



**T.C.**

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**DETERMINATION OF PROTHROMBIN  
POLYMORPHISM IN COVID-19**

**MASTER'S THESIS**

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**TOKAT - 2024**

**ETHICS CONTRACT**

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### JURY ACCEPTANCE AND APPROVAL

The defense examination of the thesis study titled "Determination of Prothrombin Polymorphism In COVID-19", which is prepared by SARBAST RAHIM OTHMAN, was held on the 06/03/2024 This Master's Thesis, having undergone evaluation by the panel of jury members, has received approval for acceptance at Tokat Gaziosmanpasa University, Graduate Education Institute Department of Medical Biology.

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## ÖZET

### COVID-19'DA PROTROMBİN POLİMORFİZMİNİN BELİRLENMESİ

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SARS-CoV-2 virüsünün neden olduğu COVID-19 pandemisi küresel sağlık, toplumsal ve ekonomik kaygılara yol açmıştır. Virüs, çeşitli dokulardaki ACE2 reseptörlerine bağlanarak hastalığı başlatır. Artan bağışıklık tepkisi çok önemli bir rol oynar ve ciddi vakalarda sitokin fırtınası görülebilir. Kesin patofizyolojik mekanizmalar ve genetik polimorfizmin hastalığın ilerlemesi üzerindeki etkisi belirsizliğini korumaktadır. Klinik belirtiler, önemli bir pıhtılaşma bileşeni olan protrombindeki genetik polimorfizm Faktör II G20210A ile bağlantılı trombotik olaylarla değişmektedir. Bu çalışmanın amacı, protrombin genindeki G20210A polimorfizmi (rs1799963) ile SARS-CoV-2 ile enfekte bireylerde şiddetli semptomların gelişimi arasındaki ilişkiyi araştırmaktır. Bu araştırma için 100 COVID-19 hastasından oluşan bir kohort kaydedilmiş ve protrombin G20210A polimorfizminin genotiplemesi Polimeraz Zincir Reaksiyonu Kısıtlama Parçası Uzunluk Polimorfizmi (PCR-RFLP) analizi kullanılarak gerçekleştirilmiştir. G20210A polimorfizminin sıklığı üç grup arasında karşılaştırıldı ve klinik sonuçlarla ilişkileri incelendi. Çalışmamızın sonuçları, tüm hastaların GG genotipi sergilediğini ortaya koymuştur. Bu gözlem, bu özel hasta kohortunda protrombin G20210A polimorfizminin varlığının COVID-19'un ciddiyeti ile ilişkili olmadığını ima etmektedir.

**Anahtar Kelimeler:** COVID-19, Protrombin, Polimorfizm

## ABSTRACT

### DETERMINATION OF PROTHROMBIN POLYMORPHISM IN COVID-19

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The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has led to global health, societal, and economic concerns. The virus attaches to ACE2 receptors in various tissues, initiating illness. A heightened immune response plays a crucial role, and severe cases may involve a cytokine storm. The exact pathophysiological mechanisms and the impact of genetic polymorphism on disease progression remain unclear. Clinical manifestations vary, with thrombotic events linked to the genetic polymorphism Factor II G20210A in prothrombin, a key coagulation component. The aim of this study was to investigate the correlation between the G20210A polymorphism (rs1799963) of the prothrombin gene and the development of severe symptoms in individuals infected with SARS-CoV-2. A cohort comprising 100 COVID-19 patients was enrolled for this investigation, and genotyping of the prothrombin G20210A polymorphism was performed using Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP) analysis. The frequency of the G20210A polymorphism was compared among the three groups, and associations with clinical outcomes were examined. The results of our study revealed that all patients exhibited the GG genotype. This observation implies that, within this particular patient cohort, the presence of the prothrombin G20210A polymorphism is not associated with the severity of COVID-19.

**Keywords:** COVID-19, Prothrombin, Polymorphism.

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## ABBREVIATION

|            |                                                              |
|------------|--------------------------------------------------------------|
| ACE2       | Angiotensin-Converting Enzyme 2                              |
| ACS        | Acute Coronary Syndrome                                      |
| ALI        | Acute Lung Injury                                            |
| ARDS       | Acute Respiratory Distress Syndrome                          |
| AST        | Aspartate aminotransferase                                   |
| BUN        | Blood Urea Nitrogen                                          |
| CAD        | Coronary Artery Disease                                      |
| CNS        | Central Nervous System                                       |
| CRP        | C-reactive protein                                           |
| CT         | Computed Tomography                                          |
| DNA        | Deoxyribonucleic Acid                                        |
| DIC        | Disseminated Intravascular Coagulation                       |
| ER         | The Endoplasmic Reticulum                                    |
| ERGIC      | The Endoplasmic Reticulum-Golgi intermediate compartment     |
| HDL        | High-Density Lipoprotein                                     |
| HE         | Hemagglutinin-Esterase                                       |
| HLA        | Human Leukocyte Antigen                                      |
| HR         | Heptad Repeat                                                |
| IBV        | Infectious Bronchitis Virus                                  |
| IL         | Interleukin                                                  |
| LDH        | Lactate Dehydrogenase                                        |
| LDL        | Low-Density Lipoprotein                                      |
| MERS-CoV   | Middle East respiratory syndrome coronavirus                 |
| MHC        | Major Histocompatibility Complex                             |
| MHV        | Mouse Hepatitis Virus                                        |
| MI         | Myocardial Infarction                                        |
| NSP        | Non-Structural Proteins                                      |
| PCR        | Polymerase Chain Reaction                                    |
| RFLP       | Restriction Fragment Length Polymorphism                     |
| RNA        | Ribonucleic acid                                             |
| rRT-PCR    | Real-Time reverse Transcription Polymerase Chain Reaction    |
| RTC        | Replication Transcription Complex                            |
| RT-LAMP    | Reverse Transcription Loop-mediated isothermal amplification |
| SARS-CoV-2 | Severe Acute Respiratory Syndrome Coronavirus 2              |
| SP         | Signal Peptide                                               |
| ssRNA      | single-stranded RNA                                          |
| TG         | Triglycerides                                                |
| TM         | Transmembrane                                                |
| TNF        | Tumor Necrosis Factor                                        |
| VLDL       | Very Low-Density Lipoprotein                                 |
| VTE        | Venous Thromboembolism                                       |
| WHO        | World Health Organization                                    |

## 1. INTRODUCTION

Coronaviruses are categorized within the Coronaviridae family, which falls under the Nidovirales order. The term "coronavirus" is attributed to the distinct crown-like projections that embellish its outer surface, these viral entities are characterized by relatively modest dimensions, featuring diameters that fall within the range of 65 to 125 nanometers. A single-stranded RNA molecule that is between 26 and 32 kilobases long makes up the genetic material of corona viruses, coronaviruses are classified into four distinct subgroups based on taxonomy, the viral entities, denoted as alpha (a), beta (b), gamma (c), and delta (d), have been implicated in various viral infections capable of inducing acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). Instances of such infections include SARS-CoV, H5N1 influenza A, H1N1 2009, and Middle East respiratory syndrome coronavirus (MERS-CoV), presenting a plausible risk of pulmonary compromise and life-threatening ramifications. Initially, the prevailing notion was that these viruses primarily impacted animals. Nevertheless, a paradigm shift in perspective transpired in 2002 with the advent of severe acute respiratory syndrome (SARS), linked to the SARS-CoV virus and initially identified in Guangdong, China. This marked a significant evolution in our understanding of viral infections (Zhong et al., 2020). A decade later, the Middle East respiratory syndrome coronavirus (MERS-CoV), an exceptionally virulent coronavirus, instigated an outbreak across multiple Middle Eastern nations (Wang et al., 2019), increased coagulation abnormalities have been linked to COVID-19, which increases morbidity and death in those with severe illness (Helms et al., 2020; Al-Samkari et al., 2020), numerous research inquiries have demonstrated an elevated incidence of venous thromboembolism (VTE) and various other coagulation irregularities among severely ill individuals suffering from COVID-19. Furthermore, a connection has been established between heightened circulating D-dimer levels and the severity of the illness or mortality rate. Autopsies have uncovered the existence of fibrin clots within dilated small vessels and capillaries, alongside a significant occurrence of concealed thrombosis in the pulmonary vasculature (Wichmann et al., 2020).

Angiotensin-converting enzyme 2 (ACE2), extensively distributed across various tissues with notable prevalence in myocytes and vascular endothelial cells, serves as the entry point for the SARS-CoV-2 virus into human cells. Following viral infiltration, the innate immune system is activated with the intent of eradicating the infection. Nevertheless, an excessively vigorous initiation of this response results in an inflammatory response characterized by an upsurge in cytokines, such as IL-6 (interleukin 6), IL-8 (interleukin 8), TNF- $\alpha$  (Tumor Necrosis Factor), IL-1 (interleukin-1), and IL-2R (interleukin-2 receptor), among other cytokines. As a consequence, this sequence of events culminates in detriment to the microvascular system (Cui et al., 2020).

The principal aim was to determine if the prothrombin G20210A polymorphism represented a notable risk factor for COVID-19 within the designated cohort of participants., stratified based on severity and demographic characteristics, underwent thorough analysis to elucidate any potential correlation between the prothrombin polymorphism and susceptibility to COVID-19.

## 2. LITERATURE REVIEW

### 2.1 Definition of SARS-CoV-2

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly transmissible and pathogenic coronavirus that emerged in late 2019 and has caused a pandemic of acute respiratory disease, named ‘coronavirus disease 2019’ (COVID-19), which threatens human health and public safety. In this Review, we describe the basic virology of SARS-CoV-2, including genomic characteristics and receptor use, highlighting its key difference from previously known coronaviruses (Hu et al.,2021).

### 2.2 Morphology of COVID-19

Genetic affinities are evident between SARS-CoV and SARS-CoV-2. The emergence of the novel coronavirus, SARS-CoV-2, has accentuated the significant impact a virus can wield on the human populace. This viral entity is responsible for the ensuing condition known as COVID-19, characterized by an RNA virus containing single-stranded RNA molecules ranging in size from 50 to 200 nanometers. Taxonomically, it is classified within the Coronaviridae family, positioned in the Nidovirales order. The structural composition of the virus is delineated by four distinct proteins, namely the spike protein, envelope protein, membrane protein, and nucleocapsid protein. Importantly, the virus is identified as a coronavirus due to the conspicuous crown-like spikes observed on its exterior. Significantly, the outer membrane's spike protein of SARS-CoV-2 bears resemblance to that of other coronaviruses (see Figure 2.1) (Shereen et al., 2020).

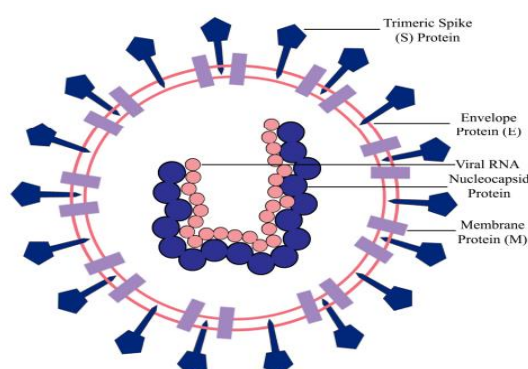


**Figure 2.1:** The architectural composition of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) delineated in the account provided by Shereen et al. (2020).

Several polyproteins, nucleoproteins, and membrane proteins have been recognized in relation to the virus. These encompass entities such as RNA polymerase, 3-chymotrypsin-like protease, papain-like protease, helicase, glycoprotein, and various ancillary proteins (Shereen et al., 2020).

### **2.3 Etiology of COVID-19**

The unequivocal determination of the coronavirus responsible for COVID-19 through thorough genetic sequencing and the application of phylogenetic analysis confirms its classification within the beta coronavirus category. More precisely, it falls under the same subgenus as certain bat coronaviruses and the severe acute respiratory syndrome (SARS) virus, albeit with a distinct evolutionary lineage (Edgar, 2004). Notably, the receptor-binding domain of this viral entity shares significant similarity with that of the SARS coronavirus. Thorough and meticulous scientific inquiries have elucidated its dependence on the angiotensin-converting enzyme 2 (ACE2) receptor as the primary mechanism for cellular entry. The virus, officially denominated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the Coronavirus Study Group, a sector of the International Committee on Virus Taxonomy, displays reduced genetic resemblances to the virus responsible for Middle East respiratory syndrome (MERS), notwithstanding their shared membership in the beta coronavirus family (Waterhouse et al., 2018).

The RNA sequence of SARS-CoV-2 displays its most notable similarities with two bat coronaviruses, suggesting bats as the probable primary reservoir. Nevertheless, the precise mode of transmission, whether directly from bats or through an intermediary host, remains uncertain (Hwang et al., 2006). A study utilizing phylogenetic techniques to analyze 103 SARS-CoV-2 strains originating in China identified two distinct viral variants. Seventy percent of the strains were categorized as type L, while the remaining thirty percent were identified as type S. During the early phases of the pandemic, type L predominated in China, with its prevalence comparatively lower outside of Wuhan in contrast to within the city. The clinical implications of these findings are yet to be ascertained (Ren et al., 2020).

## 2.4 Epidemiology

According to the World Health Organization (WHO), the inception of the COVID-19 epidemic is attributed to "wet markets" in Southern China, where the trade of exotic animal meat was prevalent. This assertion is corroborated by studies conducted by (Rothe et al. 2020), (Lai et al. 2020), and (Wang et al. 2020a). The epidemic was first reported in Wuhan, China, on December 31, 2019, and swiftly propagated across the country, reaching various other nations, including the United States, Germany, and Italy, within a month, by March 30, 2020, COVID-19 had escalated into a global pandemic, affecting 199 countries. At that juncture, there were 722,389 confirmed cases and 33,982 fatalities worldwide, constituting approximately 5% of the total reported cases (Wang et al., 2020a).

Excluding Antarctica, the escalation of COVID-19 cases is rapidly unfolding across continents, surpassing the initially documented levels in China. Initially attributed to the movement of individuals who contracted the virus in China to various global regions (Corman et al., 2020), there has been a noteworthy surge in local transmission within several nations, including France, Germany, Italy, Spain, the United Kingdom, the United States, South Korea, and Japan. This localized transmission has been instrumental in fostering more extensive outbreaks beyond mainland China, ultimately culminating in the emergence of a widespread global pandemic (Wang and Jin, 2020).

As of March 30, 2020, approximately 11% (81,470) of confirmed COVID-19 cases had been documented within mainland China, while the remaining 89% (640,919) originated from regions beyond China's borders. The share of cases in China has exhibited a consistent decline, decreasing from 99.22% on February 10 to 11% on March 30, 2020, concerning the total case count. Conversely, the infection rate in other nations has witnessed exponential expansion, surging from 0.78% to 89% over the same timeframe. Corman et al. (2020) provide an extensive global case distribution up to March 30, 2020, as delineated in Table 2.1. At the outset, the cumulative global cases closely mirrored the cumulative cases within China, but recent developments indicate that Chinese cases have diminished significantly and are effectively managed (Worldometer, 2020). In contrast, the occurrence of cases in countries beyond China has been experiencing a rapid surge, surpassing the overall global case count, as depicted in Figure 2.2. It provides a

comprehensive data analysis of reported COVID-19 cases and fatalities across various countries globally (Worldometer, 2020).

**Table 2.1:** Global dispersion of documented instances of COVID-19 viral infections (Corman et al., 2020).

| Date                         | Total Global | China | Other Countries | No of affected countries |
|------------------------------|--------------|-------|-----------------|--------------------------|
| 22 <sup>th</sup> of January  | 314          | 309   | 5               | 4                        |
| 28 <sup>th</sup> of January  | 4593         | 4537  | 56              | 14                       |
| 3 <sup>rd</sup> of February  | 17391        | 17238 | 153             | 23                       |
| 9 <sup>th</sup> of February  | 37558        | 37251 | 307             | 24                       |
| 15 <sup>th</sup> of February | 50580        | 50054 | 526             | 25                       |
| 21 <sup>th</sup> of February | 76769        | 75569 | 1200            | 26                       |
| 27 <sup>th</sup> of February | 82294        | 78630 | 3664            | 46                       |
| 4 <sup>th</sup> of March     | 93091        | 80422 | 12669           | 76                       |
| 10 <sup>th</sup> of March    | 113702       | 80924 | 32778           | 109                      |
| 16 <sup>th</sup> of March    | 167515       | 81677 | 86438           | 150                      |
| 22 <sup>th</sup> of March    | 292142       | 81498 | 210644          | 193                      |
| 26 <sup>th</sup> of March    | 462684       | 81961 | 380723          | 196                      |
| 30 <sup>th</sup> of March    | 693224       | 82447 | 610777          | 199                      |

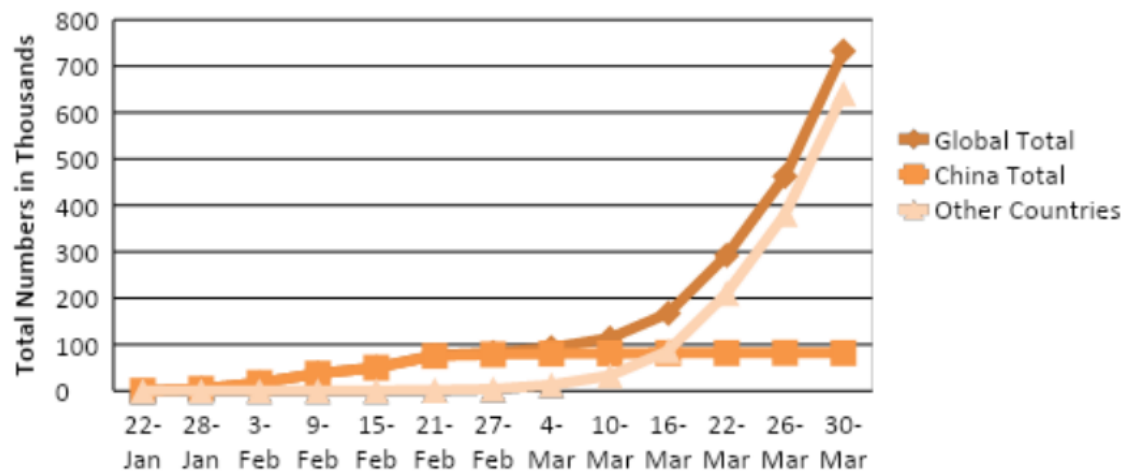


**Figure 2.2:** Worldwide infection trend of COVID-19 (Worldometer, 2020).

As of March 31st, the United States has documented the highest incidence of COVID-19 cases, totaling 164,266, accompanied by 3,170 fatalities. Subsequently, Italy follows with 101,739 cases and 11,591 deaths, China with 81,518 cases and 3,305 deaths, Spain with 87,956 cases and 7,716 deaths, Germany with 67,051 cases and 650 deaths, France with 44,550 cases and 3,024 deaths, Iran with 41,495 cases and 2,757 deaths, the United Kingdom with 22,141 cases and 1,408 deaths, Switzerland with 15,922 cases and 859 deaths, the Netherlands with 11,750 cases and 864 deaths, and Belgium with 11,899 cases and 513 deaths, Italy has recorded the highest mortality rate, totaling 11,591 deaths,

followed by Spain with 7,716 deaths, and then China with 3,305 deaths. Figure 2.3 elucidates the correlation between the cumulative count of reported cases and the cumulative count of fatalities in the prominent countries with over 10,000 cases (Wang et al., 2020b).

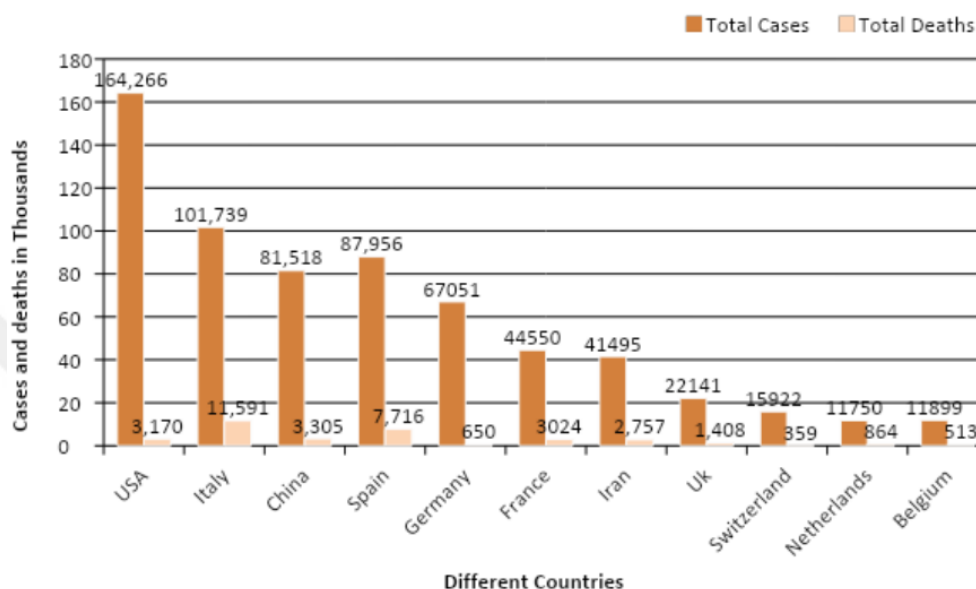


**Figure 2.3:** Total Number of Cases and Deaths (Wang et al., 2020).

As of March 30, 2020, the cumulative worldwide tally of confirmed COVID-19 cases has approached an estimated 722,389 since the commencement of the pandemic (Worldometer, 2020). Among these cases, 185,775 (26%) have reached a resolution, with 151,793 (21%) individuals experiencing recovery and subsequent discharge, while 33,982 (5%) have sadly resulted in fatalities. Subsequent reports by (Gao et al., 2020) and (Wang et al., 2020b) indicate that there are presently 536,614 active cases (74%), with 509,933 (95%) categorized as mild cases and 26,681 (5%) classified as severe or critical. The growth factor for new cases, recorded on March 21, stands at 1.17, signifying an exponential upsurge in infections (greater than 1). Notably, the number of newly recovered cases (3,411) on March 20 is far lower than the number of new infections (30,665), highlighting the pandemic's fast spread outside of China, especially in Europe and the US (Gao et al., 2020).

## 2.5 Pathogenesis

According to Dhama et al. (2020), an examination of structural similarity reveals that SARS-CoV-2 exhibits a binding configuration akin to that of SARS-CoV-1,

suggesting potential similarities in the pathophysiological characteristics between COVID-19 and SARS-CoV-1. Thus, despite variations in the disease's severity, insights gained from the comparative analysis of MERS-CoV and SARS-CoV may offer valuable perspectives in comprehending the pathophysiological features of SARS-CoV-2 infection. Consistent with the findings of Li et al. (2020a), a hypothesis posits that the primary route of viral transmission occurs through mucosal membranes, specifically in the nasal and throat regions, before the virus traverses the respiratory system to reach the lungs. Within the respiratory system, alveolar epithelial cells are identified as the principal cellular targets of the SARS-CoV-2 virus. The viral spike (S) protein plays a crucial role in facilitating the virus's entry into host cells. To elaborate, the envelope spike glycoprotein predominantly interacts with ACE2, a receptor predominantly situated in lung tissue in close proximity to alveolar epithelial cells. It is noteworthy that the spike proteins of SARS-CoV-2 exhibit approximately a tenfold higher affinity for ACE2 compared to those of SARS-CoV, thereby augmenting the virus's transmissibility. Additionally, there is some indication, that CD209L and CD209 might potentially serve as alternative receptors for the virus during cell binding, although the precise mechanistic process remains undisclosed (Dhama et al., 2020; Rothe et al., 2020 and Li et al., 2020a).

It was determined that following the virus's entry into host cells, its RNA genome is released into the cellular cytoplasm. Subsequently, this RNA genome undergoes translation processes, leading to the synthesis of two polyproteins and structural proteins. Concurrently, the viral genome initiates the replication process, and concomitantly, newly formed envelope glycoproteins become integrated into the membranes of the endoplasmic reticulum or Golgi apparatus, the formation of nucleocapsids occurs through the interaction between the genomic RNA and the nucleocapsid protein. Following this stage, viral particles initiate the budding process within the intermediate compartment between the endoplasmic reticulum and Golgi apparatus ultimately, vesicles containing these viral particles merge with the plasma membrane, facilitating the virus's release through the exocytosis mechanism ( Li et al., 2020a), postulated the potential for the virus to enter the bloodstream from the pulmonary region, potentially leading to viremia. Consequently, there is a possibility that the virus may possess the capability to impact other organs where ACE2 is expressed, such as the kidneys, heart, and gastrointestinal system (Prompetchara et al., 2020).

Viral particles, once inside a cell, are presented to antigen-presenting cells through the MHC/HLA system. These antigens are recognized by specific cytotoxic T-cell responses. However, as shown in Figure 4, the presence of these antigens might cause an abnormal T-cell response that leads to T-cell death. Additionally recent investigations conducted by (Li et al.,2020b), (Sohrabi et al.,2020), and (Rothe et al.,2020), have revealed a significant reduction in peripheral blood CD4+ and CD8+ T cells in individuals afflicted with COVID-19 (Prompetchara et al.,2020).

## **2.6 Transmission**

The primary route of COVID-19 transmission involves respiratory droplets generated during coughing, sneezing, or speaking by infected individuals, thereby facilitating person-to-person transmission. Recent research suggests that asymptomatic individuals, those not exhibiting symptoms, might also contribute to the dissemination of the virus (WHO, 2005). Currently, there is not enough data to say with certainty that animals have been a primary source of the COVID-19 virus. The limited existing evidence indicates that the likelihood of animals transmitting the virus to humans is deemed minimal. However, isolated cases have indicated the potential for the virus to transfer from humans to animals in specific scenarios (Arksey and O'Malley, 2005).

A tiger at a New York City zoo showed respiratory symptoms, marking the first known instance of a COVID-19-positive domesticated animal in the US. Subsequent to the onset of respiratory signs in multiple lions and tigers, samples were collected and analyzed from the affected tiger. Health authorities have raised the possibility that these large felines fell ill due to contact with a zoo staff member who had a confirmed diagnosis of the virus. The Centers for Disease Control and Prevention (CDC) collaborates with both human and animal health experts to vigilantly track developments and disseminate updates as fresh insights emerge. It is critical to conduct more study to determine how COVID-19 affects a variety of animal species, an epidemiological study carried out in Wuhan during the early phases of the epidemic revealed a preliminary correlation with a seafood market engaged in the live animal trade. It was disclosed that a significant portion of the patients either had employment at or visited the market before its closure for sanitation measures. However, as the pandemic spread, it became clear that interpersonal transmission had replaced animal transmission as the main method of infection. The

predominant mode of transmission for the severe acute respiratory syndrome coronavirus 2 is through interpersonal contact SARS-CoV-2 (Li et al., 2020).

Close physical contact is thought to be the main way that viruses spread, mostly through the spread of respiratory droplets. The respiratory secretions harboring the virus within infected individuals can be transmitted to others through direct contact, primarily affecting their mucous membranes, in scenarios such as coughing, sneezing, or speaking. Additionally, contamination can arise when an individual touches a surface previously contaminated and then touches their nose, eyes or mouth. Respiratory droplets generally do not disperse beyond a distance of 6 feet (approximately 2 meters), scholarly discussion has focused on the prospect of SARS-CoV-2 spreading through the air as particles smaller than usual droplets, which may stay suspended for extended periods of time and traverse significant distances. A specific research investigation disclosed that the virus exhibited viability in aerosols produced through controlled experimental methods for a minimum duration of three hours. While certain investigations have identified viral RNA in hospital room ventilation systems and air samples from locations with COVID-19 patients, there remains uncertainty regarding the feasibility of cultivating viable live viruses from these sources. Additional inquiries utilizing advanced imaging methods to observe respiratory emissions suggest the possibility of respiratory droplets converting into aerosols or combining with a gaseous plume, thereby leading to horizontal pathways exceeding the recommended 6 feet (2 meters) distance, especially during activities like coughing, speaking, or sneezing. Nonetheless, it remains uncertain how these findings might impact the epidemiological characteristics of COVID-19 and its clinical implications (Huang et al., 2020).

## **2.7 Symptoms**

Typically, manifestations of COVID-19 symptoms become apparent after a 5.2-day incubation period, with a range of 6 to 41 days and a median of 14 days between symptom onset and eventual demise. This timeframe is subject to variation influenced by factors such as age and the individual's immune system status, with those aged 70 and older exhibiting a shorter duration compared to those under the age of 70. Common early symptoms include fever, cough, and fatigue, while additional clinical presentations may involve sputum production, hemoptysis, headache, dyspnea, gastrointestinal disturbance, and decreased lymphocyte count (Ren et al., 2020), clinical observations indicative of

pneumonia are revealed through chest computed tomography (CT) scans, accompanied by distinctive anomalies such as RNAemia, acute respiratory distress syndrome, acute cardiac injury, and the emergence of ground-glass opacities, which carry the potential for severe consequences on occasion, multiple peripheral ground-glass opacities have been discerned in the subpleural regions of both pulmonary lobes, these opacities can trigger both localized and systemic immune responses, thereby exacerbating the inflammatory process, regrettably the administration of interferon inhalation as a therapeutic intervention exhibited no clinical amelioration and appeared to exacerbate the situation by expediting the progression of pulmonary opacities (Figure 2.4) (Lei et al., 2020) .

Recognizing the imperative nature of acknowledging the shared clinical manifestations between COVID-19 and preceding beta coronaviruses is crucial. These common symptoms include fever, non-productive cough, dyspnea, and the identification of bilateral ground-glass opacities in chest CT scans, (Huang et al., 2020), it is essential to discern, however, that COVID-19 manifests distinctive clinical attributes that differentiate it from other diseases. Notably, COVID-19 demonstrates a pronounced predilection for the lower respiratory system, as evidenced by the presentation of upper respiratory tract symptoms such as rhinorrhea, throat irritation, and sneezing. Furthermore, some patients may exhibit infiltrations in the upper lung lobe upon admission, correlating with the progression of breathlessness and oxygen insufficiency, as observed in chest radiographs (Phan et al., 2020).

It is noteworthy that COVID-19 patients may also experience gastrointestinal issues, including diarrhea. In contrast, a comparatively lower proportion of individuals infected with MERS-CoV or SARS-CoV report similar gastrointestinal problems. Consequently, there is an imperative to conduct examinations on urine and fecal samples to eliminate the potential for alternative transmission routes, particularly within the healthcare worker and patient populations (Figure 2.4). Hence, there is an urgent need to devise approaches for the detection of diverse transmission routes, incorporating the analysis of fecal and urine samples, with the objective of formulating strategies to mitigate or halt transmission and developing therapeutics for the effective management of the disease (Phan et al., 2020).

Other indications may consist of:

- Sore throat
- Shortness of breath or difficulty breathing
- Pink eye (conjunctivitis)
- Muscle aches
- Chills
- Chest pain
- Runny nose
- Headache
- Nausea
- Rash
- Diarrhea
- Vomiting

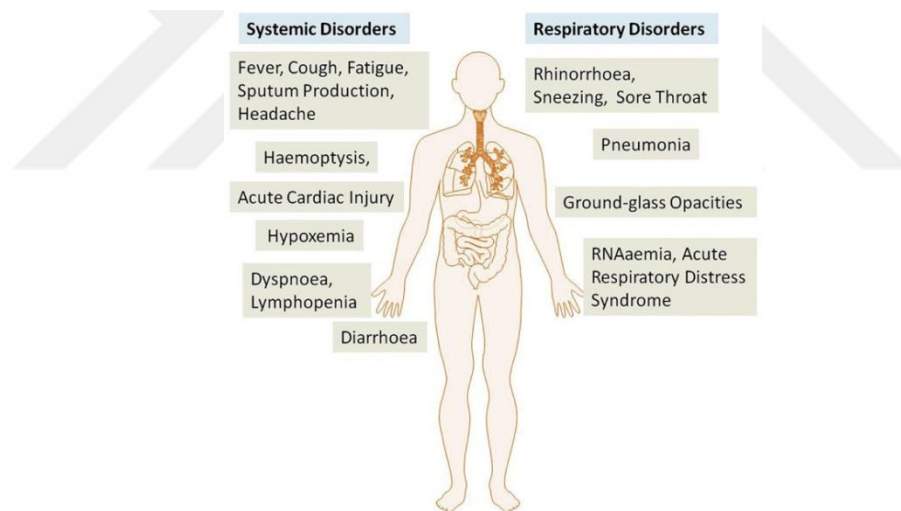


**Figure 2.4:** COVID-19 infection leads to systemic and respiratory disorders (Phan et al., 2020).

## 2.8 Mechanism of action for COVID-19

Considering that coronaviruses are responsible for causing common cold infections, human susceptibility to such illnesses has endured for an extended period, the primary modes of transmission for this contagious viral ailment include inhaling or ingesting droplets containing the virus, which are emitted during coughing and sneezing, as well as direct contact with contaminated surfaces. The genome of the coronavirus comprises numerous non-structural proteins (nsp), totaling 30,000 nucleotides, along with four

structural proteins: the Membrane (M) protein, the Envelope (E) protein, the Nucleocapsid (N) protein, and the Spike (S) protein, as illustrated in Figure 5. Enclosed within the protective protein shell known as the capsid, the nucleocapsid, or N-protein, holds significant importance, by participating in interactions with the virus's single positive-strand RNA, the N-protein facilitates the intrusion into the human host cells. Facilitating the transformation of cellular centers into sites for viral replication and subsequent virus generation is a process undertaken by the Nucleocapsid (N) protein. Its functional role is pivotal, primarily involving the envelopment of the viral RNA genome, thereby assuming a crucial function in both transcription and replication processes. Examination of Murine Hepatitis Virus (MHV) and Infectious Bronchitis Virus (IBV) virions reveals that the N-terminal region of the N protein engages in interactions with both genomic and sub-genomic RNAs, enhancing viral transcription and replication processes. The pursuit of a targeted therapeutic strategy, specifically aiming to disrupt the interaction between the N-terminal portion of the N-protein and the single positive RNA strand, holds significant research importance. Notably, identified theophylline and pyrimidone compounds as potential agents capable of inhibiting the binding of RNA to the N-terminal segment of the N protein in coronaviruses, offering an opportunity for subsequent in vitro validation (Sarma et al. 2020).

The central protein situated on the viral surface, known as the M-protein, plays a crucial role in orchestrating the assembly of coronaviruses. In contrast, the S-protein is evenly distributed across the viral surface, facilitating the virus's adherence to host cell surface receptors and subsequently fostering the fusion of viral and host cell membranes. (Kirchdoerfer et al., 2016), this fusion event holds paramount importance for the virus to efficiently enter the host cell and initiate the infection process, the Envelope (E) protein governs the assembly of viral particles, characterized by its relatively modest dimensions (76–109 amino acids), and functions as a minor constituent within the viral structure. Furthermore, this protein regulates the permeability of the host cell membrane and modulates the interactions between the virus and the host cell (Gupta et al., 2020).

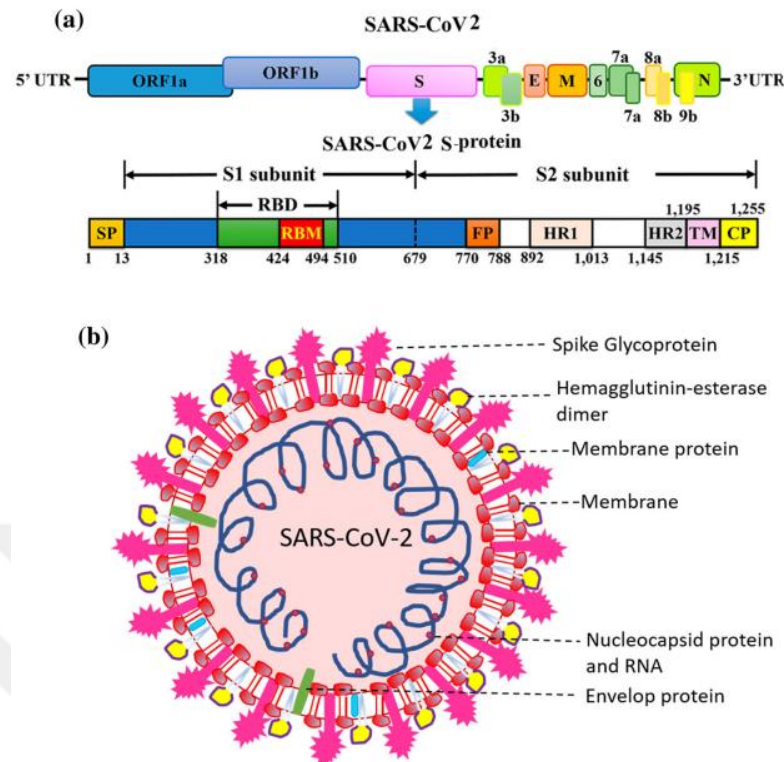


**Figure 2.5:** Visual representation portraying the genomic structure and functional domains of the Spike (S) protein in the context of COVID-19 (Gupta et al., 2020).

The lipid envelope functions as a protective casing for the viral genetic material. Dimeric Hemagglutinin-Esterase (HE) proteins are situated on the viral surface, and although not essential for viral replication, they may potentially assist in virus entry, particularly when infecting the native host cell (Lissenberg et al., 2005). As demonstrated in Walls et al.'s 2020 study, advancements in cryo-electron microscopy (cryo-EM) have enabled a thorough examination of the structural arrangement of the Spike (S) protein, encompassing both its closed conformation (identified as PDB ID: 6VXX) and its open (prefusion) state (designated by PDB ID: 6VYB) this glycoprotein, consisting of three identical chains, comprises a total of 1273 amino acids. The S1 and S2 subunits each play distinct roles in cellular recognition and the fusion of viral and cellular membranes during the process, the subsequent protein conformational changes involved in this step are currently not well understood in the present state of research (Wrapp et al., 2020).

The schematic diagram presented in Figure 6 delineates the sequential progression of processes associated with viral entry, replication, and RNA packaging within human cells. It is noteworthy that various human cells, including those in the respiratory system,

harbor ACE2 receptors. The interaction between the coronavirus's spike (S) protein and these ACE2 receptors is crucial for facilitating viral entry. Host proteases, such as trypsin and furin, play a pivotal role in the proteolytic cleavage of the coronavirus S protein at two distinct locations: the S1/S2 site, located between the S1 and S2 subunits, and the S2' site, positioned within the S2 domain. This enzymatic cleavage event releases the fusion peptide, initiating the process of membrane fusion. A potential therapeutic approach to impede viral entry involves the use of antibodies, which can obstruct the binding site of the ACE2 receptor through the application of structural insights and amino acid sequence information (Wrapp et al., 2020).

Endocytosis is the primary mechanism by which the virus typically enters a human cell. In the context of COVID-19, it is postulated that a distinct tripartite membrane fusion sequence transpires during its ingress into the cytoplasm. This sequence encompasses initial receptor engagement, subsequent structural alterations in the spike (S) glycoprotein, the catalytic action of intracellular proteases such as cathepsin L, and the initiation of the membrane fusion apparatus within endosomal compartments (Simmons et al., 2005), after the endosome has undergone activation, the proteasomes commence the process of viral nucleocapsid (N) de-capsidation by hydrolyzing host and foreign proteins, notably including the SARS nucleocapsid protein, this de-capsidation event leads to the liberation of the virus within the cytoplasm, it is noteworthy to acknowledge the existence of an alternative two-stage mechanism (Li in, 2016), in this alternative pathway, host proteases cleave the spike protein, leading to the disruption of its structural integrity, the S1 component of the virion attaches itself to a particular receptor on the host cell. Then, in an acidic environment, the S2 subunit functions to promote the viral membrane's fusion with the host's target membrane. This binding is followed by the activity of the S2 subunit. These subsequent occurrences cause the viral single-stranded RNA genetic material to get liberated and enter the host cell's cytoplasm ( Hasan et al.,2020 ), replication and transcription activities occur within the replication transcription complex (RTC), a structural scaffold composed of non-structural proteins (nsp) encoded by the viral genome. According to the hypothesis widely (Van Hemert et al.,2008), the RTC is believed to initiate the formation of double-membrane structures in the cytoplasm of infected cells. Following this, replicase proteins are synthesized from the open reading frame 1a/b (ORF 1a/b) located on the positive RNA genome (depicted in Figure 2.5), guided by the viral

genome, these proteins facilitate the synthesis of full-length negative-sense RNAs, which, in turn, serve as templates for generating additional complete genomes. In the subsequent phase, the structural viral proteins M, S, and E, produced within the cytoplasm, are incorporated into the endoplasmic reticulum (ER) and then transported to the endoplasmic Reticulum-Golgi intermediate compartment (ERGIC), as illustrated in Figure 6 (Masters, 2006).

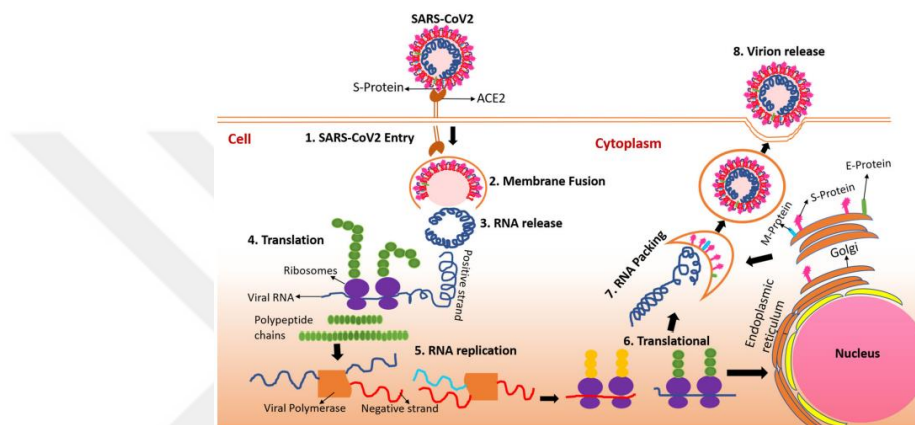


**Figure 2.6:** The schematic illustration portraying the sequence of events involved in the entry of COVID-19 (Masters, 2006).

## 2.9 Pathophysiology

SARS-CoV-2 exhibits a broad tropism, allowing it to invade various cell types and impact multiple organ systems within the human body, while its primary impact is observed in the lower respiratory system, including the lungs and trachea, it can also affect the upper respiratory tract, comprising the sinuses, throat, and nasal passages. It is essential to note that COVID-19 can have deleterious effects on the lungs, as detailed by Harrison and colleagues in their 2020 study, the Angiotensin-Converting Enzyme 2 (ACE2) receptor, predominantly located on the membranes of type II alveolar cells in pulmonary tissue, serves as the entry point for the virus into host cells. The "spike" protein, a specific surface glycoprotein binding to the ACE2 receptor, plays a crucial role in facilitating the virus's attachment and fusion with the host cell, following this interaction, the COVID-19 virus proceeds to invade the ciliated epithelium lining the upper respiratory passages, including the nasopharynx (Letko et al., 2020).

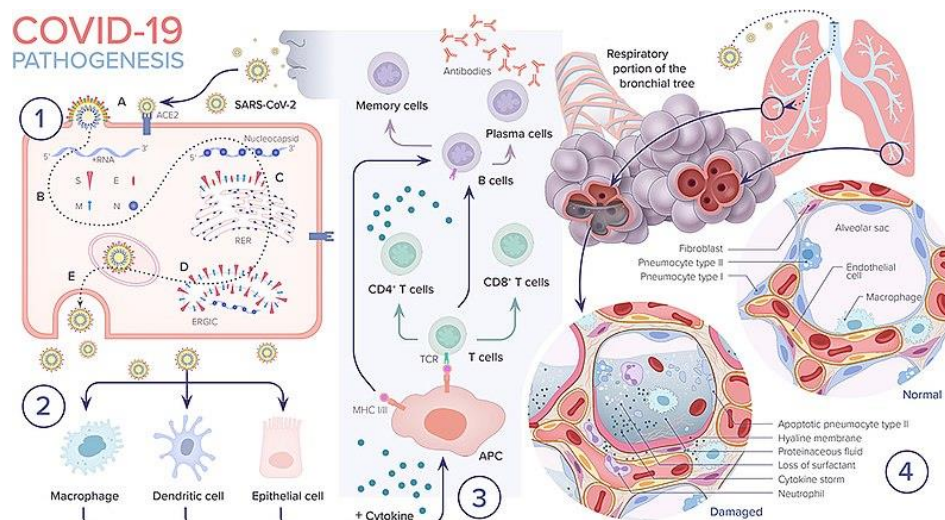


**Figure 2.7:** COVID-19 pathogenesis (Letko et al., 2020).

**Involvement of the Nervous System:** SARS-CoV-2 has the potential to impact the nervous system through diverse mechanisms, manifesting in neurological symptoms such as anosmia, ageusia, and encephalopathy. The virus can infiltrate the central nervous system (CNS), causing direct neuronal damage or eliciting an inflammatory response. Additionally, thrombotic events and the cytokine storm may contribute to neurological complications (Ellul et al., 2020).

**Implications for the Cardiovascular System:** The cardiovascular system emerges as a prominent target in the pathophysiology of COVID-19, observable outcomes include myocardial injury, arrhythmias, and thrombotic events. Direct infection of myocardial cells by the virus can induce inflammation and myocarditis. Dysregulation of the renin-angiotensin-aldosterone system (RAAS) and endothelial dysfunction play roles in fostering cardiovascular complications (Zheng et al., 2020).

**Immunopathology and Cytokine Storm:** Immunopathology assumes a pivotal role in determining the severity of COVID-19, dysregulated immune responses, including the occurrence of a cytokine storm, contribute significantly to conditions such as acute respiratory distress syndrome (ARDS) and multi-organ dysfunction. Noteworthy features include Th1/Th2 imbalance, lymphopenia, and hyperactivation of immune cells, while adaptive immune responses are essential for virus clearance, an overly robust response can lead to tissue damage (Siddiqi and Mehra, 2020).

## 2.10 Prothrombin Gene

Prothrombin assumes a pivotal role in the coagulation cascade, acting as the precursor to thrombin—a serine protease endowed with pro-coagulant, anticoagulant, and anti-fibrinolytic capabilities. Located on chromosome 11 at position 11p11-q12, the prothrombin gene spans 21 kb, encompassing 14 exons and non-coding regulatory sequences. A recent discovery unveiled a G to A transition at nucleotide position 20210 in the 3' untranslated region of the prothrombin gene, linked to a threefold heightened risk of deep vein thrombosis (DVT). The significance of the G20210-A allele as a predisposing factor for venous thrombosis has been substantiated by multiple independent studies. This allele was detected in 4-8% of patients with a history of venous thrombosis, with heterozygous carriers exhibiting a relative risk ranging from 2 to 7. Some investigations reported a milder impact of the G20210-A allele, the overall prevalence of the G20210-A allele in the general population approximates 2.0%, with a higher frequency in southern Europe. Demonstrated an association between the presence of the G20210-A allele and elevated plasma prothrombin levels, a finding corroborated in various studies involving both symptomatic and asymptomatic carriers. A family-based study utilizing linkage analysis revealed that the G20210A mutation impacted both plasma prothrombin levels and the susceptibility to venous thrombosis (Ceelie et al., 2001).

### 2.10.1 Molecular Structure and Function of Prothrombin

The structure of prothrombin has eluded investigators for decades but recent efforts have succeeded in revealing the architecture of this important clotting factor. Unanticipated features have emerged outlining the significant flexibility of the zymogen due to linker regions connecting the  $\gamma$  carboxyglutamic domain, kringles and protease domain. A new, structure-based framework helps in defining a molecular mechanism of prothrombin activation, rationalizes the severe bleeding phenotypes of several naturally occurring mutations and identifies targets for drug design. Prothrombin is a vitamin K-dependent zymogen composed of a gamma carboxyglutamic (Gla) domain (residues 1–46), kringle-1 (residues 65–143), kringle-2 (residues 170–248) and the protease domain with the A chain (residues 285–320) and the catalytic B chain (residues 321–579). The prothrombinase complex converts prothrombin to thrombin by cleaving at R271 and R320, generating the intermediates prethrombin-2 and meizothrombin, respectively, under conditions most relevant to physiology, that is, on the surface of platelets,

prothrombinase activates prothrombin along the prothrombin-2 pathway (Pozzi and DI Cera, 2014).

### **2.10.2 Factor II (Prothrombin G20210A) Mutation**

Given thrombin's pivotal role in the coagulation system, deficiencies in prothrombin, whether stemming from genetic or acquired factors, invariably result in compromised clotting mechanisms, manifesting as bleeding disorders such as hemophilia. Conversely, an augmented presence of prothrombin in the circulatory system is anticipated to heighten the proclivity for blood clot formation, a condition termed "hypercoagulability," clinically often presenting as venous thromboembolism (VTE). Empirical evidence from studies indicates that prothrombin levels exceeding 115% of the norm confer a twofold escalated risk of developing VTE, the prothrombin gene, situated on chromosome 11, was scrutinized in individuals exhibiting unexplained VTE, this investigation unveiled a singular missense mutation (guanine to adenine; G>A) at nucleotide position 20210, localized in the 3' untranslated region of the prothrombin gene. The Prothrombin G20210A mutation, positioned external to the coding region for prothrombin, does not induce alterations in the actual structure or functional attributes of the prothrombin molecule when activated into thrombin. Nonetheless, it engenders an elevation in blood prothrombin levels, approximately one-third beyond the norm (133%), surpassing the additional 15% threshold implicated in the initiation of VTE. Moreover, the Prothrombin G20210A mutation has been demonstrated to augment both mRNA and protein expression for prothrombin (Jadaon, 2011).

Furthermore, the increased levels of prothrombin associated with the mutation may lead to an elevation in thrombin-activatable fibrinolysis inhibitor (TAFI), an antagonist of the fibrinolysis process, which is the physiological mechanism responsible for clot removal. The heightened presence of TAFI has the potential to impede fibrinolysis, permitting clot accumulation and potentially contributing to venous thromboembolism (VTE), this introduces another plausible pathophysiological pathway for the heightened risk of VTE in individuals with the Prothrombin G20210A mutation, with studies indicating a 2 to 4-fold increased risk associated with this genetic variant, Prothrombin (G20210A) signifies a prevalent genetic point mutation linked to thrombosis, representing a shared genetic etiology (McGlennen and Key, 2002), Individuals harboring the Prothrombin G20210A mutation exhibit an elevation in prothrombin levels in the

bloodstream. This elevated prothrombin concentration amplifies the susceptibility to both venous and arterial thrombosis (Jadaon, 2011). The concurrent presence of an FVL mutation in these individuals further amplifies the thrombotic risk (Tirado et al., 2001).

The Prothrombin 20210A mutation manifests as an autosomal dominant disorder resulting in a 3–10 times increased risk of thrombosis in heterozygotes. Although homozygosity is infrequent, it entails a potential thrombotic risk (Athar et al., 2021), additionally, hereditary deficiencies, such as cystathionine-beta synthetase, methionine synthetase, and methylene tetrahydrofolate reductase, along with conditions like Heparin cofactor II deficiency, plasminogen deficiency, dysfibrinogenemia factor XII deficiency, and an elevation in factor VIII coagulant activity, contribute to an augmented predisposition towards thrombosis ( Litvinov et al., 2021).

### **2.10.3 Relation Between Polymorphism and Function of Prothrombin**

Protein structure change due to genetic mutation is one of the important factors of embolism during the process of coagulation and fibrolysis. The position of the prothrombin gene on human chromosome is 11p11-12. Total length of the gene is 21kb, position of G20210 is at or near the split point of the untranslated region of the 3' end of mRNA, G20210A mutation can result in the intruding of nucleotide at or near the point, and lead to alternation of genetic sequence. The change brings higher translation efficiency of the gene or higher stability of transcript mRNA, and leads to the elevation of plasma prothrombin level. Research on hereditary thrombophilia has discovered that the 20210 G-to-A mutation of the 3' untranslated region increases the risk of VTE by 3 times. In spite of age or gender, risk of VTE elevates in the population with 20210A allele (Seligsohn and Lubetsky,2001).

#### **2.10.4 Role of Prothrombin in the Coagulation System.**

Upon blood vessel injury, a cascade of chemical reactions is initiated, instigating blood coagulation and culminating in the formation of a blood clot that functions to occlude the injured vessel and forestall blood loss, this intricate process involves the collaboration of numerous enzymes and proteins collectively referred to as blood clotting factors, with thrombin (clotting factor II) serving as a pivotal constituent. Typically synthesized in an inert form termed prothrombin within the liver, thrombin circulates in the bloodstream until activation is triggered by another clotting factor, activated factor X, in response to the injury, the primary function of thrombin lies in the conversion of fibrinogen (clotting factor I) into a fibrin clot, effectively obstructing the wounded blood vessel, thrombin, being a potent enzyme, exerts substantial influence on the coagulation system by activating various clotting factors and other components, including blood platelets. Significantly, the activity of thrombin is meticulously regulated by an inhibitor known as antithrombin (AT), which downregulates thrombin post-clot formation, thereby averting inadvertent thrombin generation in locations distal to the injured vessels (Jadaon,2011).

#### **2.10.5 Thrombophilia**

Thrombophilia refers to an elevated propensity for blood to form clots, leading to hypercoagulability (Hassouna, 2009), hypofibrinolysis, characterized by a diminished ability to break down thrombin, is another factor contributing to hypercoagulability. The venous system is susceptible to thrombosis due to stasis and coagulation system disorders, while the arterial system is influenced by endothelial damage and platelet functional disturbances, these conditions result in reduced compliance and vasomotion in major organ system blood vessels (Smalberg, 2011).

Thrombophilia, implicated in clot formation within critical organs such as the heart, lungs, and brain, can ultimately result in sudden death (Libby and Lüscher, 2020), thrombophilia is characterized by two distinct mechanisms hereditary and acquired, although either hereditary or acquired factors may autonomously contribute to the manifestation of thrombophilia, it is prevalent for both to concurrently exist in the majority of cases (Nakashima and Rogers, 2014).

### **2.10.6 Antithrombin (AT) Deficiency**

The cooperative effect of AT III with heparin or heparin-like molecules results in an approximate 1,000-fold increase in its effectiveness. Consequently, when measuring antithrombin-heparin cofactor levels, especially in the context of heparin treatment, it is advisable to conduct the assessment at least two weeks after discontinuation of heparin (Avidan et al., 2005), synthesized in the liver, AT III is a 432-amino acid protein released into the bloodstream, naturally impeding the formation of blood clots (thrombus), antithrombin-III exists in plasma in latent and active states, with temperature and pH influencing the equilibrium between these two phases (Hepner and Karlaftis, 2013; Gissel et al., 2016).

Two distinct forms of AT deficiency are recognized: Type I deficiency involves diminished AT molecule synthesis, resulting in impaired functional tests due to reduced AT synthesis. On the other hand, Type II deficiency does not affect synthesis but instead hinders functionality. Type II deficiency encompasses three subtypes: (1) "reactive site" defect of AT, (2) Type II RLS with a "heparin-binding site" defect, and (3) Type II PE associated with a "pleiotropic effect" and multifunctional defect, heterozygous individuals with defects in the heparin-binding domain have reported a 6% incidence of venous thromboembolism (VTE) events (Patnaik and Moll, 2008), homozygous individuals may experience more severe VTE events. Antithrombin-III deficiency has been documented in approximately 50% of individuals with VTE (Anderson et al., 2003; Refaei et al., 2017).

### **2.10.7 Venous thromboembolism**

Venous thromboembolism stands as a significant global health burden, witnessing an annual occurrence of around 10 million cases and ranking as the third most prevalent vascular disease following acute myocardial infarction and stroke (Raskob et al., 2014). In six European countries with a collective population of 300 million, just under half a million instances of deep vein thromboses and 300,000 pulmonary embolisms are reported annually (Cohen et al., 2007), the incidence of venous thromboembolism is on the rise due to factors such as population aging, an increased prevalence of comorbidities associated with this condition (e.g., obesity, heart failure, cancer), and the enhanced sensitivity and widespread use of imaging tests for its detection (Raskob et al., 2014; Huang et al., 2014).

The yearly average incidence exhibits an exponential increase with age, reaching up to one case per hundred individuals aged over 80 years (Raskob et al., 2014; Jensvoll et al., 2015; Heit, 2015), starting from the age of 45, the lifetime risk of developing venous thromboembolism is 8%. While the incidence is higher in black individuals compared to white individuals, it is lower in Asian individuals, and the reasons for this disparity remain unclear. Risk does not vary by gender, although it appears to be twice as high in men compared to women when considering venous thromboembolisms related to pregnancy and estrogen therapy (Roach et al., 2014).

Venous thromboembolism (VTE) is associated with substantial morbidity and mortality. Although the 30-day mortality rate following pulmonary embolism is on a declining trend, approximately 20% of patients succumb to pulmonary embolism before or shortly after diagnosis, particularly in instances marked by hemodynamic instability, over the extended term, VTE evolves into a chronic condition, with approximately 30% of all patients experiencing a recurrence within a decade, the repercussions of VTE are linked to significant disability, notably the emergence of post-thrombotic syndrome, observed in 20–50% of deep vein thrombosis patients, and chronic thromboembolic pulmonary hypertension, complicating 0.1–4.0% of pulmonary embolisms (Hoeper et al., 2014), despite advancements in comprehending risk factors in recent decades, a substantial proportion, ranging from a third to half, of VTE episodes lack identifiable provoking factors and are categorized as unprovoked (Kearon et al., 2016).

Prominent risk factors for VTE encompass surgical procedures, immobilization, and cancer. Notably, patients undergoing major orthopedic surgery face a particularly elevated risk, with postoperative rates of around 1% despite receiving pharmacological thromboprophylaxis. Cancer-related incidents account for approximately 20% of all VTE cases, while surgery and immobilization contribute to 15% each (Jensvoll et al., 2015). Among the most prevalent heritable risk factors, aside from non-O blood group, are factor V Leiden and prothrombin gene mutations, with a prevalence in the European population of 3–7% and 1–2%, respectively, however, as heritable risk factors offer limited predictive value for recurrent VTE, the relevance of thrombophilia testing in the long-term management of VTE appears restricted (Morange et al., 2015).

### 3. MATERIAL AND METHODS

#### 3.1 Working process and storage of samples

The research was carried out at the Department of Medical Biology, Faculty of Medicine, Tokat Gaziosmanpasa University. A total of 141 participants were involved, comprising 100 individuals diagnosed with COVID-19 and 41 in the control group. The participants were categorized into four groups: Control Group, Mild Group, Inpatient Group, and Intensive Care Group (refer to Table 3.1). The study cohort consisted of 58 male and 83 female participants, spanning ages from 17 to 94 years. Among the participants, 37 were identified as smokers, and 27 reported alcohol consumption. Samples obtained from the patients were preserved at +4°C.

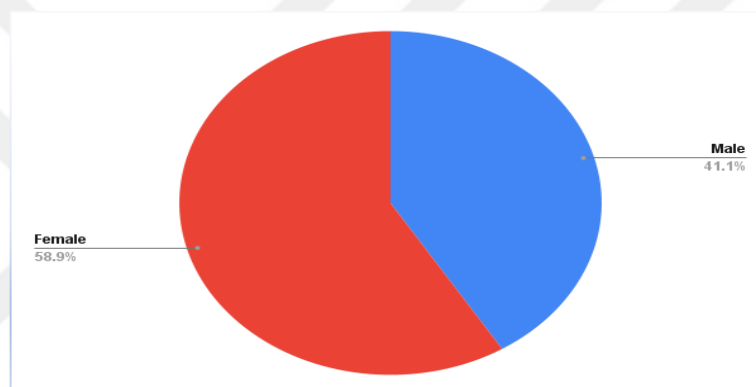


**Figure 3.1:** Individuals participated in the study.

Approval for the study was obtained from the ethics committee for clinical research at the Faculty of Medicine, Tokat Gaziosmanpasa University, with decision number 23-KAEK-006. Financial support for the study was provided by the scientific research center of Tokat Gaziosmanpasa University, designated as project number 2023/60.

**Table 3.1:** Sample size for different groups in our study.

| Symptoms       | Samples |
|----------------|---------|
| Mild           | 42      |
| Inpatient      | 15      |
| Intensive care | 43      |
| Control        | 41      |
| Total          | 141     |

### 3.2 Chemical materials, Kits and Kit contents

The DNA extraction was performed using the GeneAll® Exgene™ Blood SV mini kit (105-101/105-152), involving the utilization of the following components:

- 1- Buffer BL
- 2- Buffer BW
- 3- Buffer TW
- 4- Buffer AE
- 5- RNase A
- 6- Proteinase K
- 7- Tris-Borate-EDTA (TBE) electrophoresis buffer Lot no: 01041879
- 8- Prona agarose Lot no: 000422PR
- 9- Nusive prona agarose Lot no: 00002PR
- 10- dNTP-100 1 kit Lot no: 104K6024

### 3.3 Devices used in the study

- 1- Refrigerator (Arcelik, 570 465 MB, Turkey).
- 2- Vortex (Velp Scientifica, F20220176, Italy)
- 3- -20 ° C freezer (Arcelik, 2052DY, Turkey)
- 4- -80 ° C Deep Freezer (Nuair, NU-9483E, USA)
- 5- Microwave (Arcelik, MD554, Turkey)
- 6- PCR Thermal Cycler (Analytik Jena's Biometra, Germany)
- 7- Centrifuge (Hettich, D78532, Germany)
- 8- Kern ABJ 220-4NM Analytik Terazi 220 gr /0,0001 gr
- 9- Power Supply (Biorad, 1645070, USA)

### 3.4 Methods

#### 3.4.1 DNA Isolation

DNA extraction from blood samples was carried out using the GeneAll® Exgene™ Blood SV mini kit (105-101/105-152) with the following protocol:

1. A sterile microcentrifuge tube was used to introduce 200  $\mu$ L of blood.
2. Proteinase K (20  $\mu$ L) was added to the tube.
3. RNase A (20  $\mu$ L) was introduced into the tube.
4. The resulting mixture was thoroughly vortexed and then incubated at room temperature for 2 minutes.
5. To the tube, 200  $\mu$ L of Buffer BL was added, thoroughly mixed through vortexing, and incubated at 56°C for 10 min. After a brief spin to remove any drops from the lid, 200  $\mu$ L of 96% ethanol was added to each sample, followed by vortexing.

Purification Protocol:

6. The mixture was carefully transferred to Column Type G (mini), centrifuged at 6,000 x g (>8,000 rpm) for 1 min, and the collection tube was replaced.
7. Buffer BW (600  $\mu$ L) was added, centrifuged at 6,000 x g (>8,000 rpm) for 1 min, and the collection tube was replaced.
8. Buffer TW (700  $\mu$ L) was applied, centrifuged at 6,000 x g (>8,000 rpm) for 1 min, and the pass-through was discarded. The mini column was reinserted into the collection tube.
9. Full-speed centrifugation for 1 min removed residual wash buffer. The mini column was placed in a fresh 1.5 ml microcentrifuge tube.
10. Buffer AE (200  $\mu$ L) or sterilized water was added, incubated for 1 min at room temperature, and centrifuged at full speed for 1 min.

#### 3.4.2 Preparation of materials

##### 1- Preparation of Marker

Our study involved the application of the PUC Mix marker. During the preparation phase, we combined 80  $\mu$ L of distilled water, 10  $\mu$ L of dye, and 10  $\mu$ L of PUC. The mixture was then centrifuged for 5 seconds, resulting in a marker that was ready for use.

## **2- Gel preparation**

To prepare the gel, we introduced a mixture of 2 g (2%) of Agarose and 1 g of nusive (Gamma Micropor) into a 300 ml conical flask containing 100 ml of Tris-Borate-EDTA (TBE). The flask was then placed in a microwave set to a temperature range of 450°C-500°C for 3 minutes, with intermittent swirling to ensure complete dissolution of the Agarose. This resulted in a clear gel solution. After cooling the solution for a minute, we added 9 µL of ethidium bromide, which was evenly distributed by swirling. Finally, the gel solution was poured into the electrophoresis tray for further cooling and solidification.

## **3- dNTP preparation**

In the context of Polymerase Chain Reaction (PCR), the need for deoxynucleoside triphosphate (dNTP) necessitates a rigorous preparation process. To be specific, 200 µl of distilled water was combined with 50 µl each of dGTP, dCTP, dTTP, and dATP in a designated tube. The resulting mixture was then subjected to centrifugation for a duration of 5 seconds to achieve homogeneity.

### **3.4.3 PCR**

The Prothrombin gene polymorphism was assessed using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique. Specifically, a well-defined gene segment was amplified through Polymerase Chain Reaction (PCR) with both forward and reverse primers. Subsequently, the resulting PCR products underwent digestion using an appropriate restriction enzyme. The fragments were then separated through gel electrophoresis, enabling the identification of polymorphisms based on distinct DNA fragment patterns.

The PCR procedure was conducted in a 25 µL reaction volume, comprising 16 µL of sterile distilled water (dH<sub>2</sub>O), 2.5 µL of PCR buffer, 1.5 µL of Magnesium Chloride (MgCl<sub>2</sub>), 0.3 µL of deoxyribonucleotide triphosphates (dNTPs), 0.8 µL of each primer (forward and reverse), and 0.1 µL of Taq DNA polymerase. This complete mixture was meticulously added to a freshly labeled Eppendorf tube, supplemented with 3 µL of the DNA specimen under analysis.

**Table 3.2:** Primer sets.

| Primers             | Sequences                                  |
|---------------------|--------------------------------------------|
| Prothrombin Forward | <b>5' GCA CAG ACG GCT GTT CTC TT 3'</b>    |
| Prothrombin Reverse | <b>5' ATA GCA CTG GGA GCA TTG AAG C 3'</b> |

**Table 3.3:** PCR protocol.

|           | Temperature (°C) | Time       | PCR steps              |
|-----------|------------------|------------|------------------------|
| 40 cycles | 95 °C            | 2 minutes  | Initial denaturation   |
|           | 94 °C            | 30 seconds | Denaturation           |
|           | 63 °C            | 15 seconds | Annealing              |
|           | 72 °C            | 1 minute   | Extension (Elongation) |
|           | 72 °C            | 5 minutes  | Final Extension        |

After the Polymerase Chain Reaction (PCR) was completed, random samples were selected to undergo gel electrophoresis to verify the presence of DNA before proceeding to the next step.

#### 3.4.4 RFLP

Restriction Fragment Length Polymorphism (RFLP) is a molecular biology technique used to identify disparities in genetic sequences. It operates on the principle that variations in DNA sequences often lead to the creation or deletion of restriction enzyme recognition sites, which are places in DNA where a particular restriction enzyme can cut. In the determination of prothrombin polymorphism in COVID-19 patients, RFLP plays a crucial role in identifying and differentiating between various forms of the prothrombin gene.

In the RFLP process, after the Polymerase Chain Reaction (PCR) process was completed, a mixture was prepared using 8.5 µL of distilled water (dH<sub>2</sub>O), 1.2 µL of colored buffer, and 0.3 µL of HindIII enzyme. This mixture was then added to 10 of each of the PCR products. After that, all the samples were transferred into a 90-minute incubation interval at a controlled temperature of 37°C, enabling effective enzymatic digestion of the amplified DNA via restriction enzymes. Upon conclusion of the incubation, the treated samples were subjected to rigorous examination involving gel

electrophoresis techniques using 10  $\mu$ L of each sample, ultimately allowing us to visually inspect and interpret the distinctive banding configurations associated with varied allelic or genetic compositions within the prothrombin gene

### **3.5 Statistical analysis**

The study analyzed the demographic data using the SPSS programming and determined statistical significance using the p-value when it was less than 0.05. The research found a consistent “GG” genotype in both the COVID-19 affected group (n=100) and the control group (n=41), as identified through PCR-RFLP analysis of the prothrombin gene. However, this genetic configuration does not show a significant association with increased thrombotic risks related to SARS-CoV-2 infection. Due to limited sample size and homogeneity, statistical validation is absent. Nonetheless, these observations highlight the intricate interplay between genetics and COVID-19, suggesting a potential basis for tailored risk assessments and personalized preventive approaches in future medical practice. Larger, diverse studies are essential to validate and expand our understanding of how genetic variations impact COVID-19 progression and health outcomes.

#### 4. RESULT

The study encompassed 141 participants, comprising 100 (71%) COVID-19 patients and 41 (29%) individuals in the control group selected from Tokat Government Hospital. Gender distribution is different between the two groups, in patient group 71 (71%) were Female and 29 (29%) were Male, in control group 18 (44%) were Female and 23 (56%) were Male, and the mean age of patients was 49.5 years, The participants were categorized into four groups: 41 in the Control Group, 42 in the Mild Group, 15 in the Inpatient Group, and 43 in the Intensive Care Group. The findings of the study suggested that the prothrombin G20210A polymorphism might not represent a significant risk factor for COVID-19 within this specific patient population.

**Table 4.1:** Characteristics of the participants.

|                          |                                                         |
|--------------------------|---------------------------------------------------------|
| Department Research Type | Faculty of Medicine Medical Biology                     |
| Participants             | Total: 141 - COVID-19 Cases: 100 -<br>Control Group: 41 |
| Gender Distribution      | (total) Male: 52 Female: 89                             |
| Age Range                | 17-94 years                                             |
| Smoking                  | Smokers: 37                                             |
| Alcohol Consumption      | Alcohol Consumers: 27                                   |

**Table 4.2:** Demographic data of patient and control group.

|                   |        | Patient         | Control         | P        |
|-------------------|--------|-----------------|-----------------|----------|
| Mean age $\pm$ SD |        | 49.5 $\pm$ 21.5 | 37.8 $\pm$ 10.7 | 0.000036 |
| Gender            | Male   | 29 (29%)        | 23 (56%)        | 0.0045   |
|                   | Female | 71 (71%)        | 18 (44%)        |          |

##### 4.1 Prothrombin rs1799963 polymorphism in COVID-19

In our analysis of 141 participants, all showed in both Control and inpatient group %100 the GG genotype (406 bp) for the prothrombin G20210A polymorphism. Unexpectedly, the absence of the rs1799963 variant suggests that, in this cohort, this

polymorphism may not play a substantial role as a COVID-19 risk factor. This challenges conventional assumptions, highlighting the intricate relationship between genetics and disease outcomes (Figure 4.1).

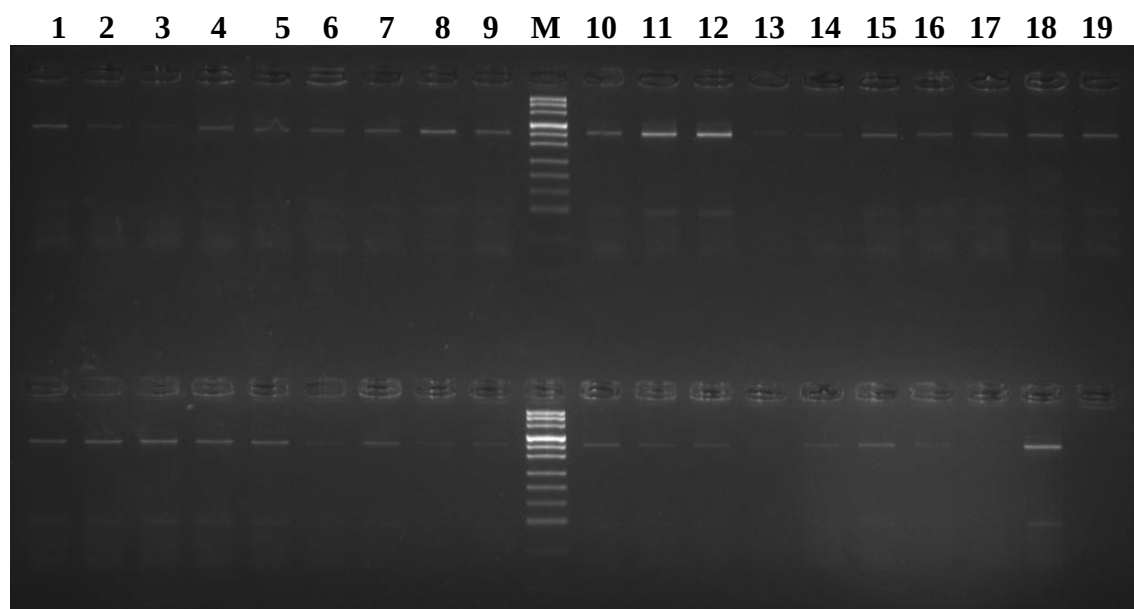


**Figure 4.1:** Agarose gel electrophoresis of prothrombin gene PCR-RFLP amplification products. all of them have a GG homozygote single band of 406 bp

The study found that the “GG” genotype in the prothrombin gene was uniformly present within the study population. No prothrombin gene polymorphism was identified among the COVID-19 patients analyzed. The study conducted laboratory tests on blood samples taken from the participants (patients and control) to find out if there is any correlation between the value of the tests with the severity of the disease (Table 4.3).

**Table 4.3:** People's Demographics and Their Distribution P-value.

|                   | P-value |       |       |        |       |        |       |        |        |          |
|-------------------|---------|-------|-------|--------|-------|--------|-------|--------|--------|----------|
|                   | Glucose | ALT   | AST   | HDL    | VLDL  | LDL    | TG    | BUN    | CRP    | Ferritin |
| Control-Mild      | 0.999   | 0.999 | 0.999 | 0.999  | 0.014 | 0.026  | 0.129 | 0.999  | 0.999  | 0.999    |
| Control-Inpatient | 0.999   | 0.999 | 0.999 | 0.567  | 0.999 | 0.158  | 0.999 | 0.450  | 0.014  | 0.999    |
| Control-IC        | <0.001  | 0.022 | 0.080 | <0.001 | 0.999 | <0.001 | 0.999 | <0.001 | <0.001 | 0.203    |
| Mild-Inpatient    | 0.679   | 0.999 | 0.999 | 0.872  | 0.975 | 0.999  | 0.999 | 0.107  | 0.038  | 0.999    |
| Mild-IC           | <0.001  | 0.009 | 0.060 | 0.062  | 0.033 | 0.005  | 0.283 | <0.001 | <0.001 | 0.229    |
| Inpatient-IC      | 0.039   | 0.804 | 0.662 | 0.999  | 0.999 | 0.126  | 0.999 | <0.001 | <0.001 | 0.999    |

The study examined six distinct categories of clinical severity, namely Control-Mild, Control-Intensive Care, Control-Inpatient, Mild-Intensive Care, Mild-Inpatient, and Intensive Care-Inpatient, and conducted a comprehensive examination of ten clinical laboratory parameters for each group. The statistical significance of the observed outcomes was established by calculating p-values, with the conventional threshold of  $p < 0.05$  serving as the demarcation for significance. Among the various clinical laboratory results being examined, 13.33% showed statistical significance, while the remaining 86.66% did not reach a statistically significant level.

The study found significant variations in VLDL and LDL levels among the Control-Mild group, with p-values of 0.014 and 0.026, respectively. In contrast, the Control-Intensive Care group did not exhibit significant differences in any parameters except for ALT (0.022). Interestingly, within the Control-Inpatient group, a singular statistical significance was observed in the CRP parameter with a p-value of 0.014, indicating a distinct elevation in CRP levels among individuals in this cohort.

The investigation of the Mild-Intensive Care group revealed that only two parameters, VLDL and LDL, showed statistically significant differences with corresponding p-values of 0.033 and 0.005. Similarly, the Mild-Inpatient group showed

statistical significance only in the CRP parameter, registering a p-value of 0.038. These findings highlight the subtle nature of clinical parameter differences within the Mild-Intensive Care and Mild-Inpatient cohorts, with emphasis on the specific parameters that exhibit statistically significant distinctions.

In the final examination of the Intensive Care-Inpatient group, statistical significance was observed only in the Glucose parameter, with a corresponding p-value of 0.039. These findings emphasize the nuanced nature of clinical variations observed among distinct severity cohorts, providing a comprehensive explanation of the significance associated with these observed differences



## 5. DISCUSSION

In December 2019, an undisclosed global health emergency emerged with the advent of SARS-CoV-2, and hitherto unidentified strain of coronavirus. This virus has been subsequently identified as the primary causative agent of COVID-19, a severe respiratory ailment (Fauci et al., 2020), consequently, this pandemic, primarily affecting the respiratory system, has posed significant challenges for governmental health authorities, scientific research endeavors, and the healthcare sector (WHO, 2022), the onset of the COVID-19 pandemic underscores the urgent need to comprehend the intricate interplay between SARS-CoV-2 infection and host immune-hemostatic responses. Mounting evidence suggests that COVID-19 exhibits distinct characteristics consistent with Disseminated Intravascular Coagulation (DIC) (Connors et al., 2020).

The human prothrombin gene, situated on chromosome 11 and spanning approximately 21 kb, consists of eight introns and nine exons among various identified polymorphisms, the G20210A transition is a noteworthy functional variant within the prothrombin gene. This variant leads to elevated plasma concentrations of prothrombin, thereby enhancing the capacity for thrombin generation (Poort et al., 1996), significantly, the G20210A polymorphism stands as the second most influential factor in inherited thrombophilia, following only the factor V Leiden mutation (Kujovich, 2005), given the accumulating evidence supporting the involvement of activated coagulation cascades in the morbidity and mortality of COVID-19 assessing the prevalence and impact of prothrombin polymorphisms becomes crucial (Spiezia et al., 2020).

Recent investigations suggest that the prothrombin G20210A polymorphism plays a role in adverse outcomes associated with COVID-19 (Alvarez-Román et al., 2021) illustrated that individuals carrying the G20210A polymorphism are at an elevated risk of pulmonary embolism (PE) during COVID-19, emphasizing the significance of considering genetic factors in PE management for this particular population. Furthermore, another study indicated that the concurrent presence of factor V Leiden and prothrombin G20210A polymorphisms increases the likelihood of thrombosis and mortality in COVID-19 patients (Zhang et al., 2020).

Within a study targeting Thromboembolism within the Pakistani populace, a comprehensive analysis encompassing 200 participants, including 100 males and 100 females aged between 20 and 40 years, was undertaken. The investigation yielded a scant presence of heterozygosity for the prothrombin gene, with only two instances detected among the cohort. This translates to an overall carrier prevalence of 1.0%, with a 95% confidence interval ranging from 0.2% to 2.4%. Notably, graphical representation of the genetic profiles of the 200 subjects vividly delineated the predominant distribution of normal genotypes (GG = 99%), with a marginal representation of heterozygous (GA = 1%) and homozygous (AA = 0.0025%) individuals within the examined population (Abdul Naeem et al., 2006).

In the study focused on a group of Turkish individuals, including 68 patients with Acute Coronary Syndrome (ACS), with the aim of evaluating hypercoagulable states. Notably, the analysis of the prothrombin gene mutation (G20210A) did not reveal a statistically significant distinction between the patient and control groups. Specifically, the prevalence of this mutation was 2.9% among patients and 1.4% among controls, resulting in a computed p-value of 0.551, indicating the absence of a noteworthy difference. Furthermore, within the overall 137 cases, the Prothrombin G20210A mutation was identified as heterozygotic in three instances, constituting 2.2% of the investigated population (Buğan et al., 2016).

In an academic inquiry, a comprehensive study involving a cohort of 803 participants in the control arm and 487 individuals diagnosed with Myocardial Infarction (MI) was conducted. The investigation uncovered noteworthy differences in genotype distribution and allelic frequency between the MI group and the control group. Specifically, individuals with myocardial infarction exhibited a prevalence of 97% for the GG genotype, with a representation of 3% for GA and AA genotypes. In contrast, the control group demonstrated a higher frequency of 99% for the GG genotype, with a lower presence of only 1% for GA and AA genotypes. These disparities in genotype distributions resulted in statistically significant outcomes ( $p = 0.031$ ), suggesting potential correlations between specific genotypic configurations and an increased susceptibility to the development of myocardial infarction. (Kallel et al., 2016).

In a 2019 investigation involving 1460 participants, the study aimed to assess the potential relationship between the G20210A polymorphism and the severity of Coronary Artery Disease (CAD). The participants were divided into two groups: those without CAD (n=465) and those with CAD (n=995). Among individuals without CAD, the majority possessed the GG genotype (95.3%), followed by GA (4.3%) and AA (0.4%), while in the CAD group, the distribution was comparable, with GG being the predominant genotype (94.9%), followed by GA (4.2%) and AA (0.9%). Upon analyzing the Gensini score, no statistically significant differences emerged between the GG and AA+GA genotypes ( $p = 0.440$ ). Similarly, examination of CAD severity, using the vessel score, revealed no notable linear trend for diseased vessels in individuals with the AA+GA genotype in comparison to those with the GG genotype ( $p = 0.765$ ). (Pourgholi et al., 2019).

In a research study conducted in Samsun, Turkey, a cohort of 90 participants diagnosed with COVID-19 was meticulously examined and categorized into three severity groups: severe/critical (n=30), mild/moderate (n=30), and asymptomatic (n=30). Among these individuals, genetic markers for hereditary Thrombophilia were assessed, revealing 57 males and 33 females with ages ranging from 19 to 95 years. Notably, the study found a predominance of the GG genotype (99%) compared to the GA genotype (1%). However, despite this genetic variation, the findings indicated an absence of statistically significant associations between the observed genotypic distribution and the severity of COVID-19 among the examined patients (Sezar et al., 2022).

There has been extensive research on the G20210A polymorphism in the literature, and a predominant occurrence of the GG polymorphism has been noted in various studies, for instance, Kallel et al. (2016) reported 97% GG polymorphism, Sezar et al. (2022) observed 99%, and Abul Naeem et al. (2006) also found 99%. However, in our study conducted on the Turkish population, every patient exhibited the GG polymorphism, constituting a 100% prevalence of GG. It's noteworthy that Turkey is not typically characterized as a region with a high incidence of prothrombin gene mutations

Investigating the prospect of prothrombin gene polymorphism in a cohort of 100 individuals diagnosed with COVID-19, we executed PCR-RFLP analysis, revealing that all patients demonstrated identical band patterns indicative of the homozygous "GG"

genotype, suggesting the absence of genetic variation in the prothrombin gene. Consequently, it appears improbable that such polymorphism considerably contributes to thrombotic challenges experienced by some COVID-19 patients within this particular group, implying that alternate factors might be implicated in influencing clotting tendencies during infection.

Although the uniformity observed in the prothrombin gene within our patient group offers valuable insights, it is crucial to acknowledge the limitations inherent in our study, particularly pertaining to the modest size of our cohort, potentially constraining its representativeness of a more extensive range of genetic diversity. Furthermore, it is essential to recognize that the interaction between genes and the environment might impact the course of disease development. Moreover, the impact of other genetic variations, such as those linked to coagulation factors or genes associated with immune response, should not be dismissed, particularly when considered in conjunction with specific environmental factors.

## CONCLUSION

In the investigation conducted at Tokat Gaziosmanpasa University, Faculty of Medicine, involving a cohort of 141 individuals comprising both COVID-19 cases and controls, a notable uniformity was observed in relation to the prothrombin G20210A polymorphism. Specifically, all participants in this specific population exhibited the GG genotype. This consistent genetic pattern suggests that, at least within the scope of this study, the prothrombin G20210A polymorphism may not exert a significant influence on the variability in susceptibility to COVID-19. These findings underscore the importance of considering population-specific genetic factors when understanding the intricate interplay between host genetics and viral infections. Further research is essential to elucidate the nuanced genetic determinants that may impact COVID-19 susceptibility across diverse populations.

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