Microbes and Infectious Diseases 2024; 5(1): 40-52

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

# Predictive role of methylenetetrahydrofolate reductase gene single nucleotide polymorphisms rs1801133 C/T and Serpin family E member 1 rs1799889 4G/5G for SARS-CoV-2 infection and severity of COVID-19 in Iraqi population

***Ghazwan Faisal Hussein*[1,2], Ali Hmood Al Saadi[1]***

*1- Department of biology College of Science, University of Babylon*

*2- Medical Services Department, Ministry of Interior, Iraq*

**ARTICLE INFO**

*Article history:*
Received 6 November 2023
Received in revised form 2 December 2023
Accepted 6 December 2023

**Keywords:**
COVID-19
PAI-1
Polymorphism
MTHFR
SERPINE-1

**ABSTRACT**

**Background:** This study aimed to detect two genetic polymorphisms, correlate each genotype of polymorphism with biomarker serum levels, and identify which genetic variation could increase susceptibility or be associated with the severity and mortality of Covid-19 infection by examining the following genetic polymorphisms: rs1801133 MTHFR, and rs1799889 SERPINE-1 (PAI-1). **Methods:** In this study, a total of 102 patients with COVID-19 (mean age 52.66±18.82) and 92 apparently healthy controls (mean age 37.88±14.19) were included. The single nucleotide polymorphisms (SNPs) in MTHFR (rs1801133) and SERPIN1 (rs1799889) were analyzed using ARMS-PCR and sequencing techniques. The levels of homocysteine (Hcy) and plasminogen activator inhibitor 1 (PAI-1) proteins were quantified using an ELISA. **Results:** Our findings suggest that MTHFR rs1801133 CT and TT genotypes are associated with increased COVID-19 risk and mortality. CT genotype is linked to higher susceptibility (OR = 1.56, $p = 0.0009$), and both CT and TT genotypes are linked to more severe illness (OR = 1.66, $p = 0.018$ and OR = 4.10, $p < 0.001$, respectively). PAI-1 rs1799889 4G/5G genotype correlates with COVID-19 risk and severity (OR = 2, $p = 0.03$ and OR = 2.7, $p = 0.04$, respectively). In a subgroup, CT/TT genotypes have elevated Hcy levels compared to CC genotype ($p = 0.00016$). Also, 4G/5G and 5G/5G genotypes have lower PAI-1 levels than 4G/4G genotype. **Conclusion:** A significant association was found between gene variations in MTHFR and SERPINE1 genes and COVID-19 infection susceptibility and severity.

## Introduction

The pandemic of Coronavirus Disease 2019 (COVID-19), also known as SARS-CoV-2, has been declared a global public health emergency. In January 2020, the disease first appeared in China [1]. Although SARS-CoV-2 shows similar transmission patterns and pathogenesis, COVID-19 distributions, and outcomes differ significantly between countries despite the same mode of transmission [2]. COVID-19 is primarily screened by reverse transcription polymerase chain reaction (RT-PCR) [3], while in some European centers using computed tomography imaging (CT) [4,5].

Despite the well-defined clinical and imaging features of COVID-19, there are still gaps in our understanding of chronic systemic diseases. It is well known that COVID-19 has the potential to cause severe thrombotic complications, including pulmonary and renal microangiopathy, deep vein thrombosis (DVT), pulmonary embolism (PE), arterial and venous thromboembolism, acute

DOI: 10.21608/MID.2023.246896.1651
* *Corresponding author:* Ghazwan Faisal Hussein
E-mail address: *ghfa2013@gmail.com*

ischemic stroke, venous and arterial catheter thrombosis, as well as disseminated intravascular coagulation (DIC) syndrome. It is believed that acute thrombophilic conditions occur due to activation of inflammatory pathways or due to endotheliitis that triggers thrombogenesis [6]. A correlation has also been found between severity of the disease and thrombotic complications [7].

There is a limited amount of data available on how certain prothrombotic risk factors may contribute to the severity of COVID-19. The Methylenetetrahydrofolate reductase (MTHFR) gene is located on chromosome 1 at 1p36.6 and is responsible for encoding the 5,10-methylene-tetrahydrofolate reductase enzyme. Polymorphisms of the MTHFR gene have been reported to increase the risk of developing DVT via homocysteine metabolism. Several common MTHFR alleles lead to MTHFR enzyme deficiency, such as C677T rs1801133. As COVID-19 causes a hyper-inflammatory response in the body, procoagulant factors are elevated in the blood and anticoagulant factors are decreased, promoting blood clots. Furthermore, the SARS-CoV-2 virus causes vascular inflammation and further increases clotting risk by directly damaging endothelial cells [8,9].

Several studies have linked the methylenetetrahydrofolate reductase gene (MTHFR) to increased plasma homocysteine levels, which were correlated to myocardial infarction (MI) [10]. Recent studies hypothesize that, besides serological and clinical biomarkers that correlate clearly with severe clinical course of COVID-19 infection [11,12], homocysteine (Hcy) is also an important prognostic indicator [13,14]. In different populations with different ethnic predominances, MTHFR gene mutations and MTHFR activity are prevalent at different rates. HCY plays an important role in several metabolic and inflammatory processes [15]. Although the recent Omicron surge has decreased mortality, disparities in age and sex persist, with AMI-associated mortality being greater among young adults [16]. It is unclear if the MTHFR rs1801133 variant is associated with COVID-19 susceptibility or severity.

The gene known as Serpin Family E Member 1 (SERPINE1), which produces the plasminogen activator inhibitor-1 (PAI-1) protein, can be found on chromosome 7 at 7q21.3-q22. Variations in the SERPINE1 gene can affect the activity of the PAI-1 protein has been linked with an increased risk of various health conditions, such as cardiovascular disease, stroke, and arterial and venous thrombosis have been linked to variant (rs1799889 -675 4G/5G) polymorphism [17–19]. In this genetic variant, there is an insertion or deletion of a guanine (G) base in the promoter region of the gene, leading to its commonly known name as the PAI-1 4G/5G variant. The presence of the 4G allele has been associated with elevated levels of PAI-1, which can disrupt fibrinolysis and contribute to the occurrence of thrombosis. A study has explored the 4G/5G gene polymorphism and identified an association with post-SARS-COV-1 osteonecrosis, which arises as a consequence of thrombotic events [19].

These genetic variants can affect the regulation of coagulation and fibrinolysis, therefore affecting the risk of thrombosis and associated conditions. These variants may affect an individual's risk of disease, although other factors such as age, sex, lifestyle, and medical history can also affect their risk. A genetic test for these variants could be recommended if there is a history of thrombosis in the family or if other factors increase the risk of thrombosis [20–22].

The aim of this study was to examine the role of specific polymorphisms in MTHFR (rs1801133) and SERPINE1 (rs1799889) in the development and severity of COVID-19.

## Material and methods

### Subjects' selection

In this study, a total of 102 patients were enrolled between February 2022 and June 2022. This group included 57 individuals who were hospitalized and 45 who were not, all of whom had tested positive for SARS-COV2 through PCR tests on nasopharyngeal swabs or lower respiratory tract samples. The study also included 92 healthy individuals as controls, who were selected based on their similar ethnic and regional backgrounds with patients. Individuals who were under 16 years old, had respiratory diseases, or had undergone chemotherapy within the past 3 months were excluded based on the study's exclusion criteria.

The WHO has established clinical criteria for assessing the severity of COVID-19 based on signs and symptoms. Severe cases are those that require hospitalization and present clinical indications of pneumonia, or other severe respiratory distress symptoms. On the other hand, non-severe cases are those that do not require

hospitalization and have no risk factors for developing severe illness [23]. The classification of COVID-19 severity was established according to the subsequent criteria:

1. Non-severe: Distinguished by mild clinical symptoms such as fever and cough, coupled with an absence of pneumonia upon lung CT scan.

2. Severe: Evidenced by the presence of respiratory distress (respiratory rate exceeding 30/min), resting oxygen saturation of 90% or below, and requiring mechanical ventilation due to respiratory failure. This category also encompasses conditions of shock and non-pulmonary organ failure, potentially leading to admission to an intensive care unit (ICU) [24]. The Participants were selected from Al Diwaniyah teaching hospital, Al-Hussein teaching hospital, and Marjan medical city located in the Provinces of the Middle Euphrates in Iraq.

**DNA extraction and genotyping assay**

EDTA-containing tubes were used to collect blood samples from all participants. Genomic DNA was then extracted from the samples using a kit (GENEAID, Taiwan) in accordance with the instructions provided by the manufacturer. The purity and concentration of DNA were measured using absorbance measurements with the NanoDrop spectrophotometer 2000 (Thermo Scientific). The genotyping of MTHFR (rs1801133) and SERPINE1 (rs1799889) polymorphisms was performed using the ARMS-PCR technique, also known as the allele-specific polymerase chain reaction (AS-PCR) method can be performed using the primers listed in **Table (1)**.

**Polymerase Chain Reaction (PCR)**

The following PCR reaction was conducted using a total volume of 25 µl: 5 µL of genomic DNA, 2 µL of each primer (10 µM) in the first reaction wild and common while in a second mutant and common, and 12.5 µL of GoTaq® G2 Green Master Mix (Promega, USA) and 3.5 µL ddH2O. **Table (2)** summarizes the PCR conditions of this study.

**DNA sequencing technique**

To confirm the accuracy and validity of genotyping information obtained from the AS-PCR method, the PCR product was shipped to Macrogen, a South Korean company, for Sanger sequencing. The resulting sequences were then analyzed using MEGA (V.11) software to identify any SNPs of present investigation.

**Enzyme-linked immunosorbent assays (ELISA) measurements**

The levels of Homocysteine and PAI-1 in the serum samples were determined using ELISA assay kits obtained from the bioassay technology laboratory (BT-lab company, China) to measure the level of each genotype with increased or decreased protein levels to impact risk of Covid-19 and severity, a sample of the patient's peripheral blood was taken and Samples were collected in tubes containing SST-Gel/clot activator and were allowed to clot for a designated period of time. the appropriate amount of time at room temperature under sterile conditions by a laboratory technician and then centrifuging the sample for 10–15 min at a speed of 1500 rounds per minute (rpm) to determine the biomarkers in this study.

**Ethical consideration**

Prior to sample collection, the study received verbal and written consent from patients after obtaining approval from a local ethics committee at the College of Science, University of Babylon. The committee reviewed and endorsed the study protocol, subject information, and consent form, following the regulations of the Ministry of Health in Iraq.

**Study sample size**

The study's sample size was determined by utilizing Raosoft online software, which considered typical reports and an acceptable margin of error ranging from 4-8%. With a margin of error of 6%, the recommended sample size was 194 individuals.

**Statistical analysis**

Statistical analysis involved presenting categorical data as numbers (percentage) and continuous variables as either mean or standard deviation (SD) or median and inter-quartile range (IQR) using SPSS 27.0 software package. Logistic regression analyses were performed to assess the odds ratios (ORs) and 95% confidence intervals (CIs) under different genetic models, aiming to explore the association between the studied polymorphisms and the risk of COVID-19 infection as well as disease severity. A p-value of less than 0.05 was considered statistically significant.

**Results**

**Characteristics that vary between patients and controls**

The analysis involved 102 confirmed COVID-19 patients, comprising 48 males (47%)

and 54 females (53%). Additionally, 92 apparently healthy individuals were part of the study, with 54 males (58.7%) and 38 females (41.3%). Age data for patients and controls were presented as mean ± SD values, indicating patients' mean age as 52.66±18.82 and controls' mean age as 37.88±14.19. The median age (IQR) for patients was 53 (36-69), while for healthy individuals, it was 31 (24-43) as shown in **Table (3)**.

The study additionally scrutinized the demographic descriptors of COVID-19 patients categorized by disease severity, with a summary provided in **Table (4)**. The findings indicated no notable gender distribution disparities between severe and non-severe cases (***p*** = 0.31). However, a statistically significant age discrepancy was identified in our study (***p***-value<0.05).

**Association between MTHFR and SERPINE1 genetic variation, risk and severity of COVID-19**

The results presented in **Table (5)** indicate significant associations for the MTHFR rs1801133 polymorphism in individuals with COVID-19 infection. Particularly, the T allele's relative risk was found to be significantly different compared to the C allele, with an odds ratio of 1.76 (95% CI: 1.15–2.69, ***p*** = 0.008). Moreover, both heterozygotes and mutant genotypes displayed odds ratios of 1.93 (95% CI: 1.04–3.6, ***p*** = 0.03) and 2.58 (95% CI: 1.07–6.22, ***p*** = 0.034), respectively. The distribution of the genotypes and allelic frequencies of SERPINE1 rs1799889 (4G/5G) were analyzed the 4G/5G genotype frequency in the patients with COVID-19 disease was compared to the control subjects (OR = 2; 95% CI = 1.06 - 3.94; ***p*** < 0.05).

Our findings indicated that in the codominant model, genotypes CT and TT of rs1801133 were associated with an increased risk of COVID-19 development (OR 2.6, 95% CI 1.06–6.38, ***p*** = 0.03 and OR 9.4, 95% CI 2.3-38.5, ***p*** = 0.0001, respectively). Similarly, in the context of the dominant genetic model for genotypes CT+TT vs. CC, rs1801133 was found to be a risk factor for COVID-19 (OR 3.53; 95% CI 1.51–8.28, ***p*** = 0.003), as well as in the recessive model TT vs. CT+CC (OR 5.46; 95% CI 1.48–20.16, ***p*** = 0.01). Additionally, our statistical analysis demonstrated a significant difference in the association of the C and T alleles in rs1801133 (MTHFR) with the severity of symptoms (p< 0.001, OR = 2.88 (1.6-5.21). While the SERPINE1 rs1799889 4G/5G polymorphism, the 4G/5G genotype was more likely to appear in patients in the codominant and over-dominant models as risk factor to increase severity of disease (4G/5G OR = 2.72, 95% CI = 1.02–7.25, ***p*** = 0.04 and 4G/4G+5G/5G vs. 4G/5G OR = 4.37, 95% CI = 1.87–10.22, ***p*** = 0.001). In the recessive inheritance model, meanwhile, the 5G/5G genotype was more frequent among severe cases (59.6%) than non-severe (35.5%) and had protective effect in patients with non-severe (OR = 0.19, 95% CI = 0.07–0.51, ***p*** = 0.001), as presented in Table (6).

To ascertain the sequence arrangement of rs1801133 bands seen in **Figures (1 and 2)**, DNA samples were PCR-amplified, followed by separation via 2% agarose gel electrophoresis. Sanger sequencing was then employed. A subset of samples underwent random selection, showcasing bands on agarose gel electrophoresis. These samples were subsequently subjected to sequencing procedures, depicted in **Figures (3 and 4)**.

**Associations of SNPs with serum levels of Hcy and PAI-1**

To assess whether the MTHFR-SNP, rs1801133, we measured mean the production of homocysteine in-vivo in the three genotypes CC, CT, and TT. The results showed carriers with TT and CT genotypes displayed significantly higher serum levels of Hcy as compared to the wild CC genotype mean ± SD (11.3 ± 4.6, 8.6±6.1, 3.9 ± 1.8 nmol/mL, respectively) ***p***< 0.001, as in **Figure (5)**. In relation to the SERPINE1 (4G/5G; rs1799889), there was a significant difference in PAI-1 serum levels with genotypes (***p***<0.05), the 5G/5G genotype exhibited decreased levels of PAI-1(3.22±0.7 ng/ml) compared to 4G/5G and 4G/4G (6.73±4.19 and 5.94±1.16 ng/ml, respectively) as shown in **Figure (6)**.

**Table 1.** Primer sequences for the SNPs of interest (rs1801133 and rs1799889) were designed using an online tool

| Polymorphisms | Primers sequences (5'-3') | Product size |
|---|---|---|
| *MTHFR*/rs1801133<br>Wild Reverse Primer<br>Mutant Reverse Primer<br>Common Forward Primer | <br>GAAGGTGTCTGCGGGAGC<br>GAAGGTGTCTGCGGGAGT<br>AGTTCTGGACCTGAGAGGAG | 266 bp |
| *SERPINE1*/rs1799889<br>Wild Reverse Primer<br>Mutant Reverse Primer<br>Common Forward Primer | <br>TCCGATGATACACGGCTGAAT<br>CCGATGATACACGGCTGAAC<br>GTCTGTGTCTGGAGGAAGAG | 255 bp |

Abbreviations: bp: bases pair, rs: reference number

**Table 2.** The PCR cycling conditions used in the experiment.

| Step | | Temperature (°C) | Time |
|---|---|---|---|
| Initial Denaturation | | 95 | 4 min |
| Denaturation | | 95 | 30 sec |
| Annealing | rs1801133 | 58 | 45 sec |
| | rs1799889 | 59 | |
| Extension | | 72 | 1 min |
| Final Extension | | 72 | 5 min |
| Cycle Number | | 34 | |

Abbreviations: °C: Celsius (°C), rs: reference number

**Table 1.** The demographic characteristics of 102 patients with COVID-19

| Characteristics | Total patient n (102) | Severe group n (57) | Non-severe group n (45) | ***p***-value |
|---|---|---|---|---|
| Age, median (IQR), year | 53(36-69) year | 67(60-75) year | 37 (28-42) year | ***p***<0.05* |
| Gender | Male/48(47.1%)<br>Female/56(52.9%) | Male/33(68.8%)<br>Female/24(44.4%) | Male/15(31.3%)<br>Female/30(55.6%) | ***p***>0.05 |

**Table 2.** Clinical attributes of the investigated COVID-19 subjects

| Characteristics | Total patient n (102) | Severe group n (57) | Non-severe group n (45) | p-value |
|---|---|---|---|---|
| Oxygen support | 47(46%) | 0 | 47(82.4%) | -- |
| Vaccination status | 41(40.1%) | 29(64.4%) | 12(21%) | P<0.05* |
| Comorbidity: | 49(48%) | 32(68.3%) | 17(34.6%) | P<0.05* |
| ▪ Hypertension | 14(13.7%) | 11(78.5%) | 3(21.42%) | P<0.05* |
| ▪ Diabetes Meletus | 15(30.6%) | 9(60%) | 6(40%) | P<0.05* |
| ▪ Cardiovascular diseases | 9(18.3%) | 4(44.4%) | 5(55.5%) | p>0.05 |
| ▪ Kidney diseases | 6(12.2%) | 5(83.3%) | 1(16%) | P<0.05* |

Abbreviations: P: probability, *: significant at the 0.05 level, IQR: Interquartile range

**Table 5.** The genotypes and alleles frequency were assessed in both the control and case groups

| Polymorphism | Genotypes | Control, n(%) | Patients, n(%) | OR (95%CI) | P |
|---|---|---|---|---|---|
| *MTHFR* rs1801133 | CC® | 49(53.3) | 36(35.2) | 1.00 (Ref.) | -- |
| | CT | 33(35.8) | 47(46.1) | 1.93(1.04-3.6) | 0.03* |
| | TT | 10(10.9) | 19(18.7) | 2.58(1.07-6.22) | 0.034* |
| | Allele frequency<br>C® | 131(71.1) | 119(58.3) | 1.00 (Ref.) | -- |
| | T | 53 (28.9) | 85 (41.7) | 1.76 (1.15-2.69) | 0.008* |

| | | | | | |
|---|---|---|---|---|---|
| *SERPINE1 rs1799889* | 4G/4G® | 39(42.4) | 29(28.4) | 1.00 (Ref.) | -- |
| | 4G/5G | 31(33.6) | 47(46.1) | 2 (1.05-3.94) | 0.03* |
| | 5G/5G | 22(24) | 26(25.5) | 1.58 (0.75-3.34) | 0.22 |
| | Allele frequency | | | | |
| | 4G® | 109(59.2) | 105(51.5) | 1.00(Ref.) | -- |
| | 5G | 75 (40.8) | 99(48.5) | 1.37 (0.91-2.04) | 0.12 |

Abbreviations: P: probability, *: significant at the 0.05 level, OR: odd ratio, ®: reference.

**Table 6.** The association between MTHFR and SERPINE1 genotypes and the severity of COVID-19 disease was examined using five different inheritance models

| Polymorphism | Genetic models | Severe, n (%) | Non-severe, n (%) | OR (95%CI) | ***p*** |
|---|---|---|---|---|---|
| *MTHFR* rs1801133 | Codominant | | | | |
| | CC® | 13(22.8) | 23(51.1) | 1.00(Ref.) | -- |
| | CT | 28(49.2) | 19(42.2) | 2.6(1.06-6.38) | 0.03* |
| | TT | 16(28) | 3(6.7) | 9.43(2.3-38.58) | ***p***<0.01* |
| | Dominant | | | | |
| | CC® | 13(22.8) | 23(51.1) | 1.00(Ref.) | -- |
| | CT+TT | 44(77.2) | 22(48.9) | 3.53(1.51-8.28) | 0.003* |
| | Recessive | | | | |
| | CC+CT® | 41(71.9) | 42(93.3) | 1.00(Ref.) | -- |
| | TT | 16(28.1) | 3(6.7) | 5.46(1.48-20.16) | 0.01* |
| | Over-dominant | | | | |
| | TT+CC® | 29(50.8) | 26(57.8) | 1.00(Ref.) | -- |
| | CT | 28(49.2) | 19(42.2) | 1.32(0.6-2.9) | 0.48 |
| | Allele | | | | |
| | C® | 54(47.3) | 60(52.7) | 1.00 | -- |
| | T | 65(72.2) | 25(27.8) | 2.88(1.6-5.21) | ***p***< 0.001* |
| *SERPINE1 rs1799889* | Codominant | | | | |
| | 4G/4G® | 15(26.3) | 14(31.1) | 1.00 (Ref.) | -- |
| | 4G/5G | 35(61.4) | 12(26.6) | 2.72 (1.02-7.25) | 0.04* |
| | 5G/5G | 7 (12.2) | 19(42.2) | 0.34 (0.015-1.57) | 0.06 |
| | Dominant 4G/4G® | | | | |
| | 4G/5G+5G/5G | 15(26.3) | 14(31.1) | 1.00 (Ref.) | -- |
| | | 42(73.6) | 31(68.8) | 1.26 (0.53-2.99) | 0.59 |
| | Recessive | | | | |
| | 4G/4G+4G/5G® | 50 (87.7) | 26(57.7) | 1.00(Ref.) | -- |
| | 5G/5G | 7 (12.2) | 19(42.2) | 0.19 (0.07-0.51) | 0.001* |
| | Over-dominant | | | | |
| | 4G/4G+5G/5G® | 22(40.4) | 33(64.4) | 1.00(Ref.) | -- |
| | 4G/5G | 35(59.6) | 12(35.5) | 4.37 (1.87-10.22) | 0.001* |
| | Allele | | | | |
| | 4G® | 65(57.1) | 40(44.4) | 1.00(Ref.) | -----0.62 |
| | 5G | 49(42.9) | 50(55.5) | (0.35-1.09) | 0.1 |

Abbreviations: P: probability, *: significant at the 0.05 level, OR: odd ratio, ®**:** reference

**Figure 1.** The rs1801133 MTHFR gene's PCR products were exhibited by performing agarose gel electrophoresis using a 2% concentration

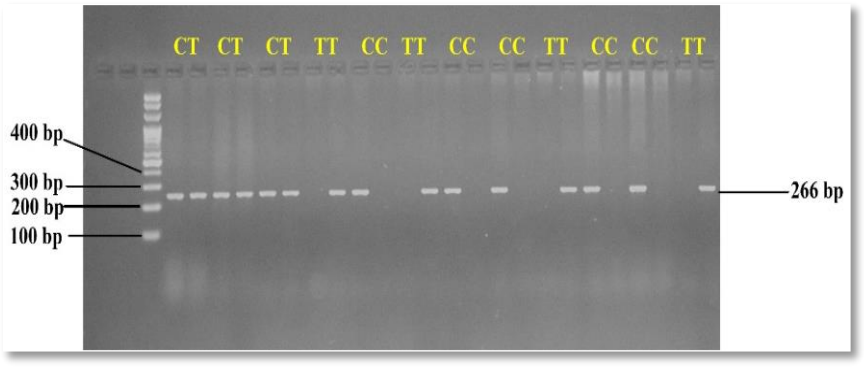


**Figure 2.** Sanger sequencing analysis was used to validate the CC, CT, and TT genotypes of the rs1801133 polymorphism

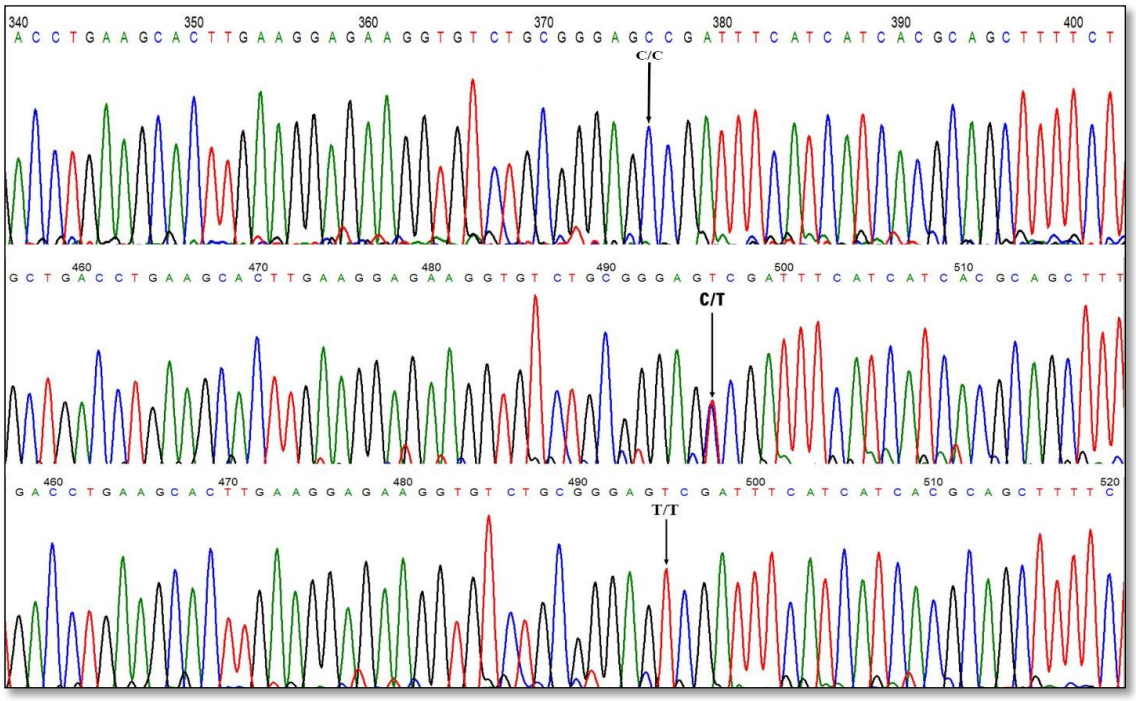



**Figure 3.** Agarose gel electrophoresis was performed with a 2% concentration to display the PCR products of the SERPINE1 gene rs1799889

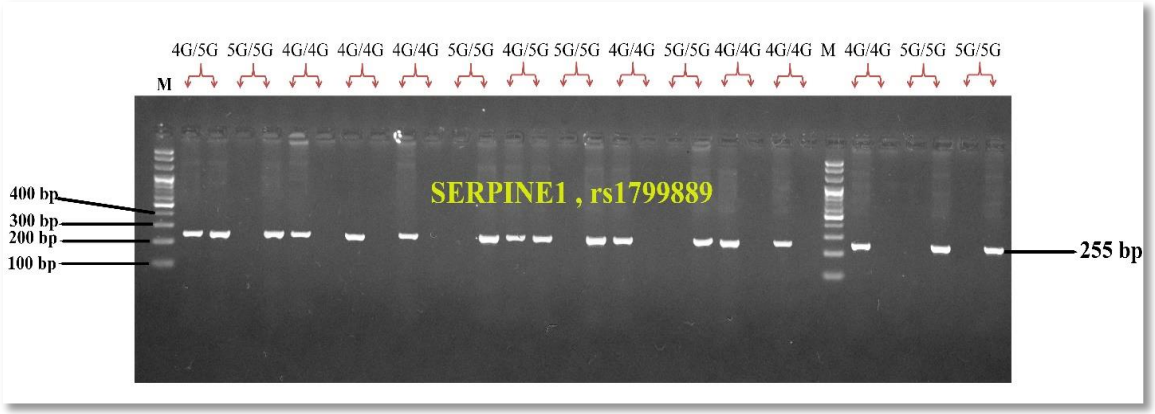



**Figure 2.** The genotypes (4G/4G, 4G/5G, and 5G/5G) of the rs1799889 polymorphism were verified using Sanger sequencing analysis of DNA

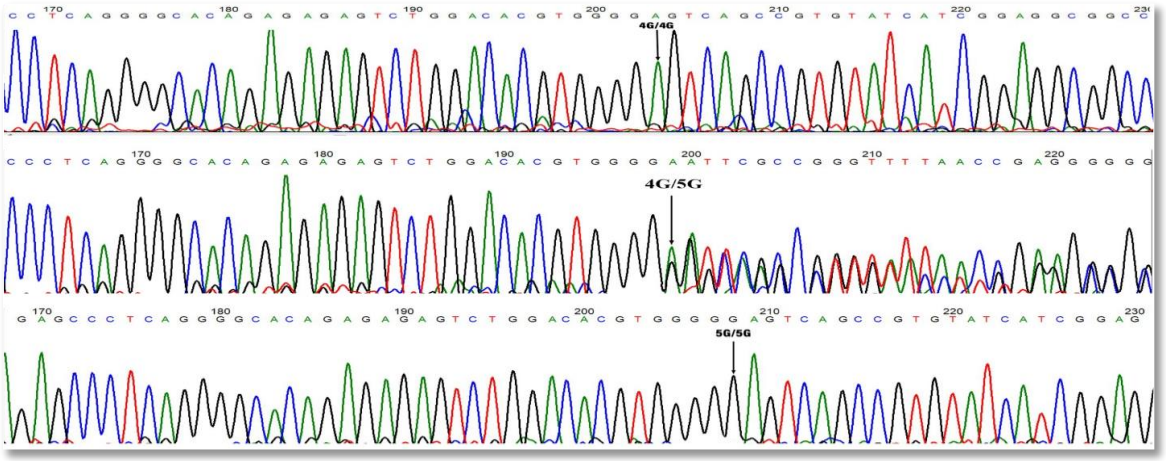


**Figure 5.** An ELISA was used to evaluate the serum concentrations of homocysteine across different genotypes of MTHFR rs1801133

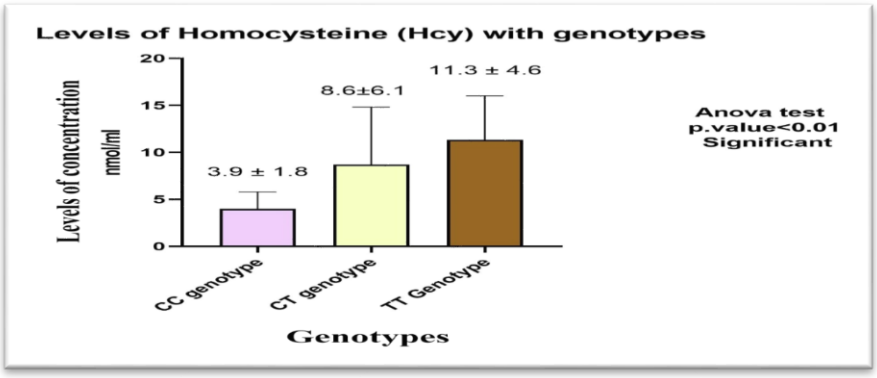
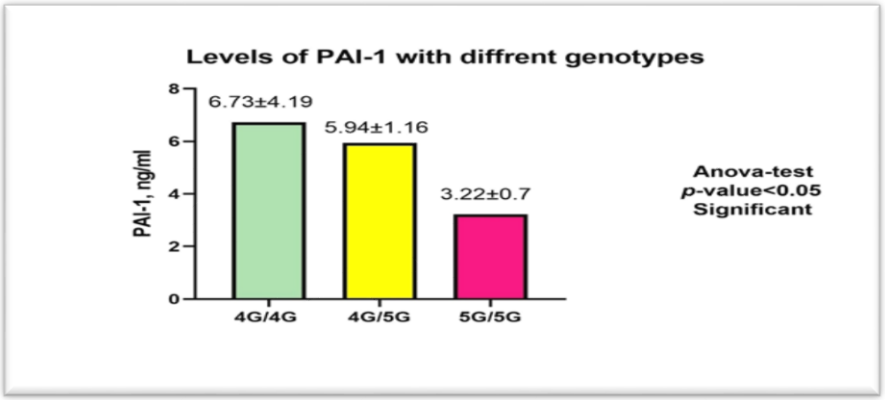



**Figure 6.** The levels of PAI-1 in the serum were determined via ELISA, while considering the genotypes of PAI-1 4G/5G




**Discussion**

The primary objective of our study was to investigate the potential correlations between the genetic polymorphisms of MTHFR (rs1801133) and SERPINE1 (rs1799889) with the risk and severity of COVID-19 infection. Additionally, we sought to explore the combined effect of these genetic variations on the serum levels of Hcy and PAI-1. Our findings revealed that the MTHFR polymorphism is linked to an increased risk and heightened severity of COVID-19 disease. On the other hand, with respect to the SERPINR1 genetic polymorphism, we identified an association with the risk of COVID-19, and our results indicated that the SERPINE1 polymorphism was related to the aggressiveness of the disease.

Our study's findings closely resemble those of Khidoyatovna et al. with respect to the association between MTHFR polymorphism and COVID-19 susceptibility. They demonstrated that having the C/T genotype in the gene increases the risk of infection by 1.9 times, which is in line with our results. Furthermore, the same study revealed that when analyzing the distribution frequency of alleles and genotypes in the gene's polymorphism, there was a significant correlation between the heterozygous C/T genotype and a 5.6-fold higher risk of severe COVID-19. This underscores the potential importance of MTHFR polymorphism in COVID-19 susceptibility and severity [25] in the Uzbek population. Furthermore, a recent study on the Turkish population revealed that the MTHFR C677T CT genotype and T allele were more commonly found in the intensive care group than in other groups, suggesting a potential association with the severity of COVID-19 disease [26].

A meta-analysis has suggested that this polymorphism may be associated with the severity of COVID-19, as the frequency of the T allele has been found to be correlated with COVID-19 mortality [27]. Another noteworthy finding is that the T allele of the rs1801133 MTHFR locus has been strongly linked to cardiovascular disease. Therefore, the increased prevalence of cardiac disease among Long Covid-19 patients cannot be solely attributed to the CC genotype [15]. A

reduction in enzymatic activity caused by hereditary enzyme defects, specifically encoded by MTHFR 677 C>T genes, is one factor contributing to elevated blood homocysteine levels. Certain research findings suggest an augmented risk of venous and arterial thrombosis, as well as an increased likelihood of coronary artery disease (CAD) and myocardial infarction (MI), in individuals with unfavorable polymorphisms of rs1801133 [28–30].

On the other hand, in multiple diseases found link between C667T and Hcy levels, in study on the young CAD group, carriers of the T allele for MTHFR 677C/T (rs1801133) genotype showed a statistically significant hyperhomocysteinemia [31]. Furthermore, second study found a significant correlation between moderately increased levels of Hcy and the MTHFR C677T polymorphism in a group of Mexican children with Legg–Calvé–Perthes disease (LCPD) [32].

Our findings were consistent with this body of evidence, as we observed higher levels of homocysteine among individuals with the TT and CT genotypes. similarly, **Van Meurs et al.** found that individuals with the mutant homozygous genotype for the C677 allele had significantly higher blood homocysteine concentrations compared to the wild homozygous genotype, while those with the heterozygous genotype also had significantly higher homocysteine levels than the wild type [33]. A study revealed Elevated levels of Hcy can serve as a useful indicator for assessing the necessity of critical care upon admission to the emergency department and also as an indicator of a poor prognosis in COVID-19 pneumonia [34]. In critically ill patients, the initial indication of coagulopathy emerges through heightened D-dimer levels, followed by abnormal prothrombin and partial thromboplastin times, and ultimately reduced platelet counts during later stages. The thrombosis arising from coronavirus infection can also trigger elevated homocysteine levels, a byproduct of methionine via methylenetetrahydrofolate reductase (MTHFR). Elevated homocysteine levels possess the potential to activate the coagulation cascade, thereby resulting in the production of D-dimer. This cumulative process culminates in the development of blood clots, which give rise to severe complications, including fatal outcomes, documented in some COVID-19 patients [35,36].

A study conducted by **Todua et al.** revealed an association coefficient of 0.557 between D-dimer levels and homocysteine levels in patients afflicted with pulmonary arterial thromboembolism [37].

Certain gene polymorphisms, particularly the 4G/5G variant of plasminogen activator inhibitor type 1 (PAI-1) and the C677T variant of methylenetetrahydrofolate reductase (MTHFR), have been reported to be associated with thrombophilia development. These genetic variations are considered significant determinants contributing to an elevated risk of thrombotic events [38,39]. To date, only one study has been conducted to investigate the potential association between the plasminogen activator inhibitor-1 (PAI-1) 4G/5G gene polymorphism and the SARS virus-1, the study found that this specific genetic variation was correlated with post-SARS osteonecrosis, which is a condition caused by blood clotting. The findings indicate that the PAI-1 -675 4G/5G polymorphism may have implications in the development of thrombotic complications in COVID-19 patients [40]. The PAI-1 rs1799889 4G/5G polymorphism (specifically the presence of 4G versus 5G) has been linked to a higher likelihood of developing conditions such as deep vein thrombosis (DVT), myocardial infarction (MI), and ischemic stroke [41,42]. Prior research has demonstrated the frequent occurrence of coagulopathy in severe COVID-19 cases. Several biomarkers including D-dimer, fibrinogen, and platelet count have been linked to disease severity and mortality [43]. The fibrinolytic system, governed by plasminogen activators and inhibitors, operates through the conversion of plasminogen to plasmin. Key players in this system encompass tissue plasminogen activator (TPA), urokinase-type plasminogen activator (uPA), and their inhibitor PAI-1. Elevated levels of TPA and PAI-1 have been identified in COVID-19 patients, particularly those with worse outcomes [44].

Collectively, these insights from (D-dimer and PAI-1) imply that distinctive alterations in coagulation markers within COVID-19 patients could aid in prognosticating changes within the fibrinolysis system and enhancing our comprehension of these modifications [44–46].

Despite numerous studies investigating the association between hereditary thrombophilia markers and thrombosis in COVID-19 patients, our understanding of this relationship remains incomplete. In our study, we observed a significant association between the SERPINE1 genetic

polymorphism and an elevated risk of COVID-19. Likewise, the presence of 4G/4G variants in the PAI-I gene (rs1799889) was found to be more common among individuals with mild COVID-19 compared to those experiencing extremely severe COVID-19. It is speculated that individuals with severe COVID-19 may experience temporary hyperfibrinolysis, leading to the progression of local pulmonary infection to sepsis. Therefore, it is possible that the 4G/4G polymorphisms confer a protective effect [47].

However, another study reported the case of an infant born with multiple abnormalities that could be attributed to the 4G/5G PAI-1 polymorphism inherited from the mother who had contracted SARS-CoV-2 during pregnancy [48]. According to the study, the presence of the PAI-1 -675 4G/5G polymorphism was observed in 64.7% of the SARS CoV-2 positive patients (case group) who carried at least one mutant allele (either rare homozygous or heterozygous). Furthermore, statistical analysis revealed that this particular polymorphism was a significant risk factor for the development of the disease. Our results align with a previous study that discovered a correlation between the PAI-1 4G/4G genotype and the 4G allele, increased serum levels of PAI-1, and and elevated disease activity scores [49]. Despite the presence of a binding site for an activator of transcription in both the 4G and 5G alleles, the 5G allele differs in that it contains an extra binding site for a repressor. As a result, the transcription rates are reduced, leading to lower levels of PAI-1 [50,51].

This study has some limitations. Specifically, we did not have access to data concerning the entirety of the treatment received by patients prior to and during their hospitalization. Furthermore, it would be advantageous to evaluate laboratory parameters specific to patients.

## Conclusions

To conclude, the results of our study suggest that the genetic polymorphism C667T in MTHFR is linked to a higher risk of COVID-19, as well as increased severity of the disease, and changes in Hcy levels among different genotypes. Furthermore, we observed a significant association between the SERPINE1 -675 4G/5G genetic polymorphism, increased disease severity, and a higher susceptibility to infection. Additionally, this polymorphism influenced PAI-1 levels, indicating its potential role in the pathogenesis of the disease. Furthermore, both polymorphisms may contribute to the risk of complications resulting from impaired coagulation activity among patients suffering from severe COVID-19.

## Author contributions

All authors have read and approved the final version of the manuscript for publication.

## Financial support and sponsorship

The study received no financial support or sponsorship.

## Conflicts of interest

The authors declare no conflicts of interest related to this research.

## References

1- **Phelan AL, Katz R, Gostin LO.** The novel coronavirus originating in Wuhan, China: challenges for global health governance. Jama 2020; 323:709–710.

2- **Tsiotas D, Tselios V.** Understanding the uneven spread of COVID-19 in the context of the global interconnected economy. Sci Rep 2022; 12:666.

3- **Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al.** Detection of SARS-CoV-2 in different types of clinical specimens. Jama 2020; 323:1843–1844.

4- **Fang Y, Zhang H, Xie J, Lin M, Ying L, Pang P, et al.** Sensitivity of chest CT for COVID-19: comparison to RT-PCR. Radiology 2020;296: E115–E117.

5- **Xie X, Zhong Z, Zhao W, Zheng C, Wang F, Liu J.** Chest CT for typical coronavirus disease 2019 (COVID-19) pneumonia: relationship to negative RT-PCR testing. Radiology 2020;296: E41–E45.

6- **Piazza G, Morrow DA.** Diagnosis, management, and pathophysiology of arterial and venous thrombosis in COVID-19. Jama 2020; 324:2548–2549.

7- **Tang N, Li D, Wang X, Sun Z.** Abnormal coagulation parameters are associated with poor

prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost 2020; 18:844–847.

8- **Henry BM, Vikse J, Benoit S, Favaloro EJ, Lippi G.** Hyperinflammation and derangement of renin-angiotensin-aldosterone system in COVID-19: a novel hypothesis for clinically suspected hypercoagulopathy and microvascular immunothrombosis. Clin Chim acta 2020; 507:167–173.

9- **Bahraini M, Dorgalaleh A**. The impact of SARS-CoV-2 infection on blood coagulation and fibrinolytic pathways: a review of prothrombotic changes caused by COVID-19. Semin Thromb Hemost 2022. p. 19–30.

10- **Slama DB, Fekih Mrissa N, Haggui A, Nsiri B, Haouala H, Gritli N.** Association of methylene-tetrahydrofolate reductase C677T and A1298C polymorphisms with myocardial infarction in Tunisian young patients. Comp Clin Path 2014; 23:1633–1638.

11- **Ponti G, Maccaferri M, Ruini C, Tomasi A, Ozben T.** Biomarkers associated with COVID-19 disease progression. Crit Rev Clin Lab Sci 2020; 57:389–399.

12- **Van Der Made CI, Simons A, Schuurs-Hoeijmakers J, van den Heuvel G, Mantere T, Kersten S, et al.** Presence of genetic variants among young men with severe COVID-19. Jama 2020; 324:663–673.

13- **Ponti G, Ruini C, Tomasi A.** Homocysteine as a potential predictor of cardiovascular risk in patients with COVID-19. Med Hypotheses 2020; 143:109859.

14- **Yang Z, Shi J, He Z, Lü Y, Xu Q, Ye C, et al.** Predictors for imaging progression on chest CT from coronavirus disease 2019 (COVID-19) patients. Aging (Albany NY) 2020; 12:6037.

15- **Liew S-C, Gupta E Das.** Methylene-tetrahydrofolate reductase (MTHFR) C677T polymorphism: epidemiology, metabolism and the associated diseases. Eur J Med Genet 2015; 58:1–10.

16- **Yeo YH, Wang M, He X, Lv F, Zhang Y, Zu J, et al.** Excess risk for acute myocardial infarction mortality during the COVID-19 pandemic. J Med Virol 2023;95: e28187.

17- **Koch W, Schrempf M, Erl A, Mueller JC, Hoppmann P, Schömig A, et al.** 4G/5G polymorphism and haplotypes of SERPINE1 in atherosclerotic diseases of coronary arteries. Thromb Haemost 2010; 103:1170–1180.

18- **Topoleanu S, Mambet C, Marutescu L, Ivan F, Cucu IA, Curici A, et al.** Carrier frequencies for thrombophilia-related genetic variants in a Romanian cohort. Biotechnol Lett 2021; 26:2275–2282.

19- **Mazzoccoli G, Pazienza V, Panza A, Valvano MR, Benegiamo G, Vinciguerra M, et al.** ARNTL2 and SERPINE1: potential biomarkers for tumor aggressiveness in colorectal cancer. J Cancer Res Clin Oncol 2012; 138:501–511.

20- **Parveen F, Tuteja M, Agrawal S.** Polymorphisms in MTHFR, MTHFD, and PAI-1 and recurrent miscarriage among North Indian women. Arch Gynecol Obstet 2013; 288:1171–1177.

21- **Fawzy MS, Toraih EA, Aly NM, Fakhr-Eldeen A, Badran DI, Hussein MH.** Atherosclerotic and thrombotic genetic and environmental determinants in Egyptian coronary artery disease patients: a pilot study. BMC Cardiovasc Disord 2017; 17:1–19.

22- **Tavares V, Neto BV, Vilas-Boas MI, Pereira D, Medeiros R.** Impact of hereditary thrombophilia on cancer-associated thrombosis, tumour susceptibility and

progression: A review of existing evidence. Biochim Biophys Acta (BBA)-Reviews Cancer 2022;188778.

23- **World Health Organization.** COVID-19 clinical management: living guidance, 25 January 2021. World Health Organization; 2021.

24- **Zayed NE, Abbas A, Lutfy SM**. Criteria and potential predictors of severity in patients with COVID-19. Egypt J Bronchol 2022; 16:1–8.

25- **Khidoyatovna IF, Chutbaevna KZ, Agzamovna BS.** Relationship between MTHFR GENE rs1801133 and rs1801131 polymorphisms with disease severity of COVID-19 and homocystein levels in Uzbek patients. J Pharm Negat Results 2022;1879–1888.

26- **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;1–15.

27- **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 methylene-tetrahydrofolate reductase (MTHFR) gene prevalence. J Clin Lab Anal 2021;35: e23798.

28- **Bozok Çetintaş V, Gündüz C.** Association between polymorphism of MTHFR c. 677C> T and risk of cardiovascular disease in Turkish population: a meta-analysis for 2.780 cases and 3.022 controls. Mol Biol Rep 2014; 41:397–409.

29- **Lewis SJ, Ebrahim S, Smith GD.** Meta-analysis of MTHFR 677C→ T polymorphism and coronary heart disease: does totality of evidence support causal role for homocysteine and preventive potential of folate? Bmj 2005;331:1053.

30- **Chrysant SG, Chrysant GS.** The current status of homocysteine as a risk factor for cardiovascular disease: a mini review. Expert Rev Cardiovasc Ther 2018; 16:559–565.

31- **Shivkar RR, Gawade GC, Padwal MK, Diwan AG, Mahajan SA, Kadam CY.** Association of MTHFR C677T (rs1801133) and A1298C (rs1801131) polymorphisms with serum homocysteine, folate and vitamin B12 in patients with young coronary artery disease. Indian J Clin Biochem 2022; 37:224–231.

32- **Buendía-Pazarán JG, Hernández-Zamora E, Rodríguez-Olivas AO, Casas-Ávila L, Valdés-Flores M, Reyes-Maldonado E. et al.** Association of MTHFR rs1801133 and homocysteine with Legg–Calvé–Perthes disease in Mexican patients. Orphanet J Rare Dis 2022; 17:1–6.

33- **Van Meurs JB, Pare G, Schwartz SM, Hazra A, Tanaka T, Vermeulen SH, et al.** Common genetic loci influencing plasma homocysteine concentrations and their effect on risk of coronary artery disease. Am J Clin Nutr 2013; 98:668–676.

34- **Kalan Sarı I, Keskin O, Seremet Keskin A, Ellidağ HY, Harmandar O.** Is Homocysteine Associated with the Prognosis of Covid-19 Pneumonia. Int J Clin Pract 2023;2023.

35- **Iba T, Levy JH, Levi M, Connors JM, Thachil J.** Coagulopathy of coronavirus disease 2019. Crit Care Med 2020.

36- **Connors JM, Levy JH.** COVID-19 and its implications for thrombosis and anticoagulation. Blood 2020; 135:2033–2040.

37- **Todua F, Akhvlediani M, Vorobiova E, Tsivtsivadze G, Baramidze A.** Homocysteine and D-dimer levels and multilayer computed

tomography for diagnosing pulmonary artery thromboembolism. Hospitals (Lond) 2017;3:5.

38- **Mannucci PM, Franchini M.** Classic thrombophilic gene variants. Thromb Haemost 2015; 114:885–889.

39- **Tang L, Wang HF, Lu X, Jian XR, Jin B, Zheng H, et al.** Common genetic risk factors for venous thrombosis in the Chinese population. Am J Hum Genet 2013;92:177–187.

40- **Sun W, Li Z, Shi Z, Wang B, Gao F, Yang Y, et al.** Relationship between post-SARS osteonecrosis and PAI-1 4G/5G gene polymorphisms. Eur J Orthop Surg Traumatol 2014;24:525–529.

41- **Jafari M, Jarahzadeh MH, Dastgheib SA, Seifi-Shalamzari N, Raee-Ezzabadi A, Sadeghizadeh-Yazdi J, et al.** Association of PAI-1 rs1799889 polymorphism with susceptibility to ischemic stroke: a huge meta-analysis based on 44 studies. Acta Medica Cordoba 2020; 63:31–42.

42- **Huang G, Wang P, Li T, Deng X.** Genetic association between plasminogen activator inhibitor-1 rs1799889 polymorphism and venous thromboembolism: Evidence from a comprehensive meta-analysis. Clin Cardiol 2019; 42:1232–1238.

43- **Fogarty H, Townsend L, Ni Cheallaigh C, Bergin C, Martin-Loeches I, Browne P, et al.** COVID19 coagulopathy in Caucasian patients. Br J Haematol 2020;189:1044–1049.

44- **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;11:1–9.

45- **Cabrera-Garcia D, Miltiades A, Yim P, Parsons S, Elisman K, Mansouri MT, et al.** Plasma biomarkers associated with survival and thrombosis in hospitalized COVID-19 patients. Int J Hematol 2022; 116:937–946.

46- **Nougier C, Benoit R, Simon M, Desmurs-Clavel H, Marcotte G, Argaud L, et al.** Hypofibrinolytic state and high thrombin generation may play a major role in SARS-COV2 associated thrombosis. J Thromb Haemost 2020; 18:2215–2219.

47- **Gorodin VN, Moysova DL, Zotov SV, Vanyukov AA, Podsadnyaya AA, Tikhonenko YV.** Role of polymorphisms of genes involved in hemostasis in COVID-19 pathogenesis. Infektsionnye Bolezn 2021;16–26.

48- **Jak B, Zanirati G, Rodrigues FV, Grahl M, Krimberg F, Pinzetta G, Borém L, et al.** Case report: placental maternal vascular malperfusion affecting late fetal development and multiorgan infection caused by SARS-CoV-2 in patient with PAI-1 4G/5G polymorphism. Front Med 2021; 8:624166.

49- **Yousef AA, Mohamed FY, Boraey NF, Akeel NE, Soliman AA, Waked NM, et al.** Association of plasminogen activator inhibitor 1 (PAI-1) 4G/5G polymorphism and susceptibility to SLE in Egyptian children and adolescents: a Multicenter study. J Inflamm Res 2020;1103–1111.

50- **Longstaff C, Kolev K**. Basic mechanisms and regulation of fibrinolysis. J Thromb Haemost 2015;13:S98–S105.

51- **Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, et al.** The metabolic syndrome and cardiovascular risk: a systematic review and meta-analysis. J Am Coll Cardiol 2010; 56:1113–1132.