/severe:[5,7–9]

(i) Severe or critical disease (n=30) was identified as patients with positive COVID-19 RT-PCR test who in addition developed significant respiratory distress (RR >30/min), arterial oxygen partial pressure (PaO2)/Fraction of inspire O2 (FiO2) <300mmHg; blood oxygen saturation <93%; shock; other organ failure need intensive care; respiratory failure with mechanical ventilation in ICU.

(ii) Mild or moderate disease (n=30) was identified as patients with positive COVID-19 RT-PCR test who had muscle/joint pain, fever, respiratory rate <30/minute, sore throat and cough, SpO2 level above 90% in room air.

(iii) Asymptomatic (n=30) cases were recruited from RT-PCR positive COVID-19 cases with contact history but no clinical signs during follow-up.

Molecular Analysis

DNA was extracted from the peripheral blood specimens of patients with COVID-19 by using a commercial spin column procedure (QIAamp® DNA Blood Mini Kit; QIAGEN, Hilden, Germany) according to the manufacturer's directions. Real-time PCR (ABI RT-PCR 7500, Applied Biosystems, USA) was applied to assess PAI-1 4G & 5G and FXIII V34L polymorphisms (Genmark Diagnosis). Genotyping for FII, FVL, MTHFR gene polymorphisms were performed by using spesific designed primers (Macrogen, Seoul, South Korea) via PCR (ABI Gene AmpPCR System 9700, Applied Biosystem) followed by restriction fragment length polymorphism (RFLP) using specific restriction enzymes (Thermo Fishcer Scientific, Waltham, MA, USA) following the manufacturer's protocol are shown on Tables 1 and 2.

Statistical analysis

Statistical analysis of the study was performed using SPSS 25.0 software (IBM SPSS, Chicago, IL, USA). Descriptive data were expressed as numbers and percentage frequencies. Comparisons between groups in terms of categorical data were made with Fisher's Exact Test and Pearson's Chi Square test. Conformity of continuous data to normal distribution was tested by Kolmogorov-Smirnov Test. Differences between groups in terms of continuous data were analyzed using the Independent Sample t-Test, and the comparison of mean levels between multiple groups was done by analysis of variance. Evaluation of the results was done at 95% confidence interval. Those with a p value below 0.05 were considered significant.

Power analysis was calculated as 0.80 using Gpower (Input: Effect size w=0.3, β/α ratio=1.069056, total sample size=90, Df=5).

RESULTS

Fifty-seven (63.3%) of the patients in the study were male. The mean age was 53$\pm$20.1 (min-max: 19-95) years.

In the PAI genotyping, 17 (18.9%) patients had homozygous 4G/4G polymorphism, 48 (53.3%) heterozygous 4G/5G polymorphism, and 25 (27.8%) had homozygous 5G/5G polymorphism. Heterozygous GA polymorphism was detected in 11 (12.2%) patients in FVL genotype analysis. Heterozygous GA polymorphism was found in only one (1.1%) patient in FII genotype examination. In MTHRF677 genotype examination, 26 (28.9%) patients had heterozygous 677CT polymorphism and 12 (13.3%) patients had homozygous 677TT polymorphism. In the MTHFR1298 genotype examination, 38 (42.2%) heterozygous 1298AC

Table 1 **Investigation of polymorphisms of hereditary thrombophilia factors by PCR and RFLP methods (stage 1).**

Factor (gene polymorphism)	PCR condition (temperature, °C)	Primers	Method
Coagulation factor II, protrombin (G20210A)	D: 95° C→ 7 min, 94 ° C→ 45 sec A: 60 ° C→ 30 sec, 72 ° C→ 45 sec (35 cycle) E: 72 ° C→ 7 min	F:5'-TCT AGA AAC AGT TGC CTG GC-3′ R:5′-ATA GCA CTG GGA GCA TTG AAG C-3′	RFLP
Coagulation factor V (FV Leiden G1691A)	D: 95° C→ 7 min, 94 ° C→ 45 sec A: 55 ° C→ 30 sec, 72 ° C→ 45 sec (35 cycle) E: 72 ° C→ 7 min	F:5'-TGCCCAGTGCTTAACAAGACCA-3′ R:5'-TGTTATCACACTGGTGCTAA-3'	RFLP
Methylenetetrahydrofolate reductase (MTHFR C677T)	D: 95° C→ 7 min, 94 ° C→ 45 sec A: 60 ° C→ 30 sec, 72 ° C→ 45 sec (35 cycle) E: 72 ° C→ 7 min	F: 5'-TGTGGTCTCTTCATCCCTCGC-3' R: 5'-CCTTTTGGTGATGCTTGTTGGC-3'	RFLP
Methylenetetrahydrofolate reductase (MTHFR A1298C)	D: 95° C→ 7 min, 94 ° C→ 45 sec A: 62 ° C→ 30 sec, 72 ° C→ 45 sec (35 cycle) E: 72 ° C→ 7 min	F: 5'-CTTTGGGGAGCTGAAGGACTACTAC -3' R: 5'-CACTTTGTGACCATTCCGGTTTG -3'	RFLP
Coagulation factor FXIII (FXIII V34L)	Pre PCR: 60° C→1 min Holding: 95° C→10 min Cycle Stage: ° C→15 min (40 cycle) 58° C→45 sec Post PCR: 60° C→1 min	Ready mastermix	Real-Time PCR
Plasminogen activator inhibitor-1 (4G and 5G)	Pre PCR: 60° C→1 min Holding: 95° C→10 min Cycle Stage:° C→15 min (40 cycle) 58° C→45 sec Post PCR: 60° C→1 min	Ready mastermix	Real-Time PCR

Sec: second, min: minute, h:hour, F: Forward, R: Reverse, bp: base pair, D: Denaturation, A: Annealing, E: Extention.

Table 2 **Investigation of polymorphisms of hereditary thrombophilia factors by PCR and RFLP methods (stage 2).**

Factor (gene polymorphism)	Enzyme (temperature)	Allele	DNA fragment (bp)
Coagulation factor II, protrombin (G20210A)	HindIII 37 °C→ 16 h, 65 °C→ 20 min	Wild type: GG Heterozygous: GA Homozygous: AA	345 345+322+23 322+23
Coagulation factor V (FV Leiden)	MnlI 37 °C→60 min, 65 °C→ 20 min	Wild type: GG Heterozygous: GA Homozygous: AA	163+67+37 200+163+67+37 200+67
Methylenetetrahydrofolat reductase (MTHFR C677T)	HinfI 65 °C → 1 h	Wild type: CC Heterozygous: CT Homozygous: TT	513 513+367+146 367+146
Methylenetetrahydrofolat reductase (MTHFR A1298C)	MboII 37 °C→ 60 min, 65 °C→ 20 min	Wild type: AA Heterozygous: AC Homozygous: CC	56+31+30+28+18 84+56+31+30+28+18 84+31+30+28+18

Sec: second, min: minute, h:hour, F: Forward, R: Reverse, bp: base pair, D: Denaturation, A: Annealing, E: Extention.

polymorphism and 17 (18.9%) patients had homozygous 1298CC polymorphism. No polymorphism was detected in (Table 3).

When only patients over 60 years old were examined, the rate of severe/critical patients with PAI 4G/5G polymorphism was higher than the other groups; The rate of critically/-severe ill patients with 4G/4G polymorphism was found to be lower than the other groups (p=0.025) (Table 3).

All genotype groups were similar in terms of COVID-19 clinical picture distribution (p>0.05 for each), (Table 3). In the examination performed by dividing clinical picture groups into asymptomatic and patient groups, all genotype groups were similar in terms of COVID-19 clinical picture distribution (p>0.05 for each) (Table 4). However, the clinical picture groups were divided into asymptomatic + moderate/mild and severe/critical, and the rate of severe/critical patients with PAI 4G/5G polymorphism was higher than the other groups, and the rate of severe/critically ill patients with 4G/4G polymorphism was found to be lower than the other groups (p=0.045). Other genotype groups were also similar (p>0.05 for each (Table 5).

When examined in terms of survival, no significant difference was found between the genotype groups in terms of the distribution of those who died and those survived (p>0.05 for each) (Table 6).

DISCUSSION

Venous thrombosis is a condition in which thrombus that occurs locally in veins causes obstruction in the form of embolism in the circulation. Although it is most common in the deep leg veins, it may develop less frequently in the brain, retina, mesentery, and liver

Table 3 **Distribution of COVID-19clinical severity by gene polymorphism groups.**

		COVID-19 clinical picture							
		Asympton		Mild/modera		Severe/criti			
Polymorphism		n	%	n	%	n	%	Total	*P*
PAI 4G & 5G									0.091
	Homozygous 4G/4G	9	52.9	6	35.3	2	11.8	17	
	Heterozygous 4G/5G	14	29.2	13	27.1	21	43.8	48	
	Homozygous 5G/5G	7	28.0	11	44.0	7	28.0	25	
	4G/4G or 4G/5G	23	35.4	19	29.2	23	35.4	65	0.412
	5G/5G	7	28.0	11	44.0	7	28.0	25	
FVL G1691A									0.902
	Heterozygous GA	4	36.4	4	36.4	3	27.3	11	
	Homozygous GG	26	32.9	26	32.9	27	34.2	79	
Prothrombin G20210A									0.364
	Heterozygous GA	0	0.0	0	0.0	1	100.0	1	
	Homozygous GG	30	33.7	30	33.7	29	32.6	89	
MTHFR C677T									0.815
	Heterozygous CT	12	46.2	8	30.8	6	23.1	26	
	Homozygous TT	4	33.3	3	25.0	5	41.7	12	
	Homozygous CC	14	26.9	19	36.5	19	36.5	52	
	CT or TT	16	42.1	11	28.9	11	28.9	38	0.32
	CC	14	26.9	19	36.5	19	36.5	52	
MTHFR A1298C									0.815
	Heterozygous AC	15	39.5	11	28.9	12	31.6	38	
	Homozygous CC	4	23.5	7	41.2	6	35.3	17	
	Homozygous AA	11	31.4	12	34.3	12	34.3	35	
	AC or CC	19	34.5	18	32.7	18	32.7	55	0.954
	AA	11	31.4	12	34.3	12	34.3	35	
Factor XIII V34L									-
	Homozygous VV	30	33.3	30	33.3	30	33.3	90	
Only for the age >60 years									
PAI 4G and 5G									0.025
	4G/4G	0	0.0	1	100.0	0	0.0	1	
	4G/5G	5	22.7	1	4.5	16	72.7	22	
	5G/5G	1	11.1	4	44.4	4	44.4	9	

COVID-19: coronavirus disease-2019,PAI: plasminogen activator inhibitor, FVL: Factor V Leiden, MTHFR: methylenetetrahydrofolatereductase.

veins.[3,10] Venous thrombosis is an important cause of mortality and morbidity. It has been stated that disorders such as coagulation disorder, abnormalities in platelet levels, increase in procoagulant factors and DIC can be seen in COVID-19 patients, and the prognosis may be much worse in patients with COVID-19 accompanying such disorders.[11] It has been noted that there is a two-way relationship between the severity of the COVID-19 clinical picture and coagulation disorders. It has been reported that the mortality risk significantly increases in COVID-19 patients who develop DIC.[12–14] Few studies have been conducted

Table 4 **Distribution of COVID-19 clinical severity by gene polymorphism groups.**

		COVID-19 clinical picture					
		Asymptomatic		Symptomatic			
Polymosrphism		n	%	nn	%	Total	*P*
PAI 4G & 5G							0.162
	Homozygous 4G/4G	9	52.9	8	47.1	17	
	Heterozygous 4G/5G	14	29.2	34	70.8	48	
	Homozygous 5G/5G	7	28.0	18	72.0	25	
	4G/4G or 4G/5G	23	35.4	42	64.6	65	0.506
	5G/5G	7	28.0	18	72.0	25	
FVL G1691A							0.82
	Heterozygous GA	4	36.4	7	63.6	11	
	Homozygous GG	26	32.9	53	67.1	79	
Prothrombin G20210A							0.477
	Heterozygous GA	0	0.0	1	100.0	1	
	Homozygous GG	30	33.7	59	66.3	89	
MTHFR C677T							0.236
	Heterozygous CT	12	46.2	14	53.8	26	
	Homozygous TT	4	33.3	8	66.7	12	
	Homozygous CC	14	26.9	38	73.1	52	
	CT or TT	16	42.1	22	57.9	38	0.131
	CC	14	26.9	38	73.1	52	
MTHFR A1298C							0.487
	Heterozygous AC	15	39.5	23	60.5	38	
	Homozygous CC	4	23.5	13	76.5	17	
	Homozygous AA	11	31.4	24	68.6	35	
	Ac or CC	19	34.5	36	65.5	55	0.76
	AA	11	31.4	24	68.6	35	
Factor XIII V34L							-
	Homozygous VV	30	33.3	60	66.7	90	

COVID-19: coronavirus disease-2019,PAI: plasminogen activator inhibitor, FVL: Factor V Leiden, MTHFR: methylenetetrahydrofolatereductase.

on the relationship between COVID-19 and congenital coagulation disorders or the presence of polymorphisms that cause thrombophilia.[4,14–17] In the present study, the relationship between the severity of the COVID-19 clinical picture and some polymorphisms that cause thrombophilia were investigated.

Factor V, one of the coagulation factors, has one of the most important roles in coagulation factors. Factor V is included in the prothrombin activator complex. The factor V gene is localized in the 1q21-25 region and contains 25 exons. Factor V Leiden polymorphism was defined as a G/A polymorphism at the 1691^{st} base in the 10^{th} exon. Factor V polymorphism is the most common inherited thrombophilia polymorphism. Factor V cannot be inactivated by Protein C in the presence of an FVL polymorphism. Consequently, a continuous increase in thrombin formation and as a result of this, hypercoagulation is observed.[17–20] It has been reported that the risk of thrombosis shows a 5-10-fold increase in the presence of

Table 5 **Distribution of COVID-19 clinical severity by gene polymorphism groups.**

		COVID-19 clinical picture					
		Asymptomatic to moderate		Sever to critical			
Polymosrphism		n	%	n	%	Total	*P*
PAI 4G & 5G							0.045
	Homozygous 4G/4G	15	88.2	2	11.8	17	
	Heterozygous 4G/5G	27	56.3	21	43.8	48	
	Homozygous 5G/5G	18	72.0	7	28.0	25	
	4G/4G or 4G/5G	42	64.6	23	35.4	65	0.506
	5G/5G	18	72.0	7	28.0	25	
FVL G1691A							0.649
	Heterozygous GA	8	72.7	3	27.3	11	
	Homozygous GG	52	65.8	27	34.2	79	
Prothrombin G20210A							0.155
	Heterozygous GA	0	0.0	1	100.0	1	
	Homozygous GG	60	67.4	29	32.6	89	
MTHFR C677T							0.397
	Heterozygous CT	20	76.9	6	23.1	26	
	Homozygous TT	7	58.3	5	41.7	12	
	Homozygous CC	33	63.5	19	36.5	52	
	CT or TT	27	71.1	11	28.9	38	0.451
	CC	33	63.5	19	36.5	52	
MTHFR A1298C							0.953
	Heterozygous AC	26	68.4	12	31.6	38	
	Homozygous CC	11	64.7	6	35.3	17	
	Homozygous AA	23	65.7	12	34.3	35	
	Ac or CC	37	67.3	18	32.7	55	0.878
	AA	23	65.7	12	34.3	35	
Factor XIII V34L							-
	Homozygous VV	60	66.7	30	33.3	90	

COVID-19: coronavirus disease-2019,PAI: plasminogen activator inhibitor, FVL: Factor V Leiden, MTHFR: methylenetetrahydrofolatereductase.

Table 6 **Distribution of survivors anddeaths by gene polymorphism groups.**

		COVID-19 clinical picture					
		Dead		Alive			
Polymosrphism		n	%	n	%	Total	*P*
PAI 4G & 5G							0.181
	Homozygous 4G/4G	0	0.0	17	100.0	17	
	Heterozygous 4G/5G	6	12.5	42	87.5	48	
	Homozygous 5G/5G	1	4.0	24	96.0	25	
FVL G1691A							0.862
	Heterozygous GA	1	9.1	10	90.9	11	
	Homozygous GG	6	7.6	73	92.4	79	
Prothrombin G20210A							0.77
	Heterozygous GA	0	0.0	1	100.0	1	
	Homozygous GG	7	7.9	82	92.1	89	
MTHFR C677T							0.2
	Heterozygous CT	0	0.0	26	100.0	26	
	Homozygous TT	1	8.3	11	91.7	12	
	Homozygous CC	6	11.5	46	88.5	52	
MTHFR A1298C							0.746
	Heterozygous AC	3	7.9	35	92.1	38	
	Homozygous CC	2	11.8	15	88.2	17	
	Homozygous AA	2	5.7	33	94.3	35	
Factor XIII V34L							-
	Homozygous VV	7	7.8	83	92.2	90	

PAI: plasminogen activator inhibitor, FVL: Factor V Leiden, MTHFR: methylenetetrahydrofolatereductase.

a heterozygous FVL polymorphism and 50-100-fold increase in the presence of a homozygous FVL polymorphism.[21,22] Stefely et al. found that there was an increase in activated Factor V level in severe patients with COVID-19 and found that the risk of thromboembolic complications was increased in these patients.[17] However, these researchers reported that on the contrary, there was a decrease in active factor V in patients with COVID-19 who died. Zhang et al. also reported that the factor V level was lower in patients who died than those who did not die but whose condition was severe.[23] However, these researchers determined that protein C activity was below the normal limit in severe COVID-19 patients. In this study, the distribution of FVL polymorphisms was similar among the COVID-19 groups in asymptomatic, mild/moderate and severe/critical clinical pictures. Kiraz et al. indicated that they found the same result in a series of 62 severe novel coronavirus pneumonia study.In addition, FVL polymorphism rates were similar between survivors and patients who died. These findings show that the presence of FVL polymorphism does not affect the clinical picture severity in COVID-19 cases.

It has been reported that the frequency of FVL polymorphism is generally around 3-10 %.[6,21,22] In the present study, heterozygous GA polymorphism was found in 12.2 % of the patients in the FVL genotype examination in COVID-19 patients. The fact that this rate is close to the general FVL polymorphism rate suggests that the FVL polymorphism is not

associated with getting COVID-19.

Prothrombin is a glycoprotein synthesized in the liver. It turns into thrombin within the coagulation system. The factor II (prothrombin) gene is localized in the 11p11-q12 region and contains 14 exons. Factor II prothrombin polymorphism is the second most common inherited thrombophilia polymorphism. Heterozygous polymorphism seen with the G-A change in the factor II gene at position 20210 causes an increase in prothrombin and thrombin levels in plasma. This tendency to clot increases the risk of venous thromboembolism. Thrombosis risk shows a 2-12-fold increase in case of factor II polymorphism.[21,24,25] Factor II polymorphism has been reported around 2-8% in Turkey and Europe.[6,21,25] In the present study, heterozygous GA polymorphism was found in only one (1.1%) COVID-19 patient. The very low number of patients with polymorphism was not sufficient to determine whether there was a relationship between factor II polymorphism and clinical picture severity in COVID-19 cases. However, Kiraz et al. indicated in their study that there were no statistically differences between the healthy population and the severe patient group regarding the factor II polymorphism.[4]

In the present study, the number of platelets in a single patient with heterozygous polymorphism (GA) in the FII genotype was found to be higher than the mean number in those without polymorphism. Detecting a polymorphism in a single patient is not sufficient for an analysis, but it is notable that the platelet count of that patient was very high. Larger studies are needed to reveal the possible relationship between factor II, platelet count and COVID-19 clinical picture.

Methylenetetrahydrofolate reductase is involved in folate metabolism. MTHFR plays a role in purine-pyrimidine synthesis in RNA/DNA synthesis, conversion of homocysteine to methionine, methylation reactions. Two important polymorphisms can be seen in the MTHFR gene. One of them is the polymorphism that MTHFR677C transforms into MTHFR677T. In the presence of MTHFR 677CT polymorphism, the enzyme becomes thermolabile and serum homocysteine level increases. This increase impairs endothelial function and increases the propensity to clot. This situation leads to an increased risk of venous thrombosis.[18,26] Plasma homocysteine level in COVID-19 has been shown to correlate with the progression of lung findings in computed tomography in COVID-19 patients.[7] Karst et al. also suggested that determination of both plasma homocysteine level and investigation of MTHFR polymorphism may be valuable in predicting severe COVID-19 clinic.[15] In the present study, the distribution of MTHFR677 polymorphisms was similar among COVID-19 groups in asymptomatic, mild/moderate and severe/critical clinical pictures. In addition, MTHFR677 polymorphism rates were similar between survivors and those who died. These findings show that the presence of MTHFR677 polymorphism does not affect the clinical picture severity in COVID-19 cases.

Another polymorphism in the MTHFR gene is the 1298AC polymorphism, in which 1298A turns into 1298C. This polymorphism causes a decrease in the activity of enzymes containing alanine and an increase in homocysteine level. This increases the tendency to coagulation.[18,26] In the present study, the distribution of MTHFR1298 polymorphisms was similar among COVID-19 groups in asymptomatic, mild/moderate and severe/critical

clinical pictures. In addition, MTHFR1298 polymorphism rates were found to be similar between those who died and those who survived. These findings show that the presence of MTHFR1298 polymorphism does not affect the clinical picture severity in COVID-19.

It has been reported that the general frequency of MTHFR polymorphisms is between 40-70 %.[15,18,26] In the present study, polymorphism was detected in 42.2 % (28.9 % heterozygous 677CT polymorphism; 13.3 % homozygous 677TT polymorphism) of the patients in the MTHRF677 genotyping. In the MTHFR1298 genotyping, 61.1 % of the patients had polymorphism (42.2 % heterozygous 1298AC polymorphism; 18.9 % homozygous 1298CC polymorphism). The fact that these rates are within the general limits indicates that the presence of MTHFR polymorphism is not directly related to get COVID-19.

PAI-1 gene polymorphism is one of the inherited thrombophilia polymorphisms. The main task of PAI-1 is to reduce fibrinolytic activity and to provide fibrin accumulation. PAI-1 gene is located in 7q21,3-q22 region on the 7^{th} chromosome. There are several polymorphic loci in the PAI-1 gene; 5G/5G, 4G/5G and 4G/4G insertion/deletion polymorphisms in the 675th position of the promoter are the most common polymorphisms. In these polymorphisms, 4 (4G) or 5 (5G) guanine sequences are formed. Individuals with one of these polymorphisms also increase plasma PAI-1 level and PAI-1 activity in platelets. These three polymorphisms have been shown to play a role in the pathogenesis of different diseases.[27–29] Matsuyama et al. stated that PAI-1 is found in high levels in the lungs and plasma of COVID-19 patients, that it was expressed in damaged lung alveolar cells, and that it might activate the signal transducer and activator of transcription 3.[30] They noted that age, cardiovascular disease, obesity, and diabetes were associated with COVID-19 risk factors. These researchers suggested that depending on this situation, the PAI-1 level might affect the clinical picture of COVID-19. Wu and Yang reported that PAI-1 levels increased in severe COVID-19 cases, and that PAI-1 could be used as a biomarker in predicting the clinical picture of COVID-19.[31] In contrast to this study, another study with data from severe novel coronavirus pneumonia cases, researchers stated that there were no statistically significant differences.[4] In the present study, in critical/severe COVID-19 cases, PAI-1 heterozygous 4G/5G polymorphism rate was found to be significantly higher, and homozygous 4G/4G polymorphism rate was lower. In the present study, in which severe COVID-19 cases were found to be associated with age, only patients over the age of 60 were analyzed separately, and the relationship between 4G/5G polymorphism and severe cases was determined in the same way. All these findings show that the clinical picture is more severe in COVID-19 cases with PAI-1 4G/5G polymorphism. Accordingly, the 4G/5G polymorphism can be used as a biomarker for predicting the course of COVID-19.

PAI-1 polymorphism rates were similar between survived and patients who died in the present study. This finding shows that although PAI-1 polymorphisms provide information about the clinical picture in COVID-19 patients, they do not directly provide information on mortality. However, the low number of patients who died in the present study may have led to this finding. Therefore, this relationship should be elucidated with larger studies. In addition, although severe clinical course in COVID-19 patients appears to be associated

with PAI-1, patient loss may be due to many different factors.

Both 5G and 4G alleles bind a transcriptional activator, but 5G additionally binds a repressor, so the presence of 4G/4G homozygotes increases transcription and consequently raises circulating PAI-1, while 5G/5G homozygous is associated with low value circulating PAI-1.[31]

There were some limitations in the present study. Although the number of patients has enabled good quality analyzes in terms of the number of polymorphisms detected in general, the fact that factor II polymorphism, which was detected in the general population at a very low rate, was detected in only one patient in the present study, did not allow an evaluation of this polymorphism. In addition, since this study was planned as a cross-sectional, the long-term prognosis of the patients was not observed, and it could not be evaluated whether the relevant polymorphisms had long-term effects.

CONCLUSIONS

The present study investigated the possible relationship between the polymorphisms that associated with hereditary thrombophilia and the clinical picture severity of COVID-19. The findings obtained in the present study showed that heterozygous 4G/5G PAI-1 polymorphism is associated with severe and critical COVID-19 picture. The present study data also revealed that FVL and MTHFR, FXIII, prothrombin polymorphisms were not directly related to COVID-19 clinical picture severity.

ABBREVIATIONS

COVID-19: coronavirus disease-2019, DIC: disseminated intravascular coagulation, FII: prothrombin, FVL: factor V Leiden, MTHFR: methylene tetrahydrofolate reductase, PAI-1: plasminogen activator inhibitor-1, RFLP: restriction fragment length polymorphism, RT-PCR: real-time polymerase chain reaction, SARS-CoV-2: severe acute respiratory syndrome coronavirus-2, VTE: venous thromboembolism.

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DECLARATIONS

Authors' contributions

Conceptualization and formal analysis: OS & OG. Data curation: OS & RA. Funding acquisition: N/A. Investigation, methodology, project administration, resources, sofware, supervision, validation, visualization, writing-original draft, review & editing: OS, OG, AK and

RA. The authors have reviewed and approved the final proof before publication.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval and consent to participate

the study protocol was endorsed by the local Ethics Committee (KAEK 2020/4/6) and the Turkish Ministry of Health (2020-05-05T16_10_59).

Data availability

The data that support the findings of this study is available from the corresponding author, upon reasonable request.

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