

The Investigation of the Relationship Between the Inherited Thrombophilia and Novel Coronavirus Pneumonia

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Research Article

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Abstract

Purpose: Novel coronavirus pneumonia (NCP) is a disease caused by severe acute respiratory syndrome coronavirus 2 virus. It was reported that there is a relationship between severe NCP and hypercoagulable conditions that predispose patients to thrombosis. Thrombophilia is a multifactor condition that can result from genetic factors, acquired factors, or a combination of both. The prothrombin gene (*F2* rs1799963 known as G20210A), Factor V Leiden (*F5* rs6025 known as G1691A) and PAI-1 (rs1799768) are important polymorphic biomarkers of thrombophilia that are investigated in severe NCP patients within this study.

Methods: NCP-diagnosed 62 previously healthy male patients (mean age 38.83±11.04) without any chronic disease were enrolled in this study for the investigation of the well-known thrombophilia-related abovementioned polymorphisms. The diagnosis of NCP was made according to the World Health Organization interim guidance and confirmed by RNA detection. SNPs were detected by real-time PCR. The frequency of genotypes was compared with healthy control group frequencies from other studies performed in the Turkish population.

Results: There were no statistically significant differences between the severe patient group and the healthy population regarding the investigated SNPs.

Conclusion: This study is the first to rule out the relationship of rs1799963 (FII), rs6025 (FV) and rs1799768 (PAI-1) with severe NCP. As there is an obvious relation between severe NCP and genetic thrombophilia susceptibility, studies focused on other thrombophilia-related genetic factors and this disease must be performed.

Introduction

Novel coronavirus pneumonia (NCP) (COVID-19) is a disease caused by the enveloped viral pathogen severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). NCP, which is a major health problem worldwide, still has no definitive treatment or vaccine. Acute respiratory distress syndrome (ARDS) and sepsis are the main complications of the disease. Additionally, disseminated intravascular coagulation (DIC) is one of the main underlying causes of death among patients [1]. A high number of thrombotic complications exist, and the incidence of thrombotic disease in individuals affected by NCP is reported to be 31% [2]. The brain and lungs were affected by the hypercoagulable state, and anticoagulant therapy should be started in these NCP patients [3].

Thrombophilia is a hypercoagulable condition that predisposes patients to thrombosis. Thrombophilia is a multifactor condition that can result from genetic factors, acquired factors, or a combination of both. The prothrombin gene (*F2* rs1799963 known as G20210A), Factor V Leiden (*F5* rs6025 known as G1691A) and PAI (rs1799768) are important polymorphic biomarkers of thrombophilia. Patients with multiple gene defects have a high risk of thrombosis [4]. Despite the hypercoagulation complications observed in NCP patients, there have been no previous investigations in terms of genetic thrombophilia predisposition. In this study, we examined thrombophilia factors that are thought to be effective in the clinical course of the disease. This study aimed to demonstrate the relationship between severe NCP and familial thrombophilia factors.

Material And Method

Adult (>18 years), previously healthy male patients treated for NCP NCP without any chronic disease who had severe complications were enrolled in this study to investigate well-known thrombophilia-related polymorphisms in Factor II (prothrombin), Factor V (F5) and the PAI gene. The diagnosis of NCP was made according to the World Health Organization interim guidance [5] and confirmed by RNA detection.

Blood samples taken from individuals were collected into tubes with EDTA and kept at 4 °C until DNA isolation. DNA isolation was performed on the MagPurix I12 (Zinext Life Science coop., New Taipei, Taiwan) device with the MAGPurix (Zinext Life Science coop., New Taipei, TAIWAN) whole blood extraction kit. After DNA isolation, measurements were made in a Nanodrop (Thermo Scientific, Massachusetts, USA) for quality control.

PCR procedures were performed for amplification after DNA isolation from the blood of individuals. Polymorphisms were investigated via PAI-1 Real-Time Hb-FRT, Factor II Real-Time HD-FRT and Factor V Leiden Real-Time HD-FRT kits (DIAGEN Biotechnological Systems Inc., Ankara, Turkey) by the Real Time PCR method on Qiagen Rotor Gene (Qiagen, Hilden, Germany) device in Detagen Genetic Diagnosis, Research And Application Center Inc., Kayseri, Turkey. The analysis was performed by using the Rotor Gene Q series software (Qiagen, Hilden, Germany).

The frequency of genotypes was compared with healthy control group frequencies from other studies performed in a Turkish population [6-8]. For comparison of groups, the chi-square test was performed by using IBM SPSS statistics 22 software with a p value of less than 0.05 considered statistically significant.

Result

Sixty-two patients who met the criteria during the study were included in the study. The mean age was 38.83 ± 11.04 (min. 18, max. 60). There was no significant difference between groups regarding rs1799963 (*FII*) and rs6025 (*FV* Leiden) (**Table 1**). While there was a statistically significant difference between groups in rs1799768 (*PAI-1*), it was considered a coincidence and non-important finding because of the small sample size; in detail, it was observed that the main cause of the difference between groups was the low ratio of 5G/5G (%12.6) in one of the control groups [6], which is not in accordance with our hypothesis (**Figure 1**). There was no significant difference between groups in the 4G/4G genotype.

Discussion

The risk of thrombosis and arterial and venous thromboembolic complications seen in 30% of hospitalized subjects due to NCP has been reported in many studies, which can be explained by the prolonged inflammatory response, decreased physical activity during infection, and reduced oxygen levels in the circulation. Some reports raise the alarm regarding this complication, such as increased thromboembolism incidence despite prophylaxis [9], sevenfold increase in large vessel stroke in some patients who experienced either no or mild COVID-19 symptoms [10], cerebral infarct occurrence in NCP diagnosed patients with thrombocytopenia, coagulopathy, and increased anticardiolipin antibodies [11], which is very worrying and needs further investigation of the molecular basis of this phenomenon.

In the literature, lung thromboembolism as well as thrombus in different localizations has been reported in NCP cases [12,13]. Sharon et al. also detected fibrin thrombi in small vessels and capillaries and argued that it would be beneficial to use agents that also treat thrombotic and microangiopathic effects caused by the virus [14].

According to the observations of some physicians in Turkey, it is suspected that genetic factors are affecting the course of NCP because of cases who are relatives and have similar severe clinical complications despite living in different provinces. Again, in these observations, the presence of young people who died at a young age and with a severe clinic was observed, although they did not have a sub-disease. Additionally, the existence of asymptomatic NCP-positive young and old people one more time confirms the genetic predisposition hypothesis.

Clinical observation of intensive care unit (ICU) physicians from all over the world indicates that NCP-diagnosed patients are very hypercoagulable and that there is a high rate of micro-pulmonary embolism between patients. There is also the possibility that some patients had already pulmonary embolism before hospitalisation who were not responsive to prophylactic doses of heparins during their hospital stay [15]. Additionally, many other studies indicate that abnormal coagulation results are (especially elevated D-dimer) common in NCP-related deaths [16-19]. Considering all of the abovementioned factors, it is possible that one of the possible susceptibility factors of severe NCP is genetic predisposition to thrombophilia, and it was considered that there can be a relation between well-known single nucleotide polymorphisms of *FII*, *FV* and *PAI-1* genes and investigated in the current study.

There are many studies showing [20] relationships between genetic thrombophilia factors (*FV*, *PAI-1*, etc.) and disseminated intravascular coagulation, which is frequently seen in severe NCP and is often associated with mortality [21]. Galinski et al. investigated the viral pathogenesis of SARS-coronavirus disease and suggested that *PAI-1* plays a protective role against infection [22].

According to Fatma Berri et al. [23], 6-aminocaproic acid can protect against influenza, as plasminogen contributes to inflammation caused by influenza. The application of aggressive anticoagulation by using inexpensive antithrombotic drugs is very attractive [15], but finding genetic markers before using it is essential, and such markers would be very important for the determination of people who are susceptible to severe NCP primary infection.

Conclusion

While investigating the frequency of suspected common SNPs of *FII*, *FV* and *PAI-1* genes in this study, there were no statistically significant differences between the severe patient group and healthy population in SNPs. As there is an obvious relation between severe NCP and

genetic thrombophilia susceptibility and this study is the first to rule out the relation of rs1799963 (*FII*), rs6025 (*FV*) and rs1799768 (*PAI-1*) with severe NCP; there is a need for studies focused on other thrombophilia-related genetic factors and disease.

Declarations

Funding: Kayseri State Hospital, Education expenses budget

Conflicts of interest: The authors state that there is no conflict of interest.

Ethics approval: The study was approved by the Ethics Committee of Kayseri State Hospital (14.05.2020/57).

Consent to participate: Informed consent was obtained from all individual participants included in the study.

Consent for publication: The participant consented to publishing their clinical data.

Availability of data and material: Not applicable

Code availability: Not applicable

Authors' contributions: AK and SG conceived the study, helped with interpretation of data, and wrote the manuscript together with AB who performed statistical analyses. SG, EE and MG provided the procurement of the study group patients. AK continuously supported the collection and analysis of samples. All authors read and approved the final version of this manuscript.

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Table

Table 1. Genotype comparison between groups.

	<i>FII</i> (rs1799963)			p	<i>FV</i> (rs6025)			p	<i>PAI-1</i> (rs1799768)			p
	WT	Het	Hom		WT	Het	Hom		4G/4G	4G/5G	5G/5G	
NCP diagnosed patients (n=62)	59 (%95)	3 (%5)	0	0.66*	56 (%90.3)	5 (%8)	1 (%1.7)	0.057*	13 _{a,b} (%21)	29 _b (%46.8)	20 _a (%32.3)	0.003**
Erten et al. 2015 (n=238) [6]	228 (%95.8)	10 (%4.2)	0		217 (%91.2)	21 (%8.8)	0		60 _a (%25.2)	148 _a (%62.2)	30 _b (%12.6)	
Yilmaz et al. 2014 (n=89) [7]	86 (%96.6)	3 (%3.4)	0		-	-	-		18 _a (%20.2)	48 _a (%53.9)	23 _a (%25.8)	
Şahin et al. 2012 (n=109) [8]	106 (%97.2)	3 (%2.8)	0		90 (%82.6)	16 (%14.7)	3 (%2.7)		-	-	-	

* Heterozygous and homozygous values combined due to some of the values not being greater than 1.

**Each subscript letter denotes a subset of Genotip categories whose column proportions do not differ significantly from each other at the .05 level.

Figures

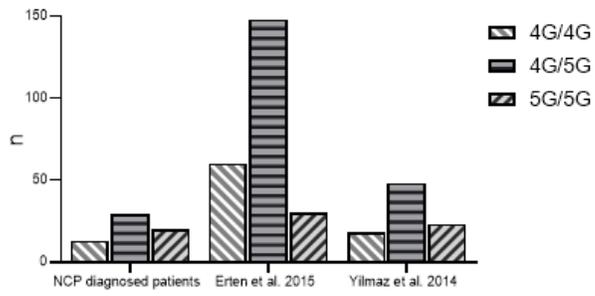


Figure 1

rs1799768 (PAI-1) ratio within groups