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Is there a role of genetic tendency in post-COVID pulmonary thromboembolism?

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ABSTRACT

Aims: COVID-19 is a multisystemic disease characterized by endothelial dysfunction. The improper activation of the coagulation cascade may lead to thromboembolic events, which are presumed to contribute to the disease's overall high morbidity and mortality. This research examines the role of thrombophilia mutations in patients diagnosed with post-COVID pulmonary thromboembolism.

Methods: Between May 2020 and December 2020, 61 patients were diagnosed with pulmonary thromboembolism (PTE). Thirty-two patients were positive in COVID-19 -RT-PCR testing, and 29 patients were identified with non-COVID PTE. All PTE diagnoses were made by thorax computed tomographic angiography. Demographic characteristics, genetic mutation results, and laboratory values of the patients were retrospectively evaluated.

Results: The median age of patients was 56 years (25-81), and most patients (n=43,70.5%) were male. There was no difference between factor 5 Leiden mutation, while prothrombin 20210A mutation was more commonly observed in post-COVID patients ($p<0.05$). Between the two groups, no difference was observed regarding MTHFR gene mutation, anticardiolipin and antiphospholipid antibodies, protein S, and protein C values. D-dimer values were statistically higher in the post-COVID PTE group ($p<0.05$). As seen in the study, we may state that patients with post-COVID PTE had a higher diagnosed prothrombin 20210A and more elevated D-dimer values compared to non-COVID-related PTE patients.

Conclusion: In our study, we found that D-dimer values were higher in patients with post-COVID PTE than in patients with non-COVID PTE, and prothrombin 20210A mutation was more common in the post-COVID PTE patient group. We believe that further studies with a larger study group are needed to elucidate this issue.

Keywords: COVID-19, D-dimer, factor 5 Leiden mutation, post-COVID pulmonary thromboembolism, thrombophilia mutations

INTRODUCTION

The novel SARS-CoV-2 virus first appeared in Wuhan, China, in December 2019. SARS-CoV-2 expanded internationally despite efforts to stop it from doing so, creating a public health emergency. Thus, the World Health Organization (WHO) designated the novel coronavirus infection as a pandemic in March 2020.[1] SARS-CoV-2 was later defined as the Coronavirus of 2019, COVID-19, and was reported as a disease correlated to coagulopathies that may cause arterial and venous thromboembolic events.[2]

Among COVID-19 patients, thrombosis is one of the most severe complications that may occur and is reported as one of the most likely causes of sudden COVID-19 death.[3] Many causes, genetic or acquired, may lead to thrombosis, however, hypercoagulation and hypofibrinolysis are among the main common pathologies observed causing thrombosis. Patients with COVID-19 typically experience severe hypercoagulability in their lungs, and pulmonary arteries are the site of most vascular thrombotic complications. Severe coagulation abnormalities, in particular fibrin and platelet-rich thrombi, were seen in post mortem lungs from SARS-CoV-2 infected individuals. These abnormalities were not seen in non-COVID-19 autopsy controls.[4] Etiologically, thrombophilia may manifest in two different pathways, acquired and hereditary. However, a clear cut cannot be made in many cases, especially in patients with already known hereditary mutations.[5]

Three factors are required for hemostatic balance: an intact vessel wall, adequate blood coagulability, and a functioning hemostasis system. Hemostasis may decline as a result of pathologies that affect anticoagulant and fibrinolytic systems. The final outcome often presents itself as deep vein thrombosis (DVT) or pulmonary thromboembolism (PTE). Many thrombophilia risk factors have been defined, with Prothrombin G20210A and factor V Leiden gene mutation

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being accepted as the most common inherited ones for venous thromboembolism.[6] Other known risk factors are deficiencies in coagulation pathways, such as protein S, Protein C, and antithrombin 3 deficiencies. The mutant form of factor V is known as factor V Leiden (FVL), which disrupts the functional interaction between factor V and protein C.[7] This leads to resistance in activated protein C, which in turn increases the risk of thrombosis. FVL is the most frequent genetic cause of unexplained venous thrombosis. As it is very common and due to being the most frequent genetic cause, patients with COVID-19 could be evaluated for the mutation, and if observed, appropriate treatment may be utilized to reduce venous thromboembolic morbidity. G20210A, on the other hand, is caused by a point mutation and is used to define a prothrombin subtype. The presence of prothrombin G20210A increases the concentration of overall prothrombin, and as a result, this autosomal dominant disease predisposes the patient to both arterial and venous thrombosis.[8]

In this study, patients diagnosed with PTE with either post-COVID-19 infection or non-COVID-19-related were evaluated to investigate the role of mutations in PTE.

METHODS

The study was performed after the approval from the Ankara Oncology Training and Research Hospital Ethics Committee (Date: 05.09.2024, Decision No: 2024-09/128). This research was carried out in conjunction with the Helsinki Declaration (as revised in 2013). Sixty-one patients diagnosed with PTE between dates of May 2020 and December 2021 were evaluated in the study, with 32 patients being positive for COVID-19 RT-PCR and the remaining defined as non-COVID-related PTE. All PTE diagnoses were made by thorax computed tomographic angiography. Demographic characteristics, genetic mutation results, and laboratory values of the patients were retrospectively evaluated. Doppler ultrasonography of the lower extremity was performed on all patients to investigate possible deep vein thrombosis as an additional risk factor for PTE. All included patients were older than 18 years old.

Inclusion criteria for the study:

- Patients over the age of 18,
- Patients diagnosed with PTE and being followed up,
- Patients whose demographic information, additional disease information, laboratory values, genetic mutation results, tomography reports and 1-year prognosis information could be accessed from the hospital system or patient files were included in the study.

Exclusion criteria for the study:

- Patients whose investigated criteria could not be accessed from the patient file or hospital information system,
- Patients diagnosed with malignancy,
- Patients with active infection other than COVID-19 infection were not included in the study.

Statistical Analysis

The initial data were first evaluated by Kolmogorov-Smirnov test for distribution pattern, with histograms used when deemed necessary. Student's t-test was used for parametric variables, whereas Mann-Whitney U test was used for nonparametric analysis, with results being given as mean with standard deviation or median with maximum and minimum values, respectively. IBM SPSS (Statistical Package for the Social Sciences) statistics (version 22) was used for statistical analysis. It was stated that if there is a p-value below 0.05, it would be taken as statistically significant.

RESULTS

All sixty-one patients evaluated in the initial period was included into the study. The mean age of the patients was 56 (25-81) years and the majority of the patients (n=43, 70.5%) were male (Table 1). Half of all patients were diagnosed and hospitalized for COVID-19 pneumonia before PTE diagnosis. None of the patients had a diagnosis of malignancy, immobilization or surgery that could have predisposed them to venous thromboembolic events. DVT incidence was lower in patients diagnosed with post-COVID PTE compared to those without COVID-related PTE. Before the diagnosis, 53.1% of all patients had been on an anticoagulant prophylaxis.

Table 1. Demographic findings and general characteristics of the patients

Parameters (n, %)		Post-COVID PTE (n=32)	Non-COVID PTE (n=29)	Total (n=61)	p value
Age (median, min-max)		56.50 (25-81)	51 (27-67)	56 (25-81)	0.333
Gender	Male	21 (48.8)	22 (61.1)	43 (70.5)	0.381
	Female	11 (61.2)	7 (38.8)	18 (29.5)	
Hospitalization before PTE due to COVID-19	Yes	16 (50)	-	16 (26.3)	-
	No	16 (50)		45 (73.7)	
Deep venous thrombosis	Yes	2 (6.3)	8 (27.6)	10 (16.4)	0.025
	No	30 (93.7)	21 (72.4)	51 (83.6)	
Anticoagulation history before PTE	Yes	17 (53.1)	-	17 (27.9)	-
	No	15 (46.9)		44 (72.1)	
Survival	Exitus	1 (3.1)	0 (0)	1 (1.6)	0.130
	Alive	31 (96.9)	29 (100)	60 (98.4)	
Hospitalization days (median, min-max)		8 (4-28)	7 (0-25)	8 (0-28)	0.337

PTE: Pulmonary thromboembolism, min: Minimum, max: Maximum

No difference was observed between two groups regarding factor V Leiden mutation, while the prothrombin 20210A mutation was significantly more common in post-COVID PTE patients ($p<0.05$). For other thrombophilia mutations; including MTHFR gene mutation, anticardiolipin and antiphospholipid antibodies, and protein S and protein C values, no significant difference was seen between two groups. The D-dimer values were higher in patients with post-COVID PTE diagnosis compared to those without, and the difference was found statsistically significant ($p<0.05$) (Table 2, 3).

DISCUSSION

Complex interactions between varying processes partake in the thrombosis development in COVID-19 patients, however exact individual components causing these complicatinos have yet to be understood. With our study, we aimed to stimulate further research to clarify the biological and clinical implications of inherited thrombophilic conditions in SARS-CoV-2 infection and to promote the idea of an optimal approach to anticoagulation in these cases.

In a study conducted in Sweden, roughly one million patients who tested positive for SARS-CoV-2 infection between the dates of February 1, 2020, and May 25, 2021, were examined and compared with over 4 million control subjects. The study reported that there was an elevated risk for pulmonary thromboembolism in the acute phase following COVID-19, along with increased incidence for DVT within three months, PTE within six months, and bleeding risk within two months following infection history. Overall, the study reported that COVID-19 was a risk factor for DVT, PTE, and hemorrhage.[9]

In a meta-analysis consisting of 20 studies, a total of 1988 COVID-19 patients were evaluated, and 30% of the patients had VTE, 20% had DVT, and 18% had PTE. Similar rates were observed when patients in intensive care units and under antithrombotic prophylaxis were evaluated as a subgroup analysis. The study also reported that a higher body weight was linked to a higher incidence of PTE, and the elderly population was more suspectable to VTE, including PTE and DVT. Male gender was not found a risk factor.[10] Other studies reported that in patients with severe COVID-19 diagnosis, a longer prothrombin time (PT), higher D-dimer values, and lower platelet counts were observed.[11-14] Currently, available literature summarizes that coagulopathy seen in severe COVID-19 would be caused by a combination of localized thrombotic microangiopathy and low-grade disseminated intravascular coagulopathy (DIC).[15]

Many studies have reported that in viral infections, levels of coagulation molecules including factor XI, factor VIII,

Table 2. Mutation and thrombophilia factor comparison between two groups

Parameters (n, %)		Post-COVID PTE (n=32)	Non-COVID PTE (n=29)	Total (n=61)	p value
Factor V leiden mutation	Heterozygote	2 (6.3)	2 (6.9)	4 (6.6)	0.315
	Homozygote	0 (0)	2 (6.9)	2 (3.3)	
	Normal	30 (93.7)	25 (86.2)	55 (90.1)	
Prothrombin 20210A mutation	Heterozygote	5 (15.6)	0 (0)	5 (4.9)	0.032
	Homozygote	0 (0)	2 (3.3)	2 (6.9)	
	Normal	29 (84.4)	27 (93.1)	56 (91.8)	
MTHFR gene 1 mutation	Heterozygote	8 (25)	5 (17.2)	13 (21.3)	0.358
	Homozygote	5 (15.6)	2 (6.9)	7 (11.5)	
	Normal	19 (59.4)	22 (75.9)	41 (67.2)	
MTHFR gene 2 mutation	Heterozygote	6 (18.8)	5 (17.2)	11 (18)	0.534
	Homozygote	5 (15.6)	2 (6.9)	7 (11.5)	
	Normal	21 (65.6)	22 (75.9)	43 (70.3)	
Anticardiolipin IgM	Positive	0 (0)	0 (0)	0 (0)	-
	Negative	32 (100)	29 (100)	61 (100)	
Anticardiolipin IgG	Positive	0 (0)	0 (0)	0 (0)	-
	Negative	32 (100)	29 (100)	61 (100)	
Antiphospholipid IgM	Positive	0 (0)	0 (0)	0 (0)	-
	Negative	32 (100)	29 (100)	61 (100)	
Antiphospholipid IgG	Positive	0 (0)	0 (0)	0 (0)	-
	Negative	32 (100)	29 (100)	61 (100)	
Parameters (mean, SD)					
Protein C (IU/dl)		74.28 (±5.58)	73.51 (±7.02)	73.91 (±6.26)	0.639
Protein S (IU/dl)		71.9 (±8.12)	71.37 (±5.24)	71.65 (±6.85)	0.767
Antithrombin 3 (%)		71.65 (±4.75)	72.48 (±9.98)	72.04 (±7.64)	0.677
Fibrinogen (mg/dl)		308.46 (±46.83)	301.72 (±44.36)	305.26 (±45.42)	0.567

PTE: Pulmonary thromboembolism, MTHFR: Methylenetetrahydrofolate reductase, SD: Standard deviation, IgM: Immunoglobulin M, IgG: Immunoglobulin G, SD: Standard deviation

Table 3. Comparison of laboratory results between two groups				
Parameters (median, min-max)	**Post-COVID PTE (n=32)**	**Non-COVID PTE (n=29)**	**Total (n=61)**	**p value**
WBC (10^3/µl)	8.6 (2.82-18.26)	9.63 (5.36-24.85)	9.23 (2.82-24.85)	0.038
NLR	3.06 (0.91-16.8)	3.69 (1.36-20.69)	3.2 (0.91-20.69)	0.239
Hemoglobin (g/dl)	13.25 (8-16.8)	14.5 (11.1-17.3)	13.7 (8-17.3)	0.054
Platelet (10^3/µl) (mean, SD)	246.03 (±77.40)	248.03 (±88.54)	246.98 (±82.19)	0.925
Uric acid (mg/dl)	4.95 (3.3-9.7)	5 (1.7-7.7)	5 (1.7-9.7)	0.448
LDH (IU/L)	244.5 (147-558)	283 (151-1134)	270 (147-1134)	0.309
Albumin (g/L)	39.9 (18.7-47)	37.5 (27.4-48)	38.1 (18.7-48)	0.319
D-dimer (µg FEU/L)	2.88 (0.23-17.85)	1.51(0.22-9.13)	1.9 (0.22-17.85)	0.030

PTE: Pulmonary thromboembolism, WBC: White blood cell, NLR: Neutrophile lymphocyte ratio, LDH: Lactate dehydrogenase, SD: Standard deviation, FEU: Fibrinogen equivalent unit

Von Willebrand factor, soluble tissue factor, prothrombin fragment 1β 2 and thrombin-antithrombin complexes, platelet activation, and fibrin degradation products were found to be increased.[16,17] The link between the release of cytokines and the coagulation cascade was assumed to be the cause of these findings.[18] Patients with severe COVID-19 disease symptoms typically have high D-dimer, fibrin degradation products, fibrinogen, and low antithrombin levels.[19] These findings could be attributed to the discussed studies, and similarly, in our study, D-dimer values were significantly higher in patients with post-COVID PTE. The prolongation of the inflammatory process and the development of endothelial dysfunction due to cytokine storm may explain microthrombus formation in critically ill patients, and a similar process could be assumed for patients with post-COVID history and their higher D-dimer levels.

Setefely et al.[20] reported in a study with 102 hospitalized COVID-19 patients that Factor V activity was considerably higher in COVID-19 patients compared to concurrent controls. Patients with a Factor V activity over 150 IU/dl had a significantly higher DVT and PTE incidence rate than those lower than the reported value.

In a study examining the association between hereditary thrombophilia and VTE due to COVID-19, patients diagnosed with hereditary thrombophilia, prothrombin G20210A and factor V Leiden mutations were investigated. Patients with hereditary thrombophilia were found to have a higher risk of VTE after SARS-CoV-2 infection than those without thrombophilia, despite all other demographic results, medication history, and comorbidities being similar within two groups.[21] In our study, in our study, no difference was observed between post-COVID and non-COVID PTE patients regarding factor 5 Leiden mutation incidence, whereas the prothrombin 20210 A mutation was observed more commonly in post-COVID PTE patients and was statistically significant. No difference was present between the two groups regarding MTHFR gene mutation, anticardiolipin and antiphospholipid antibodies, and protein S and protein C values.

A case series that suggested previously unidentified inherited thrombophilias are likely a contributing factor to mortality in COVID-19 individuals suggested that COVID-19 infection was a factor in death. Consequently, it was underlined that more investigation is required to clarify the association between thrombotic risk factors and to demonstrate that SARS-CoV-2 patients with genetic thrombophilias are more susceptible to thrombotic consequences.[22]

CONCLUSION

It is not possible to distinguish which patients may develop PTE during the post-COVID period. The assumption that those with a history of thrombosis or with known thrombotic risk factors would cause post-COVID PTE also does not have any adequate supporting evidence, compared to the general population.

In our study, we found that D-dimer values were higher in patients with post-COVID PTE than in patients with non-COVID PTE, and prothrombin 20210A mutation was more common in the patient group with post-COVID PTE. Although we obtained significant results, we believe that because of our limited number of patients and the study's single-centre nature, further studies with a larger study group are required to illuminate this topic.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of the Ankara Oncology Training and Research Hospital Ethics Committee (Date: 05.09.2024, Decision No: 2024-09/128).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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