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TOKAT GAZIOSMANPAŞA UNIVERSITY
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DEPARTMENT OF MEDICAL BIOLOGY
MEDICAL BIOLOGY MASTER'S PROGRAM

DETERMINATION OF MTHFR GENE POLYMORPHISM IN COVID-19 PATIENTS

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Department of Medical Biology
MASTER'S THESIS

Supervisor: Prof. Dr. H. Ömer ATEŞ

TOKAT - 2024

ETHICAL CONTRACT

T.C

TOKAT GAZİOSMANPAŞA UNIVERSITY

TO THE INSTITUTE OF GRADUATE STUDIES DIRECTORATE

I attest to the originality of the Master's thesis entitled "Determination of MTHFR Gene Polymorphism In Covid-19 Patients" in this document. I confirm that I followed the guidelines of Tokat Gaziosmanpaşa University – Graduate Education Institute while preparing this thesis. Moreover, I affirm that I gathered and presented all the information in accordance with academic regulations and ethical principles. To abide by these regulations and principles, I have cited all the data, thoughts, and findings that are not my own.

Tokat Gaziosmanpasa University Ethics Committee for Clinical Research decision number: 23- KAEK-080

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DILER GABRAEL SAADI

JURY ACCEPTANCE AND APPROVAL

The defense examination of the thesis study titled “Determination of MTHFR Gene Polymorphism In Covid-19 Patients’’ is prepared by DILER GABRAEL SAADI was held on 06/03/2024 it has been accepted by jury members as a Master’s Thesis at Tokat Gaziosmanpaşa University, Graduate Education Institute, Department of Medical Biology.

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ÖZET

COVID-19 HASTALARINDA MTHFR GEN POLİMORFİZMİNİN BELİRLENMESİ

Diler Gabrael Saadi

Prof. Dr. H. Ömer ATEŞ

Mart 2024, xi + 63 sayfa

Covid-19 pandemisi, koronavirüs soyuna yeni dahil olan SARS-CoV-2 virüsünden kaynaklanan olağanüstü bulaşıcı bir hastalığı ifade etmektedir. İlk olarak Aralık 2019'da Çin'in Hubei eyaletinde bulunan Wuhan şehrinde tespit edilmiştir. Bu hastalığın uluslararası sınırlar arasında hızla yayılması, Dünya Sağlık Örgütü'nün (WHO) Mart 2020'de bu hastalığı resmen küresel bir pandemi olarak ilan etmesine yol açmıştır. Bu akademik araştırma kapsamında temel amacımız, COVID-19'dan etkilenen bireylerde metilentetrahidrofolat redüktaz (MTHFR) geni C677T'deki genetik varyasyonların etkilerini incelemekti. Tokat Devlet Hastanesi'nden 99 Covid-19 hastası ve 41 sağlıklı kontrol olmak üzere 140 katılımcıdan oluşan bir kohort temin edilmiş, daha sonra Tıp Fakültesi'ne nakledilmiş ve Tıbbi Biyoloji Anabilim Dalı laboratuvar tesislerinde kapsamlı analizlere tabi tutulmuştur. Araştırma prosedürleri, Polimeraz Zincir Reaksiyonu-Restriksiyon Parça Uzunluğu Polimorfizmi (PCR-RFLP) teknikleri de dahil olmak üzere bir dizi metodolojinin kullanıldığı DNA izolasyonunu içermektedir. Elde edilen sonuçlar daha sonra Jel Elektroforezi ile görselleştirilerek genetik varyantların tanımlanması kolaylaştırılmıştır. Bu analitik çabanın birincil amacı, Metilentetrahidrofolat Redüktaz (MTHFR) geni C/T polimorfizmi (rs1801133) ile Covid-19 belirtilerinin şiddeti arasındaki potansiyel korelasyonları 0,05'ten küçük bir p-değeri ile ayırt etmekti. Sonuçlarımızın belirgin bir oranı, tam olarak %42,86'sı, Kontrol grubu ile hastalar arasında ve Türk toplumundaki hafif ve yatan vakalar arasındaki genotip karşılaştırmalarında istatistiksel anlamlılık göstermiştir. Bu ilgili genotip karşılaştırmaları için hesaplanan p-değerleri 0.0154, 0.0222 ve 0.0204'tür.

Anahtar kelimeler: COVID-19, bulaşıcı hastalıklar, genetik varyasyonlar, DNA izolasyonu, MTHFR gen.

ABSTRACT

DETERMINATION OF MTHFR GENE POLYMORPHISM IN COVID-19 PATIENTS

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The Covid-19 pandemic denotes an exceptionally transmissible illness originating from the SARS-CoV-2 virus, a recent inclusion within the coronavirus lineage. It was first identified in December 2019 in Wuhan, a city located in the Hubei province of China. The rapid spread of this illness across international borders led the World Health Organization (WHO) to officially declare it a global pandemic in March 2020. In the scope of this academic research endeavor, our main objective was to examine the effects of genetic variations within the methylenetetrahydrofolate reductase (MTHFR) gene C677T in individuals affected by COVID-19. A cohort comprising 140 participants including 99 Covid-19 patients and 41 healthy control was procured from Tokat Government Hospital, subsequently transported to the Faculty of Medicine, and subjected to comprehensive analysis within the laboratory facilities of the Department of Medical Biology. The investigative procedures involved DNA isolation, employing a series of methodologies, including Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) techniques. The obtained results were subsequently visualized through Gel Electrophoresis, facilitating the identification of genetic variants. The primary objective of this analytical endeavor was to discern potential correlations between the Methylenetetrahydrofolate Reductase (MTHFR) gene C/T polymorphism (rs1801133) with the severity of Covid-19 manifestations with a p-value less than 0.05. Significantly, a discernible proportion of our results, precisely 42.86%, demonstrated statistical significance in the comparisons of genotypes between the Control group and patients, as well as between mild and inpatient cases in Turkish population. The calculated p-values for these respective genotype comparisons were 0.0154, 0.0222, and 0.0204.

Keywords: COVID-19, infectious diseases, genetic variations, DNA isolation, MTHFR gene.

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ABBREVIATION

ALT	Alanine transaminase
AST	Aspartate aminotransferase
AGE	Agarose Gel Electrophoresis
ACE2	Angiotensin-converting enzyme 2
ARDS	Acute Respiratory Distress Syndrome
BUN	Blood urea nitrogen
CT	Computed Tomography
CRP	C-reactive protein
CTD	C-terminal domain
CDC	Centers for disease control and prevention
DNA	Deoxyribonucleic Acid
DAMP	Damage-associated molecular pattern
ER	Endoplasmic Reticulum
HDL	High-density lipoprotein
Hcy	Homocysteine
HCoV	Human Coronavirus
IL	Interleukin
IFN	Interferon
ICTV	International Committee on Taxonomy of Viruses
LDL	Low-density lipoprotein
MODS	Multiple Organ Dysfunction Syndrome
MTHFR	Methylenetetrahydrofolate reductase
MERS-CoV	Middle East Respiratory Syndrome Coronavirus
NTD	N-terminal domain
nsps	Non-structural proteins
NHC	National Health Commission
NHCPRC	National Health Commission of People's Republic of China
ORFs	Open Reading Frames
PCR	Polymerase Chain Reaction
PDB	Protein Data Bank
POCT	Point-of-care tests
PAMP	Pathogen-associated molecular pattern
PRRs	Pattern recognition receptors
RNA	Ribonucleic Acid
RBD	Receptor binding domain
ROS	Reactive oxygen species
SNPs	Single-nucleotide polymorphisms
RFLP	Restriction Fragment Length Polymorphism
RMSD	Root Mean Square Deviation

RT-PCR	Real-time reverse transcription-polymerase chain reaction
SNS	Sympathetic nervous system
+ssRNA	Positive Sense Single-stranded RNA
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus-2
TG	Triglyceride
TBE	Tris-Borate-EDTA
TLRs	Toll-like receptors
TMD	Transmembrane domains
TPR	Total peripheral resistance
TNF-α	Tumor necrosis factor-alpha
VLDL	Very-low-density lipoprotein
WHO	World Health Organization

1. INTRODUCTION

Viruses are diminutive parasitic entities incapable of independent reproduction. Upon infiltrating a host cell, they possess the ability to infect and duplicate within it. The essential components of a virus consist of its genetic material, which can exist in the form of either DNA or RNA strands, a safeguarding capsid protein envelope that shields the genetic material, and an external lipid sheath. The fundamental structural entity of an infectious virus, referred to as a virion, consists of genetic material encapsulated within an outer protein coat (Khaykelson and Raviv, 2020).

In 2019, an unprecedented and distinctive ailment emerged, subsequently recognized as COVID-19. The initiation of the epidemic was associated with a previously undiscovered variant of coronavirus, subsequently identified as Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). COVID-19 constitutes a serious respiratory disorder primarily transmitted through the respiratory route. It is triggered by a newly discovered coronavirus strain that exhibits significant dissimilarity from the more commonly associated coronaviruses responsible for causing common colds in humans. The inaugural instance of pneumonia linked to this novel coronavirus strain was initially documented by the World Health Organization (WHO) in late 2019 (WHO, 2019).

COVID-19 is an agent classified within the Coronaviridae family and positioned within the Nidovirales order. It possesses a single-stranded RNA genome and bears the designation "Corona," originating from the Latin word "Crown," which alludes to its distinctive spike proteins resembling a crown. Formally designated as SARS-CoV-2, this viral entity belongs to the lineage of coronaviruses, acknowledged for their involvement as causative agents in various maladies affecting both human and animal populations (Wu et al., 2020).

COVID-19 is classified as a zoonotic malady, indicating its initial transmission from animals to humans. Its first emergence was observed in late 2019 in Wuhan, China, coinciding with a significant rise in unexplained cases of pneumonia. In January 2020, following comprehensive genomic sequencing analyses of specimens obtained from the lower respiratory tract, a novel virus was discerned and designated as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), thereby confirming its role as the causative agent responsible for the pneumonia outbreak. The disease caused by

SARS-CoV-2 was formally designated by world health organization as "COVID-19" on February 11, 2020 (Gennaro et al., 2020).

As elucidated in the research conducted by Lu et al. in 2020, SARS-CoV-2 has been identified as a recently characterized Betacoronavirus with the ability to infect humans. Examination of the phylogenetic characteristics of the SARS-CoV-2 genome reveals its close affinity, showing an 88% similarity, with two bat-derived SARS-like coronaviruses, specifically bat-SL-CoVZC45 and bat-SL-CoVZXC21, acquired in 2018 from eastern China. It is noteworthy to underscore that this recently emerged virus displays genetic distinctiveness from both SARS-CoV, with an approximate 79% similarity, and MERS-CoV (Lu et al. in 2020).

The onset of the global COVID-19 pandemic, triggered by the emergence of the novel coronavirus, poses a significant challenge for healthcare and scientific professionals in their efforts to deliver effective medical care and identify reliable indicators associated with the progression of this disease. Moreover, it is crucial to distinguish clinical, epidemiological, and laboratory markers linked to the primary cause of mortality, particularly focusing on microvascular dysfunction. Exploring the potential for mitigating such damage through secure therapeutic interventions warrants investigation (Simons et al., 2020).

A discernible association has been established between specific serological and clinical biomarkers and the severity of the clinical trajectory in individuals afflicted with COVID-19 (Ponti et al., 2020). The participation of homocysteine (Hcy) in diverse metabolic and inflammatory processes is well substantiated. Diverse populations, marked by unique ethnic predispositions, have displayed varying incidences of mutations in the MTHFR gene and differences in its functionalities, as reported by Liew et al. in 2015. Our investigation aims to explore the relationship between the prevalence of the MTHFR C677T polymorphism and the rates of occurrence and mortality related to COVID-19. The primary objective is to establish a robust correlation between the presence of the MTHFR C677T mutation and the frequencies of both incidence and mortality in cases of COVID-19. This genetic variation intricately associates with prothrombotic events arising from disruptions in homocysteine metabolism (Ponti et al., 2020).

2. LITERATURE REVIEW

2.1 General Information on SARS-CoV-2

2.1.1 Description

SARS-CoV-2 is taxonomically categorized as a Beta coronavirus within the Coronaviridae family, distinguished by its enveloped, spherical morphology, single-stranded plus-sense RNA genome, and helical symmetry (Wassenaar and Zou, 2019). While these viruses do not inherently possess RNA-dependent RNA polymerase enzymes, their genetic material has the capacity to encode for this enzyme. The viruses exhibit distinctive elongated protrusions on their exteriors, resembling the linguistic origins of the term "corona," denoting "crown" in the Latin language. As a result, these viral entities have been classified as Coronaviruses, also referred to as Regal Viruses (Zhou et al., 2019).

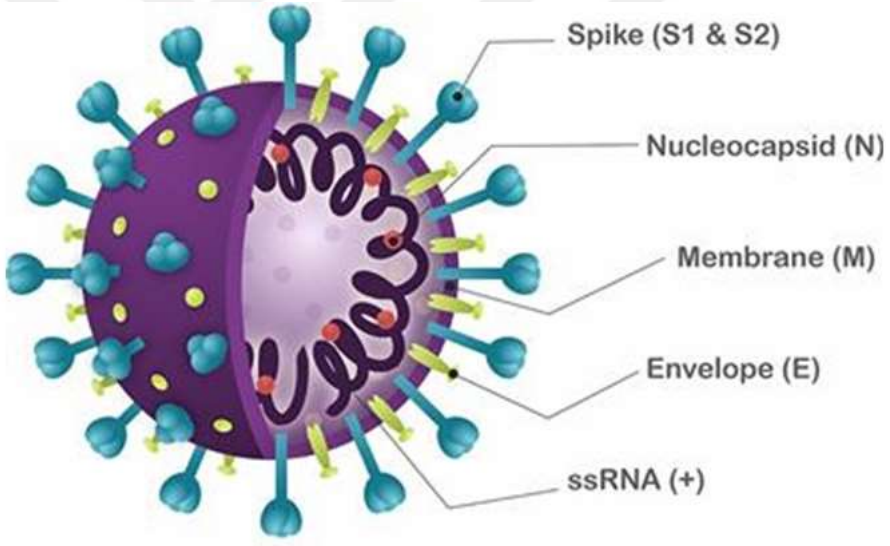

Figure 2.1: Illustration of Coronavirus Structural Composition (Zhou et al., 2019).

The structural composition of SARS-CoV-2 comprises four principal proteins, namely spike (S), membrane (M), envelope (E), and nucleocapsid (N) glycoproteins, along with diverse accessory proteins (Shereen et al., 2020) (Figure 2.1). Located on the exterior surface of the virus, the spike protein consists of two subunits. The S1 subunit plays a pivotal role in mediating the virus binding process to the receptor on the host cell surface, while the S2 subunit facilitates cell membrane fusion (Walls et al., 2020).

SARS-CoV-2 is systematically classified within the family Coronaviridae and the order Nidovirales. This taxonomic categorization encompasses two distinct subfamilies, namely Coronavirinae and Torovirinae. The Coronavirinae subfamily is

further subdivided into four genera: (a) Alphacoronavirus, encompassing human coronaviruses (HCoV)-229E and HCoV-NL63; (b) Betacoronavirus, hosting HCoV-OC43, Severe Acute Respiratory Syndrome human coronavirus (SARS-HCoV), HCoV-HKU1, and Middle Eastern respiratory syndrome coronavirus (MERS-CoV); (c) Gammacoronavirus, including viruses identified in whales and birds; and (d) Deltacoronavirus, incorporating viruses isolated from pigs and birds (Burrell et al., 2016).

The size of the viral particles, predominantly confined within vesicular compartments and various intracellular organelles, ranged between 60 and 140 nm. Considering the substantial genetic sequence resemblance, A hypothesis posits that the structural arrangement of SARS-CoV-2 bears a significant resemblance to that of SARS-CoV (Kumar et al. 2020). The architectural configuration of coronaviruses consists of a three-tiered structure that includes an outermost surface spike protein, an intermediate membranous layer, and an enveloping framework, all intricately embedded within a lipid bilayer originating from the host cell's membrane. The lipid bilayer envelops a helical nucleocapsid, thereby enclosing the viral RNA, as illustrated in (Figure 2.1) (Finlay et al., 2004). The revelation of the structural attributes of the spike protein and the specific protease inherent to SARS-CoV-2 has initiated the formulation of innovative therapeutic approaches aimed at addressing the intricacies posed by COVID-19 (Yan et al. 2020; Zhang et al. 2020).

2.1.2 Transmission

Given the lack of a targeted therapeutic approach and a prophylactic measure for the illness, it becomes crucial to comprehend its transmission dynamics and mitigate its prevalence (Sharif, 2020). From an epidemiological perspective, the onset of the SARS-CoV-2 epidemic is conceivably linked to the Hua Nan seafood and live animal wholesale market in Wuhan. Key domains facilitating the interpersonal transmission of SARS-CoV-2 include communal settings, healthcare facilities, and residential settings. Following infection, the verification of SARS-CoV-2 presence can be ascertained across various anatomical compartments, including the ocular region, nasopharynx, salivary secretions, alveolar lavage fluid, circulatory system, gastrointestinal tract, and excretory products (Li et al., 2020). In light of the swift propagation of SARS-CoV-2 and the indeterminacy of the main transmission routes, the control and reduction of the

infection pose considerable challenges. Empirical studies suggest that the predominant modes of transmission involve close interpersonal contact and the dissemination of respiratory droplets. Consequently, regulatory bodies strongly advocate the adoption of social distancing protocols and the utilization of masks as preventive measures. The transmission mode involving touching the T-zone of the face post-contact with infected surfaces further highlights the necessity for hand cleanliness and frequent handwashing. Other recognized modes of transmission encompass interaction with contaminated surfaces, airborne dispersion, and fecal-oral spread. In a broader context, the transmission of the SARS-CoV-2 virus has been attributed to droplet and contact pathways, airborne dissemination, and fecal-oral mechanisms (Falahi and Kenarkoohi, 2020).

2.1.3 Diagnosis

The clinical indicators and respiratory manifestations in patients afflicted with SARS-CoV-2 typically align with those observed in other upper respiratory infections. Thus, the commencement of the diagnostic process for COVID-19 entails a thorough examination of the patient's medical history. Subsequent confirmation of the diagnosis requires scrutiny and validation of epidemiological background, clinical presentations, computed tomography (CT) scan observations, hematological analyses, and results from biochemical tests. Nucleic acid testing techniques, including real-time polymerase chain reaction (RT-PCR) assays, are utilized, alongside serological assessments such as lateral flow tests and point-of-care tests (POCT) designed to detect the presence of IgM/IgG antibodies or antigens. Computed tomography (CT) proves highly informative for rapid initial diagnosis and prognosis evaluation, especially in instances of heightened prevalence or suspected cases of SARS-CoV-2 infection. Typical thoracic CT scans frequently display a ground glass pattern, marked by bilateral involvement of pulmonary parenchyma and consolidations. However, it is crucial to emphasize that these manifestations lack specificity and may resemble features observed in other cases of viral pneumonia. This underscores the necessity for meticulous consideration within diverse clinical contexts (Kashani et al., 2022; Li et al., 2020).

2.2 Causative agent of Covid-19

Coronaviruses represent a substantial assemblage of enveloped RNA viruses, notable for their distinctive surface features characterized by glycoprotein spikes

resembling coronal projections, thus attributing the nomenclature. These viral entities consist of a non-segmented, single-stranded RNA genetic material with positive-sense, and they are systematically categorized into four primary categories: alpha, beta, gamma, and delta. Genomic investigations indicate that the origins of alpha and beta coronaviruses can be traced back to genetic elements found in diverse animal species, including humans, bats, camels, and rodents. In contrast, the genetic lineage of gamma and delta coronaviruses is indicative of an avian provenance. Typical human coronaviruses, such as 229E, NL63, OC43, and HKU1, typically result in mild upper respiratory tract infections that resolve on their own. On the other hand, newly identified variants stemming from animal origins, exemplified by beta-coronaviruses such as SARS-CoV, MERS-CoV, and the present SARS-CoV-2, have incited far-reaching and substantial epidemics. The clinical manifestations of these viruses display a variable spectrum, encompassing conditions of mild and self-limiting nature as well as those bearing the potential to jeopardize life (Singhal, 2020).

The significant genetic heterogeneity evident within the realm of coronaviruses is chiefly attributable to their wide-ranging animal host repertoire, coupled with their proclivity for frequent genetic recombination events and mutational occurrences. Disparities in cellular tropism, limitations in host range, and variations in pathogenic potential across coronaviruses primarily stem from the particularity inherent in the viral surface attachment glycoprotein, known as the S glycoprotein (Habibzadeh and Stoneman, 2020).

Comprehensive genomic sequencing analyses conducted on RNA samples isolated from patients afflicted by COVID-19, sourced from both nasopharyngeal and sputum specimens, have revealed evolutionary affinities reminiscent of those observed in SARS-CoV. Subsequent to its initial identification as 2019-nCoV, the virus has undergone a formal nomenclatural revision and is presently officially designated as SARS-CoV-2 by the International Committee on Taxonomy of Viruses (ICTV). Despite some uncertainty surrounding the precise origin of SARS-CoV-2, the prevailing hypothesis implicates bats as the probable reservoir, suggesting a potential zoonotic transmission event from bat hosts to humans (Hasoksuz et al., 2020).

2.3 Virology

2.3.1 Coronavirus virology

Coronaviruses are defined by the presence of enveloped virions, with a particle diameter measuring 120 nm. The unique crown-shaped architecture is generated by cloverleaf formations that incorporate glycoproteins and surface proteins of the virus. The designation "coronaviruses" is derived from the characteristic crown morphology exhibited by these viral entities. Situated within these viral frameworks is an element identified as the nucleocapsid, composed of proteins encased within a capsid that envelops the genetic material of the virus (Figure 2.2) (Mittal et al., 2020).

The coronavirus accommodates a genomic RNA classification, manifesting in either a helical or circular arrangement within the virus's nucleocapsid. The genetic makeup of the coronavirus comprises a single positive-sense RNA (ribonucleic acid) strand. Significantly, the genomic RNA is marked by the existence of a methylated cap at its 5' terminus and is discernible through an increased adenine nucleotide composition at its 3' terminus. Within the genomic structure, coronaviruses incorporate the replicase enzyme, a protein tasked with transcribing the viral genome and generating new copies through the utilization of the host cell's machinery (Neerukonda and Katneni, 2020).

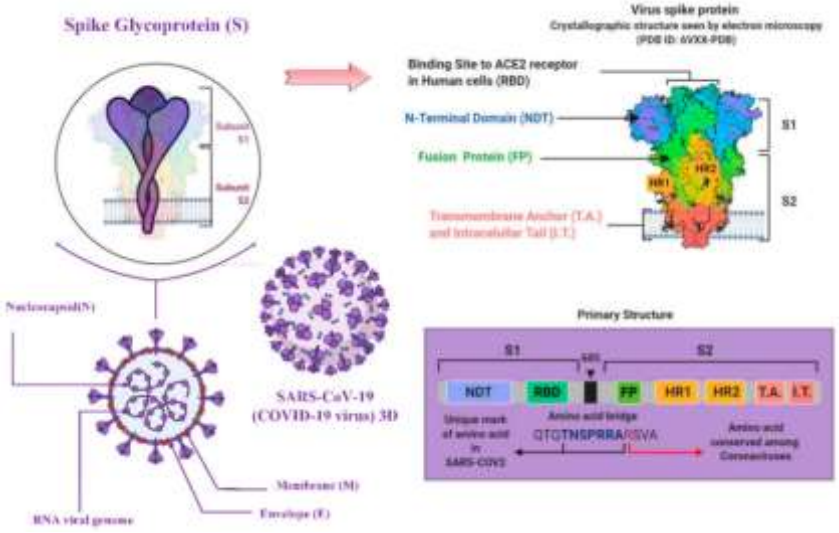

Figure 2.2: An in-depth look into the SARS-CoV-2 Spike Glycoprotein (BioRender, 2021).

The structural arrangement of the SARS-CoV-2 virus comprises four indispensable proteins: nucleocapsid (N), spike (S), membrane (M), and envelope (E), as depicted in (Figure 2.2). The M protein plays a pivotal role in mediating the virus's

entry into the host organism and actively contributes to the genesis of its envelopes. Concurrently, Protein E is accountable for coordinating processes associated with proliferation, maturation, envelope formation, and the subsequent dissemination of the virus (Schoeman and Fielding, 2019). The versatile N protein is designated with the task of amplifying transcription and overseeing virus assembly, whereas the exclusive role of the Spike (S) protein is to facilitate the virus's binding to host cells, establishing it as a unique focal point in the fields of pharmaceutical and vaccine research. It is crucial to acknowledge that, within the academic sphere, the N, M, and E proteins are considered unsuitable targets for drug development owing to their resilience against neutralizing or immune antibodies (Walls et al., 2020).

As illustrate in Figure 2.2 the S glycoprotein of the recently recognized SARS-CoV-2, portrayed as consisting of two subunits, S1 and S2, conventionally depicted as a spike resembling a sword. The precise configuration of this protein can be clarified through crystallographic analysis. The model of this glycoprotein in the Protein Data Bank (PDB) offers a thorough insight into subunit composition, unveiling specific regions pivotal to the infection process. The connection between Sl and S2 is facilitated by a polybasic amino acid bridge, a crucial feature deemed indispensable for exploring the intricacies of viral targeting mechanisms. The engagement between the virus and the host cell is orchestrated by the participation of an S-protein, thereby enabling cellular penetration. Subsequent to the penetration phase, transcription is instigated, leading to viral replication within the host cell until complete infection occurs, ultimately leading to cell destruction (McIntosh et al., 2020).

Thorough structural examination reveals that the receptor-binding domain comprises an inner core and an external subdomain (Du et al., 2009). The cellular receptor identified and specifically targeted by SARS-CoV has been elucidated as Angiotensin-converting enzyme 2 (ACE2), as explicated in the study conducted by Kuba et al. (2005). Likewise, SARS-CoV-2 utilizes ACE2 as its entry receptor in cells expressing ACE2, indicating a shared life cycle between the two viruses, namely SARS-CoV and SARS-CoV-2 (Figure. 2.3) (Kuba et al., 2005).

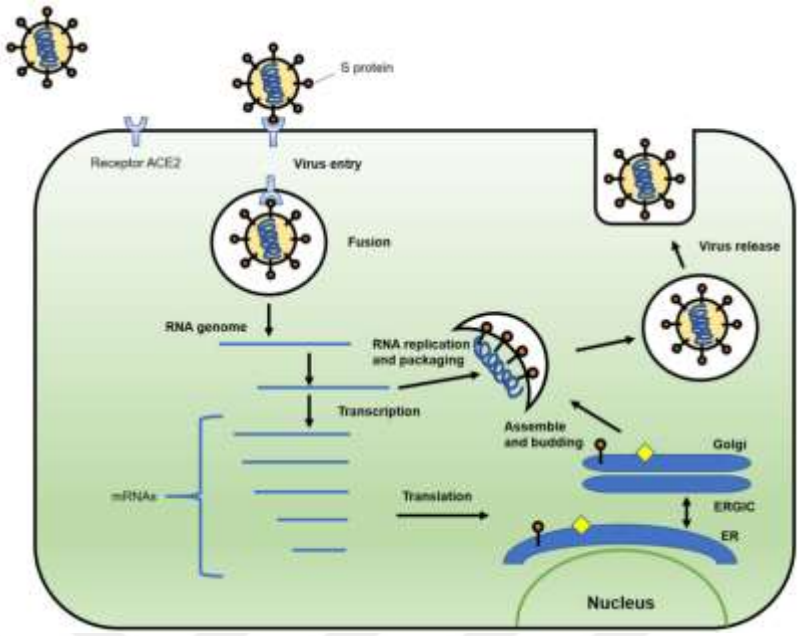

Figure 2.3: Diagrammatic Representation of the Life Cycle of SARS-CoV-2 (Lu *et al*., 2020).

2.3.2 Variants of concern

Similar to the evolutionary pattern seen in other viral entities, SARS-CoV-2 undergoes genetic alterations as time progresses. The majority of these genetic variations within the SARS-CoV-2 genome do not exert substantial influence on the virus's operational attributes. However, certain distinct iterations have attracted noteworthy scrutiny owing to their swift dissemination within communities and potential ramifications on transmissibility or clinical implications. These variants, termed as "variants of concern," assume diverse appellations based on the phylogenetic classification conventions adopted by various scientific frameworks. Moreover, (WHO) has employed Greek alphabet characters to designate prominent variants with specific nomenclatures (WHO, 2021).

1-The Omicron variant (B.1.1.529): The Omicron variant was first detected in Botswana and subsequently verified in South Africa in November of 2021. Its emergence coincided with a localized surge in infection rates within the South African region, swiftly disseminating to numerous other nations, where it was similarly associated with notable escalations in confirmed case numbers (CDC, 2021). Over time, distinct Omicron sub-lineages characterized by enhanced replication proficiency emerged, displacing the previously prevailing sub-lineage. The initial classification of the original Omicron variant was identified as sub-lineage BA.1. Subsequent to this,

several additional sub-lineages, namely BA.2, BA.4, and BA.5, have emerged. Various Omicron subvariants, such as BQ.1, BQ.11, BF.7, BA.2.75, XBB, XBB.1, and XBB.1.5, have originated from diverse preexisting subvariants, and they are presently demonstrating an escalating prevalence on a global scale. Each of these subvariants is characterized by at least one mutation in the spike protein, with the exception of BA.4 and BA.5, which share an identical spike protein configuration (Tegally et al., 2022).

Numerous sub-lineages of the Omicron variant have exhibited a heightened capacity for replication in comparison to the Delta variant, alongside an elevated resilience against immunity conferred by prior infections and vaccinations when contrasted with preceding iterations. Furthermore, these sub-lineages seem to be linked with comparatively milder clinical manifestations in contrast to alternative variants (Tegally et al., 2022).

- **Alpha (B.1.1.7 lineage)** – This specific variant was initially detected in the United Kingdom in the latter months of 2020 and subsequently spread globally until the emergence of the Delta variant. The Alpha variant exhibited a transmissibility that was roughly 50 to 75 percent higher in comparison to the viral strains that were in circulation prior to its appearance. Although consensus is not universal, certain investigations have suggested. In a study conducted by Frampton and colleagues in 2021, it was proposed that there could be a potential association between the Alpha variant and an increased level of disease severity (Xie et al., 2021).
- **Beta (B.1.351 lineage)** – Designated as 20H/501Y.V2, this variant was initially identified and attracted notice in South Africa in late 2020, as documented by Tegally et al. (2021). Despite subsequent detections in various nations, including the United States, the Beta variant did not achieve widespread prevalence on a global scale. The primary concern linked to the Beta variant revolves around its capacity to elude immune responses. Plasma derived from individuals who had recovered from infection or received vaccination displayed diminished neutralization efficacy against viral specimens featuring the Beta spike protein in comparison to those bearing the wild-type spike protein (Xie et al., 2021).
- **Gamma (P.1 lineage)** – The variant, labeled as 20J/501Y.V3, was first identified in Japan in December 2020 and subsequently gained notable

recognition in Brazil. Although it was later detected in various nations, including the United States, it did not attain global predominance. The presence of multiple mutations in this variant has raised concerns about its potential for increased transmissibility and its potential impact on immunity (Faria et al., 2021).

- **Delta (B.1.617.2 lineage)** — Designated as 20J/501Y.V3, this particular variant was initially observed in Japan in December 2020 and subsequently gained significant recognition in Brazil. Although later identified in different countries, including the United States, it did not rise to prominence as the predominant global strain. The existence of numerous mutations within this variant has elicited concerns regarding its potential for enhanced transmissibility and the implications it may have on immunity. The presence of multiple mutations within this variant has led to concerns regarding their potential to increase transmissibility and impact immune responses (Faria et al., 2021).

2.4 Genome Organization of SARS-CoV-2

The genomic structure of coronaviruses spans a sequence length ranging from 26 to 32 kilobases and incorporates between 6 and 11 open reading frames (ORFs) dedicated to the synthesis of polyproteins. In aggregate, these polyproteins encompass a total of 9680 amino acids (Guo et al., 2020). The predominant open reading frame (ORF), accounting for approximately 67% of the entire genome, is tasked with the synthesis of 16 non-structural proteins (nsps). Supplementary ORFs contribute to the generation of both structural and accessory proteins. The genomic architecture of SARS-CoV-2 lacks the hemagglutinin-esterase gene but incorporates two non-coding regions positioned at the 5' end (spanning 265 nucleotides) and the 3' end (comprising 358 nucleotides). In contrast to SARS-CoV, substantial modifications in the open reading frames (ORFs) and non-structural proteins (nsps) are conspicuously absent in SARS-CoV-2. Within the category of non-structural proteins, there are two distinct types of viral cysteine proteases: one is the papain-like protease designated as nsp3, and the other is the 3C-like or chymotrypsin-like protease, commonly recognized as the principal protease and designated as nsp5. Additionally, it is postulated that other non-structural proteins, such as the RNA-dependent RNA polymerase (nsp12) and helicase (nsp13), are presumed to play crucial roles in the transcription and replication processes

of SARS-CoV-2. Alongside the non-structural proteins, there exists a set of four essential structural proteins, comprising the spike surface glycoprotein (S), the membrane protein (M), the nucleocapsid protein (N), and the envelope protein (E), along with additional accessory proteins stemming from open reading frames (ORFs). Within the membrane protein (M), the N-terminal extremity encompasses a glycosylated ectodomain, characterized by three transmembrane domains (TMD), as well as an elongated C-terminal domain (CTD) (Figure. 2.4) (Chan et al. 2020).

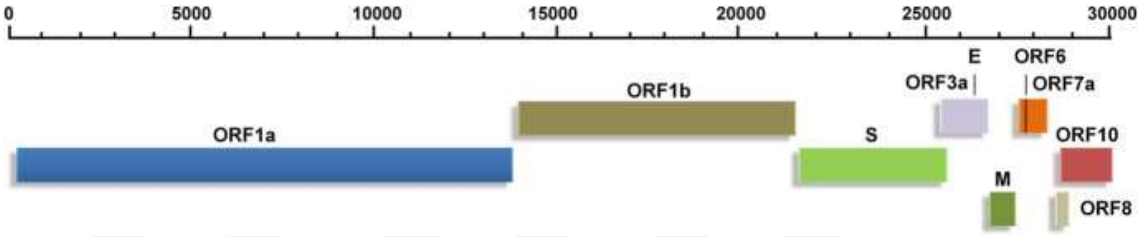

Figure 2.4: Structural Arrangement of the SARS-CoV-2 Genome (Chan et al. 2020).

The genetic composition of SARS-CoV-2 encompasses a coronavirus genome that spans a length between 26 and 32 kilobases. This genome houses between 6 and 11 open reading frames (ORFs) responsible for encoding a polyprotein consisting of 9680 amino acids. The primary open reading frame (ORF), which accounts for approximately 67% of the entire genome, is primarily tasked with generating 16 nonstructural proteins (nsps). Subsequent open reading frames (ORFs) are dedicated to synthesizing both accessory and structural proteins. The synthesis of nonstructural proteins is facilitated by two classes of viral cysteine proteases: the papain-like protease, denoted as nsp3, and the 3C-like protease, recognized as the primary protease or nsp5. These proteins, along with the RNA-dependent RNA polymerase (nsp12), helicase (nsp13), and various other proteins, are theorized to play a pivotal role in the transcription and replication mechanisms of SARS-CoV-2. In conjunction with nonstructural proteins, the viral genome generates four essential structural proteins—namely, the spike surface glycoprotein (S), the membrane protein, the nucleocapsid protein (N), and the envelope protein (E). Additionally, accessory proteins, including those encoded by open reading frames (ORFs), are produced (Chan et al. 2020).

The fundamental procedures linked to virus morphogenesis, assembly, and budding are directed by essential components inherent in the Membrane (M) and Envelope (E) proteins. Simultaneously, the viral Spike (S) glycoprotein, acknowledged as a fusion protein, manifests as a composite structure comprising two distinct subunits,

designated as S1 and S2. It is noteworthy that the S1 subunit exhibits a sequence similarity of approximately 70% with SARS-like Coronaviruses identified in bats and the human SARS-CoV. This constituent encompasses a signaling peptide, a signaling N-terminal domain (NTD), and a receptor binding domain (RBD), as delineated by Walls and colleagues in their 2020 publication. Predominantly, the observed distinctions were situated within the outer subdomain, which plays a central role in facilitating the interaction between the spike protein and the ACE2 receptor. In pursuit of a more comprehensive comprehension of the structural configuration of the SARS-CoV-2 spike glycoprotein, the ectodomain of this protein, spanning amino acid residues from 1 to 1208, was isolated, synthesized, and subsequently subjected to crystallization techniques. The structural arrangement of the spike glycoprotein in SARS-CoV-2 exhibits resemblances to the spike protein identified in SARS-CoV, as verified through an examination of Root Mean Square Deviation (RMSD) measuring 3.8 Å. It is essential to underscore that noticeable structural disparities were identified, particularly within the receptor-binding domain (RBD) (Wrapp et al., 2020). The S2 subunit, sharing a sequence identity of 99% with both bat SARS-like Coronaviruses and human SARS-CoV, comprises two distinct heptad repeat domains known as HR-N and HR-C. These domains converge to form coiled-coil structures integrated within the ectodomain of the protein. Simultaneously, a furin cleavage site (PRRARS'V) is situated at the junction of the S1 and S2 subunits, undergoing proteolytic cleavage during its formation (Coutard et al. 2020).

2.5 Entry and Replication of SARS-CoV-2 in Host Cells

Coronaviruses initiate host cell entry by engaging in the interaction between the spike glycoprotein and the cellular receptor, subsequently activating the S protein through proteolytic cleavage mediated by host cell proteases. Similar to the mechanism observed in SARS-CoV, SARS-CoV-2 utilizes the ACE2 receptor for host cell entry and instigates S protein activation with the involvement of serine proteases, specifically identified as TMPRSS2 (Hoffmann et al., 2020). The capacity of SARS-CoV-2 to access extrapulmonary tissues, mirroring the observed pattern in SARS-CoV, is presumably associated with the ubiquitous distribution of the ACE2 receptor throughout diverse bodily tissues. Additionally, research outcomes suggest a notably enhanced binding affinity between the SARS-CoV-2 spike protein and the ACE2 receptor, as

compared to the SARS-CoV spike protein. This signifies a considerable disparity, approximated to be ten to twentyfold greater (Wrapp et al., 2020). The engagement between the spike protein and the ACE2 receptor initiates a series of structural modifications within the spike protein, culminating in the fusion of the viral envelope protein with the host cell membrane through the endosomal pathway (Coutard et al. 2020). Subsequent to this series of occurrences, viral RNA is discharged into the host cell's cytoplasm. Subsequently, this viral RNA undergoes a translation phase, which initiates the synthesis of replicase polyproteins identified as pp1a and pp1b. These polyproteins are subsequently cleaved by viral proteases through proteolytic procedures. During the replication of coronaviruses, there is a phenomenon known as ribosomal frame shifting that takes place during translation. This leads to the production of both genomic and various subgenomic RNA species through a process involving discontinuous transcription. This complex transcriptional process encodes the indispensable array of viral proteins. The coordination of virion assembly encompasses the interplay between viral RNA and proteins situated within the endoplasmic reticulum (ER) and Golgi apparatus. Subsequent to this process, mature virions are discharged from the cellular milieu via vesicular exocytosis (Figure 2.5) (Hoffmann et al. 2020)

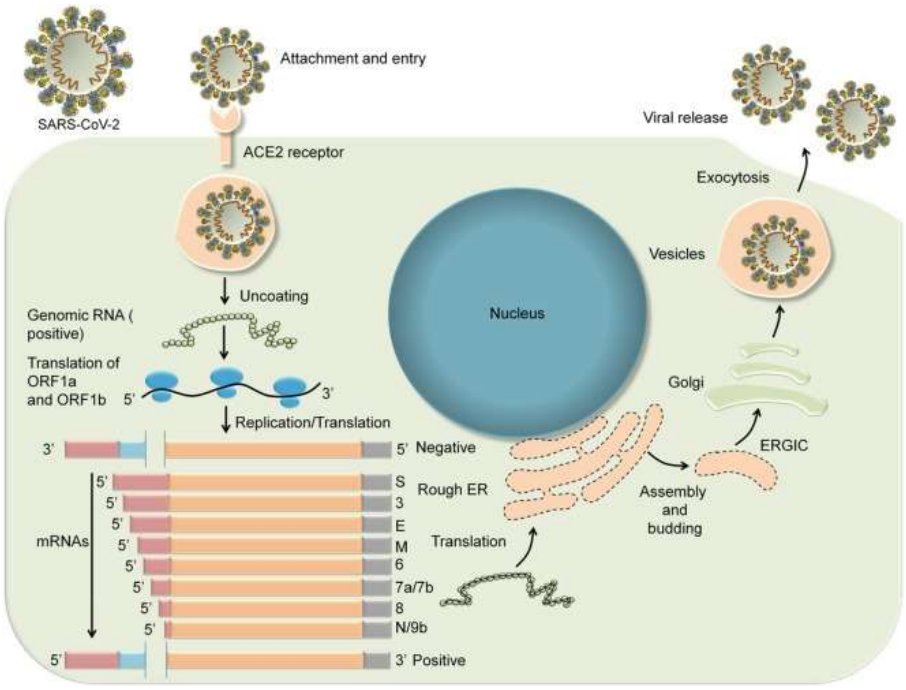

Figure 2.5: Entry and Replication of SARS-CoV-2 in Host Cells (Hoffmann et al. 2020).

The ingress of SARS-CoV-2 into host cells is contingent upon the interaction between the spike glycoprotein and the ACE2 receptor positioned on the cell surface, serving as the pivotal occurrence for viral entry. Consequently, a cascade of events ensues, culminating in the shedding of the protective envelope from the viral RNA within the cell's cytoplasm. Following this, viral RNA undergoes a translational process, giving rise to replicase polyproteins denoted as pp1a and pp1b. The viral-encoded proteases subsequently cleave these polyproteins into smaller protein constituents. The replication mechanism of SARS-CoV-2 involves ribosomal frame-shifting during translation, leading to the production of genomic RNA and diverse subgenomic RNA species through a process of discontinuous transcription. These transcriptions play a critical role in enabling the synthesis of distinct viral proteins. Virion assembly occurs through the amalgamation of viral RNA and proteins within the endoplasmic reticulum (ER) and the Golgi complex. Subsequent to assembly, these viral particles are extricated from the cells through exocytosis, a process involving the utilization of vesicles (Hoffmann et al. 2020).

2.6 Epidemiology

On December 29, 2019, the initial occurrences of an unidentifiable severe respiratory syndrome were documented, involving individuals with connections to a regional aquatic market colloquially referred to as a "wet market," situated in Wuhan City, Hubei Province, China. Ongoing initiatives are directed towards enhancing the understanding of the transmission dynamics, clinical severity, and additional attributes intrinsic to COVID-19. Preliminary indications suggest that a considerable portion of the initial cases demonstrated varying degrees of association with the previously mentioned seafood marketplace (Li et al., 2020).

Within a relatively condensed timeframe subsequent to the initial epidemic, an alternative avenue of transmission emerged, specifically, interpersonal transmission through close proximity. There was an escalating count of afflicted individuals lacking any prior record of wildlife exposure or sojourn in Wuhan. This demographic encompassed numerous occurrences of infection transmission among healthcare personnel (Gralinski and Menachery, 2020). Consequently, it was ascertained that the contraction of COVID-19 is contingent upon contact with the virus, rendering both individuals with compromised immune systems and the broader population susceptible.

Empirical investigations have delineated an age spectrum encompassing adult patients ranging from 25 to 89 years, with a substantial portion concentrated within the age bracket of 35 to 55 years. In contrast, fewer instances of infection have been documented among children and infants. An examination focusing on the initial transmission kinetics of the virus yielded findings indicating a median age of 59 years among the patients studied, spanning a range from 15 to 89 years. Notably, the male gender constituted the larger proportion, accounting for 59% of cases. The conjecture has arisen that heightened susceptibility may be manifested in individuals with compromised immune systems, particularly among the elderly population and those afflicted with renal or hepatic impairments (Wang and Wang, 2020).

The etiological factor of COVID-19, namely the SARS-CoV-2 virus, exhibits significantly increased transmissibility and possesses an elevated potential for a global pandemic when compared to the initial SARS-CoV. This statement finds validation in the elevated effective reproductive number (R) attributed to SARS-CoV-2, calculated at 2.9, surpassing the estimated R value of 1.77 for SARS-CoV, particularly evident in the initial phases of the outbreak (Wang and Wang, 2020).

Several scientific investigations examining the attributes of the SARS-CoV-2 virus have revealed a fundamental reproduction number (R0) ranging from 2.6 to 4.71. The estimated temporal incubation period of this novel pathogen has been statistically calculated to have a mean duration of 4.8 days, with a standard deviation of 2.6 days. This period spans from a minimum of 2 days to a maximum of 11 days. Furthermore, the median incubation period is approximated to be 5.2 days, with a 95% confidence interval spanning from 4.1 to 7 days. Consistent with these assessments, the most recent guidelines disseminated by Chinese health authorities suggest an average incubation period of 7 days. Nevertheless, this range encompasses a wider spectrum, extending from 2 to 14 days (Liu *et al.*, 2020).

As of the termination of January 31, 2020, within the territorial confines of China, the cumulative count of officially verified COVID-19 cases reached 11,791, accompanied by an additional 17,988 suspected cases distributed across the entirety of the 34 provinces (NHCPRC, 2020). Studies have indicated that the propagation of COVID-19 was exceedingly swift, confirming its spread to multiple countries following its initial emergence in China. As of the same date, the global mortality figure

associated with COVID-19 stood at 213. The presence of the affliction was verified in 19 nations situated beyond the geographical boundaries of China. These countries and their respective confirmed case counts were as follows: Australia reported 9 cases; Cambodia, 1; Canada documented 3; Finland, 1; Germany, 5; France, 6; Italy, 2; India, 1; Nepal, 1; Singapore, 13; Malaysia, 8; Japan, 14; the Philippines, 1; the Republic of Korea, 11; Sri Lanka, 1; the United States of America, 6; Thailand, 14; the United Arab Emirates, 4; and Vietnam, 5 (Figure. 2.6) (WHO, 2020).

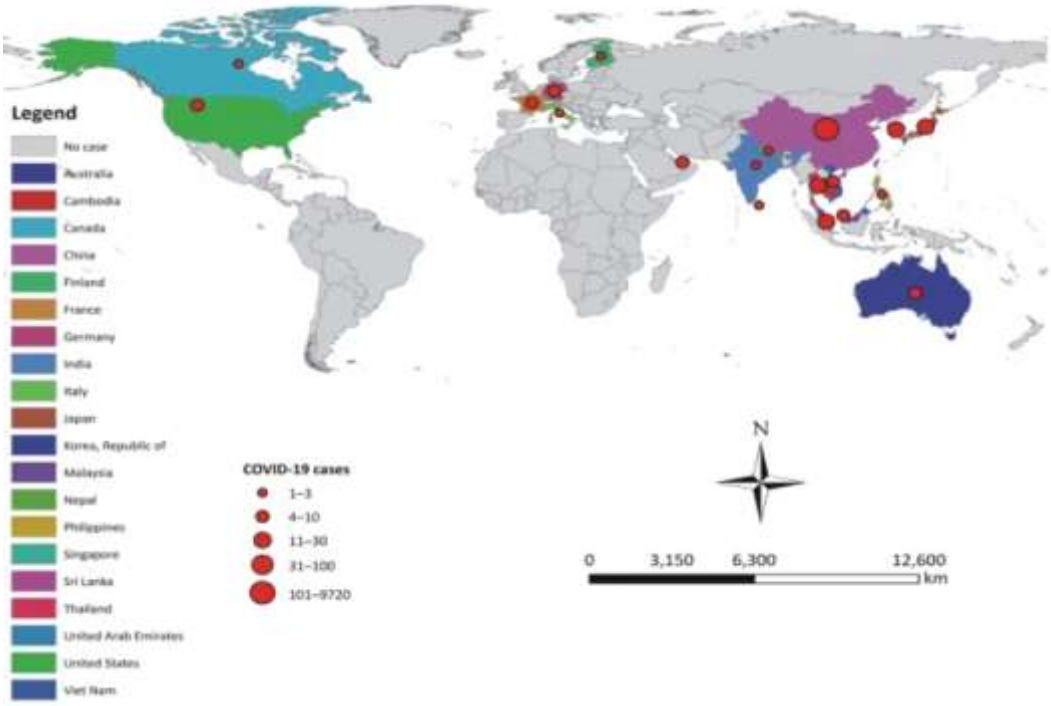

Figure 2.6: Global distribution of COVID-19 cases (WHO, 2020).

2.7 Pathogenesis of COVID-19

The predominant causative agent of COVID-19, namely the SARS-CoV-2 virus, predominantly impacts the lower respiratory system, manifesting a pronounced predilection for the bronchi and alveoli. The primary site of entry is determined by the presence of type 2 alveolar pneumocytes situated in the lower respiratory tract. This infiltration initiates a sequence of responses, including an inflammatory cascade, the initiation of interstitial edema, the formation of hyaline membranes, and significant perturbation to the alveolar architecture. The culmination of these processes ultimately gives rise to the onset of severe acute respiratory distress syndrome (Tian et al., 2020). The virus is characterized by the existence of the S-spike on its surface, which specifically interacts with the Angiotensin-Converting Enzyme type-2 receptor of the

human host cell. Following this binding event, the virus enters the endosome and subsequently releases its positive-sense single-stranded RNA (+ssRNA) into the cytoplasm of the host cell (Tian et al., 2020).

The initiation of viral RNA replication is instigated through transcription, a process facilitated by the enzyme known as RNA-dependent RNA polymerase. After successful replication, the viral RNA moves to the host cell's ribosome, where it undergoes translation to produce proteins. This series of events gives rise to an elongated protein sequence termed a polypeptide. Subsequently, this chain undergoes fragmentation into smaller protein units through the action of protease enzymes. Simultaneously with the generation of fresh positive-sense single-stranded RNA, these proteins congregate to form complete viral particles. Following their assembly, these viral particles exit the host cell and proceed to initiate infection in additional cells (Koester, 2020).

The pathological processes underlying COVID-19 are not fully elucidated. Preliminary investigations suggest that SARS may involve three distinct stages: viral replication, immune hyperactivity, and pulmonary deterioration (Navas-Martín and Weiss, 2004). Recently, Lin et al. (2020) proposed clinical phases for COVID-19, identifying them as the viremia phase, acute phase, and recovery phase. The prevailing hypothesis suggests a progression through these sequential stages, encompassing viral infiltration and replication, dysregulated immune response, multi-organ damage, and eventual recovery (Millet and Whittaker, 2015).

Initially, the virus penetrates host cells, undergoes replication and assembly, and is subsequently discharged extracellularly to infect target cells, leading directly to the damage and destruction of parenchymal cells, including alveolar epithelial cells. Concurrently, a substantial quantity of pathogen-associated molecular pattern (PAMP) and damage-associated molecular pattern (DAMP) molecules are released, initiating the innate immune response. This commencement triggers the infiltration of inflammatory cells, the substantial release of cytokines, chemokines, proteases, and free radicals, ultimately leading to conditions such as Acute Respiratory Distress Syndrome (ARDS), sepsis, and Multiple Organ Dysfunction Syndrome (MODS). Observations indicate that the pathological manifestations of pneumonia induced by COVID-19 exhibit similarities to those observed in infections caused by SARS-CoV and MERS-CoV. These

resemblances include bilateral acute changes characterized by diffuse alveolar damage and vascular congestion, intermittent inflammatory cellular infiltration, intra-alveolar edema, hemorrhage, proteinaceous exudate, denudation, and reactive hyperplasia of pneumocytes. Moreover, the presence of multinucleated giant cells is evident, although the consistent prominence of hyaline membrane formation is not universally noted (Hanley et al., 2020).

The hypothesis suggests that the primary pathological changes in crucial organs during COVID-19 could be directly correlated with the cytopathic effects induced by SARS-CoV-2 and indirectly associated with the adverse immune responses triggered by SARS-CoV-2. However, a more comprehensive investigation is imperative to ascertain the precise significance of each of these factors. There is an indication that an aberrant immune response might exert a more pronounced impact on the outcomes of COVID-19 compared to a direct viral cytopathic effect. It has been noted that individuals with COVID-19 exhibit the highest viral load in the initial phases (To et al., 2020).

2.7.1 SARS-CoV-2 invades host cells

It is widely recognized that the transmission and pathogenicity of human coronaviruses (CoV) primarily depend on the intricate interaction between the virus and specific host cells (Skariyachan et al., 2019). The initial stage of viral infection encompasses receptor identification and entry, playing a pivotal role in establishing tissue tropism. The proposition suggesting that an enhanced binding affinity between SARS-CoV-2 and ACE2 may be associated with increased virus transmissibility and heightened disease severity in humans has been postulated (Wan et al., 2020). The entry of coronaviruses (CoV) into host cells involves a multi-step process that incorporates various distinct domains within the S protein. These domains facilitate viral attachment to the target cell surface, interaction with receptors, protease processing, and membrane fusion. After this intricate sequence, the viral genome is liberated into the cytoplasm, instigating viral replication within the host cells. Remarkably, three coronaviruses (human CoV-NL63, SARS-CoV, and SARS-CoV-2) cause diseases of diverse severity while employing the same receptor (ACE2). This observation suggests the presence of additional pathogenic factors contributing to the noticeable distinctions observed among these three coronaviruses (Davidson et al., 2020).

Research has shown that the ACE2-binding mechanism displayed by the receptor-binding domain (RBD) of the SARS-CoV-2 Spike closely resembles that of the SARS-CoV RBD. However, the SARS-CoV-2 RBD assumes a more compact conformation, thereby increasing its affinity for ACE2 binding (Lan et al., 2020). Walls et al. (2020) demonstrated that the receptor-binding domains (RBD) of the SARS-CoV-2 Spike protein and the SARS-CoV Spike protein exhibit comparable affinities in binding to human ACE2 for cellular entry. However, an independent investigation noted a 10-20 times higher affinity between SARS-CoV-2 and ACE2 in comparison to SARS-CoV, potentially contributing to the heightened transmissibility observed in SARS-CoV-2 (Wrapp et al., 2020).

The distinctive distribution pattern of SARS-CoV-2 and ACE2 offers valuable insights into the fundamental pathogenic mechanisms of COVID-19. Notably, viral RNA from SARS-CoV-2 has been identified in respiratory secretions, peripheral blood, urine, and stool samples of particular COVID-19 patients, aligning with diverse transmission pathways associated with SARS-CoV-2 infection (Zhou et al., 2014). Particles released into the bloodstream from the primary infection site, such as the lungs, possess the capacity to circulate and initiate infections in distant secondary organs and tissues (Zhou et al., 2014).

On the contrary, ACE2 is present in the pulmonary, cardiac, renal, and gastrointestinal systems, demonstrating particularly abundant expression in the epithelial tissues of the human lungs and small intestines. These findings suggest a potential significance of ACE2 in the extrapulmonary manifestations of COVID-19, involving symptoms associated with the gastrointestinal system. It is crucial to underscore that the interplay between the gut and lungs may contribute to the pathogenesis of COVID-19. Nevertheless, the investigation of probiotics as a novel therapeutic approach for COVID-19 demands further investigation and exploration (Mak et al., 2020). Furthermore, ACE2 displays broad expression in vascular endothelial cells and smooth muscle cells across diverse organs, potentially resulting in significant damage to vascular endothelial cells. This molecular phenomenon could be implicated in the progression of multiple organ lesions in individuals infected with COVID-19. Cardiac injury has been noted in 7-23% of individuals diagnosed with COVID-19, a condition linked to heightened mortality rates. A recent investigation

revealed heightened ACE2 expression in individuals with pre-existing heart failure, indicating that cardiac cells exhibiting increased ACE2 levels could potentially become susceptible to infection by SARS-CoV-2 (Chen et al., 2020).

2.8 Pathophysiology

The SARS-CoV-2 virus, which is accountable for initiating COVID-19 infection, primarily inflicts significant harm to type 2 alveolar pneumocytes. The resulting cellular impairment induced by the virus leads to the release of specific inflammatory signaling molecules, subsequently triggering alveolar macrophages to produce cytokines, including interleukin-6 (IL-6), interleukin-1 (IL-1), and tumor necrosis factor-alpha (TNF-α) (Li et al., 2020). These inflammatory mediators incite the capillary endothelium to augment its permeability, facilitating the ingress of fluid into interstitial compartments. This process leads to the development of interstitial edema, alveolar edema accompanied by diffuse alveolar damage, and encourages vasodilation, thereby facilitating the extrusion of fluid into tissue spaces. As a consequence, these events disrupt the normal gas exchange process, resulting in hypoxemia and the potential development of necrosis in pulmonary tissue. Circulating in the bloodstream, Interleukin-1 and Interleukin-6 eventually reach the hypothalamus, triggering the synthesis of prostaglandins and endotoxic pyrogens. These agents elevate the body's core temperature, initiating the onset of fever. Therefore, alleviating the inflammatory response by suppressing the release of inflammatory mediators, including Interleukin-6, Interleukin-1, and tumor necrosis factor-alpha, through interventions such as Tocilizumab, may offer potential therapeutic benefits in the management of COVID-19 (Zhong et al., 2020).

In physiological circumstances, type 2 alveolar pneumocytes are responsible for synthesizing surfactant, a vital compound that reduces surface tension and enhances lung compliance. Nevertheless, impairment of these cells undermines their operational capability. An escalation in alveolar edema culminates in the dilution of surfactant, instigating a rise in lung surface tension and subsequent collapse of the alveolar units (Li et al., 2020). Subsequent to this, a decline in the partial pressure of oxygen (pO2) triggers the response of peripheral chemoreceptors, resulting in the activation of the sympathetic nervous system (SNS). In response, the SNS elevates both the heart rate and, concurrently, enhances the rate of respiration. This compensatory reaction aims to

mitigate the compromised gas exchange that has transpired. This instigates the emergence of clinical manifestations characteristic of acute respiratory distress syndrome. Inflammatory reactions draw leukocytes, particularly neutrophils, to the infection site. These neutrophils subsequently release reactive oxygen species (ROS) and protease enzymes. While effective against the virus, these agents inadvertently cause detriment to both type 1 and 2 pneumocytes. This exerts an adverse effect on gas exchange by impairing the integrity of the respiratory membrane and further obstructing the production of surfactant. The buildup of liquid, proteins, and cellular remnants resulting from the compromised type 1 and 2 alveolar cells in the alveolar space triggers a phenomenon referred to as pulmonary consolidation. If the inflammatory process extends to encompass the pulmonary circulation, it holds the potential to trigger septic shock. Consequently, individuals may experience hypotension as a result of vasodilation, a reduction in total peripheral resistance (TPR), and a subsequent decline in blood volume, potentially culminating in the onset of multiorgan failure (Arlati, 2019).

It is firmly established that, similar to other human coronaviruses, SARS-CoV-2 transmits via droplets and initiates the primary infection by attaching to ACE-2 receptors, abundant in the epithelial cells of the lower respiratory tract. The average incubation period was noted to be 4-5 days before the manifestation of symptoms, with 97.5% of symptomatic individuals exhibiting symptoms within an average duration of 11.5 days (Lauer et al., 2019). Typical presentations of COVID-19 comprise fever and a dry cough, coupled with additional symptoms such as shortness of breath, muscle and/or joint pain, headache, diarrhea, and nausea. In severe instances of COVID-19, individuals may encounter acute respiratory distress syndrome (ARDS) approximately 8-9 days after the initiation of symptoms (Huang et al., 2020). Histopathological examinations through biopsy and autopsy of specific COVID-19 patients have identified damage to the alveoli. Despite an incomplete comprehension of the virus's pathophysiology and virulence characteristics, a plausible association is noted with the structural and non-structural proteins of the virus (Xu et al., 2020).

The immune system of the host utilizes diverse antiviral defense mechanisms to counter viral infections. Intracellular pattern recognition receptors (PRRs), particularly Toll-like receptors (TLRs), assume a crucial role in identifying RNA products generated

during viral replication within host cells. Upon this recognition, the immune system is activated, resulting in the secretion of type I interferon (type I IFN) from virus-infected cells (Abbas et al., 2014). Upon this recognition, the immune system is triggered, leading to the secretion of type I interferon (type I IFN) from cells infected by the virus (Doan et al., 2017). Antigenic structures of pathogens are presented to CD8 T lymphocytes by antigen-presenting cells via Major Histocompatibility Complex class I (MHC-I). Activation of cytotoxic T-lymphocytes ensues through the stimulation of cytokines such as interleukin-12 (IL-12) and interferon-α/β (IFN-α/β). Furthermore, specific dendritic cells possess the capability to present processed antigens to both CD4 and CD8 T-lymphocytes by engulfing cells infected by the virus (Abbas et al., 2015).

2.9 Clinical Presentation

The gravity of a COVID-19 infection depends on both the virulence of the SARS-CoV-2 strain and the simultaneous immune reaction of the person. This results in a broad spectrum of clinical manifestations, encompassing cases with no symptoms as well as severe inflammatory conditions characterized by dysfunction in multiple organs. To address this variability, the National Institutes of Health has developed a classification system that delineates distinct categories for different COVID-19 scenarios, providing specific criteria for asymptomatic, mild, moderate, severe, and critical cases. It is essential to note that these classifications may undergo revisions, as a significant portion of patients often transition between these categories as the disease evolves (Khazanchi et al., 2020).

The duration between exposure to SARS-CoV-2 and the appearance of symptoms can exhibit variability, typically encompassing a period of 2 to 14 days. Various viral strains have been identified to introduce some level of diversity in this regard. A comprehensive analysis of 181 documented COVID-19 cases in China determined a median incubation period of approximately 5.1 days, with a 95% confidence interval ranging from 4.5 to 5.8 days. Notably, infections linked to the Delta and Omicron variants have showcased briefer incubation durations compared to preceding significant variants, demonstrating a median period of around 4 days (Wang et al., 2021).

2.9.1 Asymptomatic Infection

Asymptomatic cases are characterized by individuals testing positive for SARS-CoV-2 but remaining devoid of evident symptoms. It is hypothesized that approximately half of individuals with a confirmed viral diagnosis do not display any discernible symptoms. Notably, a particular meta-analysis approximated that around 25% of infections are characterized by an absence of symptoms. Nevertheless, the prevalence of asymptomatic infections tends to be elevated among individuals with preexisting immunity (Syangtan et al., 2021).

Instances of asymptomatic cases are predominantly identified among individuals younger than 50, female individuals, and those without preexisting comorbidities. An initial investigation analyzing 55 cases of asymptomatic infections unveiled a median age of diagnosis at 49 years. It's essential to highlight that in this study, a substantial portion of the participants (37 out of 55) displayed signs of pneumonia upon undergoing computed tomography scans, even though they did not exhibit apparent symptoms (Meng et al., 2020). Crucially, certain individuals who were initially designated as asymptomatic may later exhibit symptoms of infection, often within 48 hours of the detection of SARS-CoV-2 RNA in the nasopharynx. Consequently, they are reclassified as presymptomatic cases. Nevertheless, a substantial cohort of patients persist in remaining asymptomatic during further observation (Meng et al., 2020).

2.9.2 Mild Disease

Individuals manifesting slight symptoms of COVID-19 commonly show signs of elevated body temperature, throat irritation, muscular soreness, and a prevailing sense of malaise. This is in contrast to the absence of any observable signs of breathlessness or anomalous findings in chest imaging that could potentially imply an affliction of the lower respiratory tract. Empirical evidence has indicated that a substantial proportion, comprising 81%, of symptomatic infections give rise to a moderate manifestation of the ailment. Additional clinical manifestations, such as gastrointestinal issues like diarrhea, nausea, and vomiting, have been observed, although they occur less frequently (below 20%). This stands in contrast to the prevalence observed in cases of SARS or MERS infections, as elucidated in the investigation conducted by (Bleibtreu et al., 2020). The presentation of indications such as compromised olfactory or gustatory perceptions exhibits a discernible heterogeneity throughout scholarly investigations, encompassing

frequencies spanning from 10% to 40%. The deprivation of olfaction or taste senses, denoted as anosmia and ageusia correspondingly, is frequently recognized by individuals afflicted with COVID-19 as a distinguishing hallmark. These sensory changes often appear before the emergence of other symptoms akin to those of influenza. This unique array of symptoms has become a focal point in scientific investigations. One theory posits that particular cells situated in the olfactory bulb and the olfactory epithelium, harboring ACE2 receptors, could facilitate viral entry and consequent infection. This hypothesis gains support from observed changes in brain imaging in individuals who have experienced anosmia, as documented by Kandemirli et al. (2021).

2.9.3 Moderate, Severe, and Critical Disease

Individuals identified as having a moderate form of the ailment display indications of infection affecting the lower respiratory tract, discernible through either physical assessment or radiographic evaluation of the chest. Nonetheless, their arterial oxygen saturation levels remain equal to or surpass 94% during the act of inhaling air under normobaric conditions at sea level. In contrast, individuals with a severe manifestation of the disease demonstrate arterial oxygen saturation levels below 94% under normal conditions at sea level with regular air, an arterial partial pressure of oxygen to the fraction of inspired oxygen ratio falling below 300 mm Hg, a respiratory rate exceeding 30 breaths per minute, or the presence of pulmonary infiltrates exceeding 50% The critical iteration of the ailment is distinguished by the onset of respiratory insufficiency, septic shock, and/or the failure of multiple organs, as expounded upon by (Wu and McGoogan, 2020).

During the initial stage of the pandemic, information from the Chinese Center for Disease Control and Prevention indicated that about 14% of cases presented severe forms of the illness, with approximately 5% falling into the critical condition category. Nevertheless, the frequency of severe disease expressions is subject to fluctuations influenced by multifarious factors, encompassing prior exposure to infections, an individual's immunization history, the specific viral variant responsible for the infection, and the adequacy and efficacy of healthcare provisions. Illustratively, the Omicron variant evidently induces a less severe clinical course when contrasted with the Delta variant. The likelihood of advancing towards severe and critical disease states is notably

diminished in individuals possessing acquired immunity, particularly among those who have undergone vaccination, as proposed by (Berger et al., 2022).

The predominant hallmark of COVID-19 resides in respiratory impairment, stemming from pronounced inflammation and substantial disruption of pulmonary tissue. The intricacies associated with the progression of COVID-19 remain the focus of comprehensive and in-depth investigations. The replication of the virus within the respiratory epithelium triggers an elevated proinflammatory reaction, giving rise to the production of chemokines and cytokines. These include significant elements such as IL-1, IL-6, and tumor necrosis factor-alpha, as outlined in the research by Azkur et al. (2020). The predominant factor associated with lung damage in COVID-19 cases is recognized as diffuse alveolar injury. This pathogenic process, facilitated by fibrin deposition and inflammatory processes, precipitates the occurrence of pulmonary edema and vascular thrombosis. The advancement of this process has the capacity to trigger substantial harm and the formation of alveolar edema, characterized by the accumulation of proteinaceous substances and hyaline membranes. This localized inflammatory response within the pulmonary parenchyma manifests as the production of mucus, bouts of coughing, and respiratory distress. Imaging modalities like radiography have the potential to unveil intermittent areas of increased opacity, whereas computed tomography scans have the capacity to identify regions exhibiting ground glass opacities. These discernible areas of increased opacity are chiefly localized in the peripheral or subpleural domains and commonly manifest bilaterally. Severe pneumonia has the potential to result in hypoxemic respiratory failure and, in specific cases, may progress to Acute Respiratory Distress Syndrome (ARDS), ultimately leading to a fatal outcome. Several factors have been recognized as contributing to an increased likelihood of progressing to ARDS. These factors include older age, specifically over 65 years, the presence of comorbid conditions like diabetes mellitus, hypertension, obesity, and the absence of SARS-CoV-2 vaccination, among other variables detailed in the research conducted by (Chen et al., 2020).

2.10 Detection of COVID-19 infection

Documented instances of contact or close proximity to individuals suspected or confirmed to be afflicted by the ailment substantially contribute to the diagnostic process. Nonetheless, in cases where a clear history of exposure is lacking, clinical

manifestations and imaging results can provide indications suggestive of a potential COVID-19 infection. Consequently, in such scenarios, the performance of a real-time reverse transcription-polymerase chain reaction (RT-PCR) test is considered imperative as a benchmark, as outlined by (Zu et al., 2020).

In accordance with the directives outlined by the National Health Commission (NHC) of China, the diagnostic criteria are as follows:

1- An established account of contact with individuals manifesting respiratory symptoms originating in Wuhan or other affected urban areas during the two-week period preceding the onset of the ailment.
2- Clinical indications include elevated body temperature, a white blood cell count within the normal range or slightly reduced, decreased lymphocyte count, and radiological findings indicative of pneumonia.
3- The confirmation of positive results in the detection of COVID-19 through real-time PCR analysis mandates that any confirmed case of COVID-19-associated pneumonia requires hospitalization for treatment and isolation (Pan et al., 2020).

In accordance with the guidelines established by the National Health Commission (NHC) of China, which were developed following the recommendations provided by the World Health Organization for dealing with SARS and MERS, protocols for diagnosing and handling COVID-19 pneumonia were implemented. An individual presenting two clinical indicators and a documented history of exposure is regarded as a potential case. When a definite history of contact is absent, individuals with suspected cases should exhibit three distinct clinical characteristics. The criteria for discharge encompass: 1. Maintaining a normal body temperature for at least three consecutive days, and 2. Experiencing substantial alleviation in respiratory symptoms and improvements in abnormalities observed in chest imaging. A fundamental necessity is the establishment of a conclusive etiological diagnosis of the infection, a validation that can be bolstered by employing PCR analysis using blood or pulmonary samples, or through the process of viral gene sequencing. Predicated on clinical assessments, eligible individuals are stratified into distinct classifications, encompassing critical, severe, moderate, and mild categories (Zu et al., 2020).

2.11 MTHFR gene

The Methylenetetrahydrofolate reductase (MTHFR) gene, situated on chromosome 1p36.3, has been a focal point of extensive scientific investigations due to its pivotal role in human physiology and general well-being. Structural mapping studies have elucidated that the human MTHFR gene is composed of 11 exons and 10 introns (Gaughan et al., 1994; Goyette et al., 1994).

Exons are the coding regions of a gene that are transcribed and translated into proteins, while introns are non-coding regions that are transcribed but not translated. The MTHFR gene encodes the methylenetetrahydrofolate reductase (MTHFR) enzyme, a critical protein in human physiology. This enzyme plays a pivotal role in the metabolic pathway governing the conversion of homocysteine to methionine, a fundamental process in amino acid metabolism. The resultant protein, MTHFR, possesses a molecular weight of approximately 77 kDa (kilodaltons), substantiating its identity as a substantial molecular entity with functional significance. The intricate regulatory mechanisms and potential genetic variations of the MTHFR gene underscore its physiological importance and susceptibility to polymorphic alterations that may contribute to various health conditions (OMIM Entry - 607093, 2023). One particular genetic variation is MTHFR C677T. A genetic variation denotes a modification in the DNA sequence that diverges from the expected or standard DNA sequence (Gaughan et al., 1994)

Figure 2.7: Location of the MTHFR gene (Goyette et al., 1994).

Methylenetetrahydrofolate reductase functions as a crucial enzymatic component in the methyl cycle, its regulatory role in the rate-limiting process being dictated by the genetic encoding within the MTHFR gene. This enzyme assumes a

crucial role in folate metabolism by facilitating the transformation of 5,10-methyl-THF into 5-methyl-THF. The latter serves as a vital cofactor in the methylation process of homocysteine, culminating in the synthesis of methionine. Compromised enzymatic activity or reduced levels of MTHFR may be implicated in the development of hyperhomocysteinemia (Goyette et al., 1994). Two notable genetic variations have been identified within the MTHFR gene, specifically the single-nucleotide polymorphisms C677T (rs1801133) and A1298C (rs1801131). The single nucleotide polymorphism C677T involves the substitution of a cytidine residue with thymidine at position 677 in the gene, leading to an alteration from alanine to valine in the resulting protein. This variant exhibits thermolability, resulting in a 35% reduction in enzymatic activity among individuals with the heterozygous genotype C/T relative to those with the wild-type genotype C/C. Furthermore, the homozygous genotype T/T is linked to a more pronounced reduction, with a 70% decrease in enzyme activity compared to the reference C/C genotype (Wouters et al., 1993; Şahin et al., 2012).

The alternative single nucleotide polymorphism A1298C, situated in the regulatory domain of the enzyme, induces a substitution from glutamate to alanine, resulting in a modest decrease in MTHFR enzyme activity (Calero et al., 2002). A plethora of investigations have delineated an association between genetic variations in MTHFR and gene deficiency, implying potential ramifications for an individual's susceptibility to occlusive vascular diseases, notably thrombosis and atherosclerosis. Moreover, these genetic variations have been associated with the development of various health conditions, including neural tube defects, mental disorders such as depression and schizophrenia, neurodegenerative disorders like Alzheimer's disease and Parkinson's disease, and a range of malignancies such as colon cancer, prostate cancer, and breast cancer (Efrati et al., 2014; Liu et al., 2015).

2.11.1 Role of MTHFR in Homocysteine

Homocysteine, a sulfur-containing amino acid, exhibits limited incorporation into proteins. It functions as a metabolic intermediate arising from the transformation of the amino acid methionine into homocysteine and possesses the capacity for renal excretion (Hou and Zhao, 2021). Homocysteine has the capacity for methylation, leading to the production of methionine, or it can traverse the transsulfuration pathway, undergoing conversion into cystathionine and subsequently cysteine. The methylation

pathway is contingent upon the presence of vitamin B12, folate, and the enzyme methylenetetrahydrofolate reductase (MTHFR). As a consequence, deficiencies or significant insufficiencies in these essential vitamins may contribute to the accrual of homocysteine in the circulatory system, particularly in individuals harboring specific mutations in the MTHFR gene (Raghubeer and Matsha, 2021).

Methylenetetrahydrofolate reductase (MTHFR) plays a crucial role in the metabolic pathways of homocysteine by facilitating the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the primary circulating form of folate. The MTHFR gene harbors a minimum of two functional polymorphisms, specifically identified as 677C>T and 1298A>C. The presence of the MTHFR 677T allele is associated with reduced enzymatic activity, diminished folate levels in serum, plasma, and red blood cells, coupled with a slight increase in plasma total homocysteine concentrations (Frosst et al., 1995; Jacques et al., 1996).

The MTHFR variant 1298A>C exerts an impact on MTHFR activity without manifesting a correlation with elevated plasma homocysteine or reduced folate levels, as evidenced by Gabreels et al.'s (1998) study. Sustaining optimal MTHFR activity is imperative for upholding the reservoirs of circulating folate and methionine, thereby mitigating the accrual of homocysteine (Frosst et al., 1995).

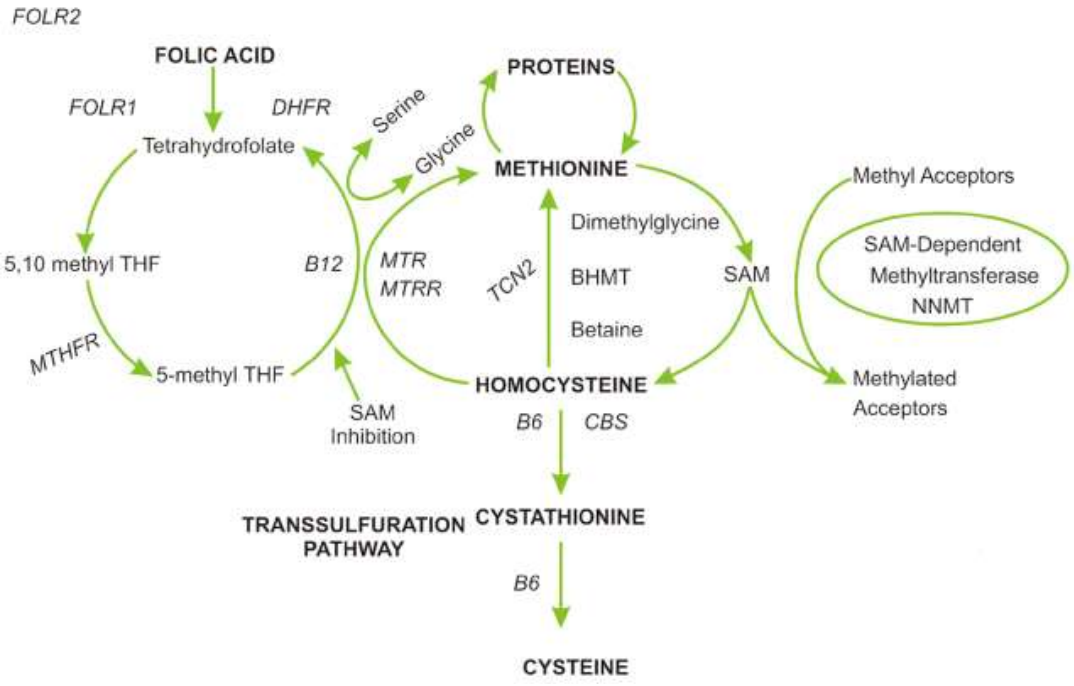

Figure 2.8: Homocysteine metabolism (Selhub and Miller, 1994).

The state of possessing dual heterozygosity for the MTHFR 677C>T and 1298A>C polymorphisms is linked to a decline in MTHFR activity compared to the condition of being heterozygous for either MTHFR variant in isolation (Gabreels et al., 1998). Individuals with the 677TT genotype exhibit MTHFR enzyme activity at approximately 30% in comparison to those with the 677CC genotype, while individuals with the 677CT heterozygous genotype display an enzymatic activity level of around 65% (Rozen, 1997).

The principal factor believed to contribute to elevated homocysteine (Hcy) levels is attributed to the C677T polymorphism (rs 1801133) in the MTHFR gene. This polymorphic variation entails the substitution of cytosine with thymine at position 677, resulting in a modification in the amino acid composition from alanine to valine within the enzyme (Frosst et al., 1995). This prevalent mutation within the MTHFR gene exerts an impact on homocysteine (Hcy) levels and is hypothesized to be a contributing factor to hyperhomocysteinemia, diminished folate levels, and various cardiovascular disease (CVD)-related conditions (Rosenberg et al., 2002). The C677T mutation has been documented to compromise thermostability, leading to impaired enzyme function. This mutation is designated as thermolabile owing to a decrease in enzyme activity at temperatures surpassing 37 °C. In individuals homozygous for C677T (TT), MTHFR enzyme activity demonstrates a notable reduction, ranging from 50% to 60%, and further diminishes by 65% at 46 °C (Rozen, 1997). A frequently encountered polymorphism is located at position 1298 of the MTHFR gene, marked by the substitution of adenine with cytosine. This modification leads to a shift in the amino acid composition from glutamate to alanine within the enzyme. Although causing a reduction in enzyme activity, this mutation does not reach the same magnitude as observed with the C677T polymorphism (Botto and Yang, 2000).

2.11.2 MTHFR gene and Hyperhomocysteinemia

The MTHFR gene (methylenetetrahydrofolate reductase) participates in the conversion of homocysteine to methionine, a crucial amino acid. Alterations in the MTHFR gene may impact the efficacy of this conversion, potentially resulting in increased levels of homocysteine in the bloodstream, referred to as hyperhomocysteinemia. Two frequently examined variations in the MTHFR gene concerning hyperhomocysteinemia are C677T and A1298C. Individuals with certain

variants of these polymorphisms may have reduced enzyme activity, impacting the body's ability to process homocysteine properly (Wilcken et al., 2003).

Elevated homocysteine levels, known as hyperhomocysteinemia, are linked to an heightened susceptibility to diverse health conditions, encompassing cardiovascular disease, stroke, and neural tube defects. It is crucial to acknowledge that, although there is evidence supporting the connection between MTHFR gene variants and hyperhomocysteinemia, the association is intricate, and the gene variants in isolation may not adequately predict these health outcomes. Other genetic and environmental factors also contribute to this relationship (Hu et al., 2013).

2.11.3 The Association Between MTHFR Gene Polymorphisms and COVID-19

Following the onset of the pandemic caused by the novel coronavirus, COVID-19, the medical and scientific sectors encounter complexities in patient management and the identification of dependable biomarkers related to disease advancement. The goal is to promptly classify individuals based on their vulnerability to severe manifestations. Moreover, it is essential to discern clinical, epidemiological, and laboratory indicators associated with a significant risk of mortality, with a particular emphasis on microvascular damage. The identification of such indicators may facilitate the development of secure therapeutic interventions (Simons et al., 2020).

Beyond serological and clinical biomarkers demonstrating a discernible correlation with the severe clinical trajectory of COVID-19 infection, recent hypotheses underscore the significance of Homocysteine (Hcy) as a notable prognostic indicator (Ponti et al., 2020). The involvement of Homocysteine (Hcy) in numerous metabolic and inflammatory processes has been previously validated. Different populations, characterized by diverse ethnic compositions, demonstrate varying frequencies of MTHFR gene mutations and fluctuations in MTHFR activities (Liew and Gupta, 2015). The prevalence of C677T homozygosity varies across populations, ranging from 1% or less in individuals of African descent and the Finnish population to 20% or more in Latino and U.S. Hispanic populations. Notably, the worldwide distribution of the MTHFR 677 T polymorphism displays marked heterogeneity, delineating a geographic gradient from southern to northern and western to eastern regions, encompassing both Europe and the Americas (Yadav et al., 2017).

The Methylenetetrahydrofolate Reductase (MTHFR) gene, a crucial factor influencing folate metabolism and homocysteine concentrations, exhibits correlations with various health conditions due to its distinctive genetic variants, notably the C677T and A1298C mutations. Amidst the prevailing COVID-19 pandemic, substantial scientific inquiry has concentrated on elucidating potential connections between MTHFR gene polymorphisms and their implications for susceptibility to or the severity of COVID-19 (Delgado-Roche and Mesta, 2020).

Genetic modifications in the MTHFR gene have been linked to an elevated vulnerability to various health conditions, encompassing cardiovascular diseases, specific cancer forms, and neurological disorders. Furthermore, these genetic variations could potentially impact the immune response of the body, potentially influencing an individual's vulnerability to different infections. The potential rationale for the link between MTHFR gene mutations and COVID-19 outcomes is grounded in the intricate interplay between the MTHFR gene, the immune system, and the impact of hyperhomocysteinemia on vascular health. Genetic variations within the MTHFR gene could plausibly impact an individual's response to the SARS-CoV-2 virus, potentially leading to disparities in the severity and progression of the disease (Delgado-Roche and Mesta, 2020).

The selection of the MTHFR gene as the focus of investigation is motivated by the pursuit of understanding potential associations between this genetic factor and the severity of COVID-19 manifestations. This study aims to elucidate any discernible correlation between the MTHFR gene and the severity of illness in COVID-19 patients.

3. MATERIAL AND METHODS

The research was conducted in the Faculty of Medicine at Tokat Gaziosmanpaşa University, Department of Medical Biology. The research involved a total of 140 participants, that were collected from Tokat Government Hospital. Including 41 control group and 99 patients which divided into 3 distinct groups: the, Mild Group, Inpatient Group, and Intensive Care Group (Table 3.1). The participants' ages ranged from 17 to 94 years and the average of the age was 46.1±19.8 years. It was noted within the sample that 28 participants were smokers, and 12 admitted to consuming alcohol. Patient Blood samples were preserved at a temperature of +4 degrees Celsius for the duration of the research.

Approval from the Ethics Committee for Clinical Research at Tokat Gaziosmanpaşa University's Faculty of Medicine was obtained with decision number 23.KAEK-080 for the current study.

Table 3.1: sample size for different groups in our study.

Symptoms	Number of samples
Mild	42
Inpatient	14
Intensive care	43
Control	41
Total	140

3.1 Chemical materials, Kits and Kit contents

GeneAll® ExgeneTM Blood SV mini (105-101/105-152).

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

3.2 Equipment used in the study

1- Water bath (Electronic balance type ABJ 220-4M, No: WB0750631).
2- -20 ° C freezer (Arcelik, 2052DY, Turkey)
3- -20 ° C freezer (Profilo)
4- Power Supply (Biorad, 1645070, USA)
5- Microwave (Arcelik, MD554, Turkey)
6- qPCR Thermal Cycler (Analytik Jena‘s Biometra, Germany)
7- Centrifuge (Hettich, D78532, Germany)
8- Vortex (Velp Scientifica, F20220176, Italy)
9- Kern ABJ 220-4NM Analitik Terazi 220 gr /0,0001 gr
10- Gel imaging device (Prizmalab).
11- Incubator (Memmer P.G. Box 1720 D 91 107 Schwabach).

3.3 DNA Isolation

DNA extraction from blood samples was performed using the GeneAll® ExgeneTM Blood SV mini kit (105-101/105-152) following the prescribed manufacturer's protocol.

1. A volume of 200 µL of blood was introduced into a sterile microcentrifuge tube.
2. A quantity of 20 µL of Proteinase K was incorporated into the tube.
3. A volume of 20 µL of RNase A was introduced into the tube.
4. The resultant blend was thoroughly mixed through vortexing and subsequently incubated at room temperature for a duration of 2 minutes.
5. Add 200 µl of Buffer BL to the tube, ensuring thorough mixing by vortexing. Incubate the mixture at 56˚C for a duration of 10 minutes. Subsequently, briefly centrifuge the tube to eliminate any residual drops adhering to the inside of the lid.
6. Each sample was treated with 200 µl of 96% ethanol, and thorough mixing was achieved through vortexing.
7. Carefully transfer the blend to Column Type G (mini), apply centrifugal force at 6,000 x g or beyond (>8,000 rpm) for 1 minute, and then substitute the collection tube with a fresh one.
8. Add 600 µl of Buffer BW, centrifuge the specimen at 6,000 x g or higher (>8,000 rpm) for 1 minute, and replace the collection tube with a new one.

9. Administer 700 µl of Buffer TW, conduct centrifugation at 6,000 x g or higher (>8,000 rpm) for 1 minute, discard the pass-through, and subsequently reinsert the mini column back into the collection tube.
10. Perform centrifugation at maximum speed for 1 minute to eliminate any remaining wash buffer. Transfer the mini column to a new 1.5 ml microcentrifuge tube.
11. Introduce 200 µl of Buffer AE or sterile water. Allow incubation at room temperature for 1 minute. Conduct centrifugation at maximum speed for 1 minute.

3.4 Preparation of Components

3.4.1 Preparation of Marker

Our study involved the application of the PUC Mix marker. In the preparatory phase, we combined 80 µl of distilled water, 10 µl of dye, and 10 µl of PUC. Subsequently, the mixture underwent centrifugation for a period of 5 seconds, yielding a prepared and ready-to-use marker.

3.4.2 Gel preparation

To formulate the gel, a blend comprising 2 g of Agarose and 1 g of nusive (Gamma Micropor) (3%) was introduced into a 300 ml conical flask, along with 100 ml of Tris-Borate-EDTA (TBE) solution. The conical flask was then positioned in a microwave for 3 minutes. Intermittent swirling during the microwave process was implemented to guarantee the thorough dissolution of Agarose, leading to the formation of a transparent gel solution. After the microwave treatment, the solution was permitted to cool for one minute prior to the incorporation of 8.5 µl of ethidium bromide, which was uniformly dispersed through swirling. Subsequently, the gel solution was poured into the electrophoresis tray to undergo additional cooling and solidification.

3.5 PCR

The Polymerase Chain Reaction (PCR) technique, widely utilized in the realm of molecular biology, enables the amplification of specific DNA sequences, simplifying their analysis and manipulation. In the context of a confirmed diagnosis of COVID-19, PCR can play a crucial role in detecting specific MTHFR polymorphisms that could potentially influence the severity of the disease. Through the amplification of precise segments within the MTHFR gene, it becomes possible to examine single-nucleotide

polymorphisms (SNPs), including C677T, which have been linked to a range of health-related conditions.

The Polymerase Chain Reaction (PCR) method can be integrated with supplementary methodologies, such as Restriction Fragment Length Polymorphism (RFLP) analysis, to genotype particular polymorphisms. Within the framework of PCR-RFLP, the PCR-generated product is subjected to cleavage by a restriction enzyme, which possesses the ability to recognize and cut DNA at a distinct sequence altered by the SNP. Subsequently, the resulting DNA fragments are distinguished through gel electrophoresis. The configuration of the fragments discloses the existence (or non-existence) of the polymorphism.

3.5.1 Components of PCR

The PCR reactions were executed within a 25 µl reaction volume using a MiniAmp™ Plus Thermal Cycler from Applied Biosystems™. The primer sets employed in both polymerase chain reactions are detailed in (Table 3.2).

Table 3.2: Primer sets.

Primers	**Sequences (5′ to 3′)**
MTHFR Forward	TGA AAG GAG AAG GTG TCT GCG GGA
MTHFR Reverse	GGA CGG TGC GGT GAG AGT G

We prepared a mix for each of the samples which contains 16 µl of distilled water (dH_2O), 2.5 µl of PCR buffer, 1.5 µl of $MgCl_2$, 0.3 µl of dNTP, 0.8 µl each of forward and reverse primers, and 0.1 µl of Taq DNA polymerase and then add to a tube that has 3 µl DNA.

3.5.2 PCR protocol

Table 3.3: PCR program applied for DNA synthesis (38 cycle).

	PCR steps	**Temperature (°C)**	**Time**
38 cycles	Initial denaturation	94°C	2 minutes
	Denaturation	94°C	30 seconds
	Primer annealing	61°C	30 seconds
	Extension	72°C	30 seconds
	Final extension phase	72°C	7 minutes

3.6 RFLP

RFLP which stands for Restriction Fragment Length Polymorphism is a molecular technique that detects variations in DNA sequences. The MTHFR gene is known to contain specific polymorphisms, notably the C677T variant. This variant, a Single Nucleotide Polymorphism (SNP), can be identified using the RFLP approach.

After the PCR process, we performed the RFLP process, for which we prepared a mixture using 8.5 µl distilled water, 1.2 µl colored buffer, 0.3 µl Hinf I enzyme and after vortexing the mixture we added it to 10 µl for each PCR products. Subsequently, the products were subjected to 1.5-hour incubation at 37^{o}C, which is crucial for the precise and selective cleavage of DNA by restriction enzymes, HinfI. Then the results were observed on Gel Electrophoresis.

3.7 Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 27 to compare the frequencies of alleles and genotypes between the investigated cases and the control cohort. This analytical methodology enabled the evaluation of the potential association between the MTHFR C677T polymorphism (rs1801133) and the susceptibility to Covid-19. The comparison of categorical variables utilized the Chi-square test or Fisher's exact test. To ascertain the p-value between laboratory parameters and MTHFR genotypes, one-way ANOVA was employed. Multivariate analyses were conducted for pairwise comparisons between groups and laboratory parameters. Statistical significance was defined as a p-value less than 0.05.

4. RESULTS

In the current study, an evaluation was conducted on a total of 140 participants (52 Male and 88 Female) including 99 patients and 41 control group. The study involved participants of both genders, female individuals constituted approximately 62.86% of the participants, while male individuals accounted for approximately 37.14% of the total study population (Figure. 4.1), including 99 individuals diagnosed with COVID-19.

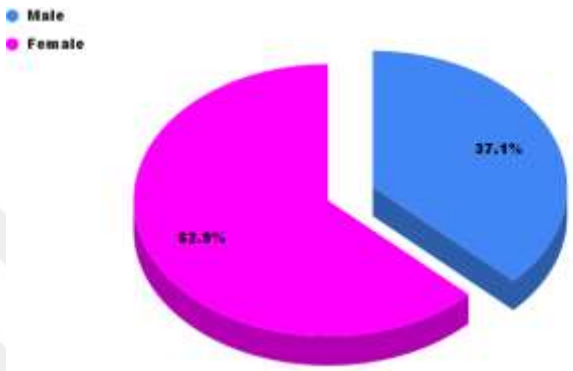

Figure 4.1: Female and Male individuals participated in the study.

4.1 MTHFR gene C677T polymorphism in Covid-19

After performing the PCR and then RFLP for the participants, we placed the samples on the agarose gel and waited for 40 minutes for the bands to appear then the genotyping result was visualized under UV light transilluminator (Figure. 4.2).

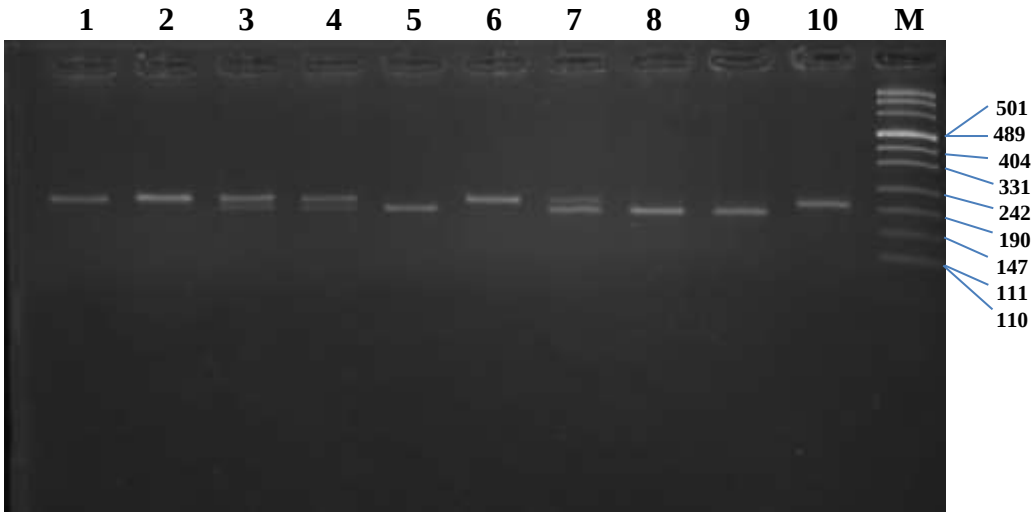

Figure 4.2: Agarose gel electrophoresis of the MTHFR gene PCR-RFLP amplification products.

CC homozygote had a single band (1,2,6,10) of 198 bp. CT heterozygote had three bands of 198, 175 and 23 bp (3,4,7) and TT homozygote had a two fragment of 175 and 23 bp (5,8,9). Marker: PUC mix.

4.2 Genotype and allele comparison

We performed a comprehensive analysis to compare genotypes across diverse cohorts in our study. As illustrated in Table 4.1, seven discrete groups were delineated according to their genotypic variations (CC, CT, and TT). Each group was systematically contrasted with an assigned control group, resulting in associated p-values consistently below the significance threshold of 0.05 for their respective comparisons. Importantly, within the purview of the scrutinized Groups and Genotypes, 42.86% demonstrated statistically significant associations, while the remaining 57.14% did not achieve statistical significance.

Table 4.1: Genotypes Comparison

	Groups	CC	CT	TT	P Value
Control –Patient	Control	19	22	0	0.0154
	Patient	39	45	15	
Control-Mild	Control	19	22	0	0.0222
	Mild	16	19	7	
Control-Intensive Care	Control	19	22	0	0.0902
	Intensive care	17	21	5	
Control-Inpatient	Control	19	22	0	0.0204
	Inpatient	6	5	3	
Mild- Intensive Care	Mild	16	19	7	0.7997
	Intensive Care	17	21	5	
Mild-Inpatient	Mild	16	19	7	0.7454
	Inpatient	6	5	3	
Intensive Care – Inpatient	Intensive Care	17	21	5	0.4629
	Inpatient	6	5	3	

Based on the groups and their corresponding p-values the comparison in Control-Patient Group, Control-Mild Group and Control-Inpatient Group are statistically significant with the p-value of 0.0154, 0.0222 and 0.0204 while in Control-Intensive Care Group, Mild-Intensive Care Group, Mild-Inpatient Group and Intensive Care-Inpatient Group with the p-value of 0.0902, 0.7997, 0.7454 and 0.4629 emphasizing the lack of a statistically significant difference between these groups

respectively. This higher p-value suggests that any disparities observed between these groups are likely attributed to chance occurrences rather than a statistically meaningful connection.

Table 4.2: Alleles comparison.

	Groups	C	T	P Value
Control-Patient	**Control**	60	22	0.086
	Patient	123	75	
Control-Mild	**Control**	60	22	0.085
	Mild	51	33	
Control-Intensive Care	**Control**	60	22	0.216
	Intensive Care	55	31	
Control-Inpatient	**Control**	60	22	0.195
	Inpatient	17	11	
Mild-Intensive Care	**Mild**	51	33	0.695
	Intensive Care	55	31	
Mild-Inpatient	**Mild**	51	33	0.912
	Inpatient	17	11	
Intensive Care-Inpatient	**Intensive Care**	55	31	0.739
	Inpatient	17	11	

The p-values furnish data regarding the statistical significance of the detected variances among the groups under consideration. Comparisons with high p-values (e.g., 0.695, 0.912, 0.739) indicate that the observed differences are likely due to random chance and not a true association (Table 4.2)

4.2.1 Analysis of MTHFR C/T Allele frequency

The allelic distribution of homozygous and heterozygous polymorphisms for the MTHFR gene in the control group manifested as 46.35% CC, 53.65% CT, and 0% TT. The corresponding allele frequencies indicated 73.1% for the C allele and 26.9% for the T allele. Conversely, among COVID-19 patients, the observed frequencies were 39.3% CC, 45.4% CT, and 15.1% TT, with allele frequencies of 62.2% for the C allele and 37.8% for the T allele (Table 4.2). This table delineates the MTHFR C/T polymorphism distribution in COVID-19 patients and the control group. Although a marginal discrepancy existed in the prevalence of the T allele between the two groups (26.9% in controls versus 37.8% in patients), this disparity lacked statistical significance, as evidenced by a p-value of 0.086.

4.3 Genotype-Phenotype association

The essential traits of the COVID-19 patients under investigation are expounded upon in this discourse. Of particular significance is the dearth of apparent heterogeneity among these characteristics, encompassing a spectrum of genotypes. Additionally, the observed variance between these cohorts did not achieve statistical significance (Table 4.3).

Table 4.3: Distribution of Patients Social Demographic Characteristics.

		Patients			
		CC	CT	TT	p value
Gender	M	15	10	4	0.257
	F	24	35	11	
Smoking	Yes	5	6	3	0.777
	No	34	39	12	
Alcohol	Yes	2	2	0	0.681
	No	37	34	15	
Hypertension	Yes	3	3	1	0.981
	No	36	42	14	

As shown in Table 4.3 within the scope of this research, the subjects were segregated into four distinct clusters according to their genotypes: CC, CT, and TT. Each cluster underwent individual analysis, and p-values were derived to evaluate the statistical relevance of the outcomes.

When comparing the genders (male and female) within each genotype category, the computed p-value is 0.257 ($p > 0.05$).

Upon examining the correlation between smoking habits and genotypes, the resulting p-value is 0.777. This implies that there isn't a substantial link between the various genotypes and smoking tendencies. The disparities in smoking prevalence among the CC, CT, and TT groups are probably attributable to random fluctuations.

The examination regarding alcohol consumption in relation to genotypes resulted in a p-value that is 0.681. This signifies that there is no substantial association between genotypes and alcohol consumption.

Lastly, when examining the relationship between hypertension and genotypes, the calculated p-value is 0.981. The elevated p-value indicates that there is no

noteworthy correlation between the genotypes and hypertension. The differences in hypertension prevalence among the CC, CT, and TT groups are likely due to random variation.

To encapsulate, the derived p-values do not provide substantial proof to affirm significant correlations between factors such as gender, smoking habits, alcohol intake, hypertension, and the genotypes CC, CT, and TT. The disparities noted within these genotype groups in relation to the aforementioned factors are more plausibly attributable to random variation as opposed to a consequential correlation.

Table 4.4: Mean and standard deviation between genotypes and laboratory paramaters in patients.

		CT	CC	TT	Total	p-value
Glucose	Mean	124.6	120.8	122.9	122.8	0.932
	S.D	±52.5	±40.5	±39.7	±45.9	
AST	Mean	54.3	51.5	47.1	52.1	0.980
	S.D	±150.1	±94.6	±81.7	±120.6	
ALT	Mean	38	33.8	38.9	36.5	0.940
	S.D	±74.7	±41.6	±63.9	±61.3	
TG	Mean	167.6	224.3	298.2	209.7	0.01
	S.D	±57.8	±135.6	±309.2	±156.1	
HDL	Mean	40.4	40.1	40.5	40.3	0.988
	S.D	±8.7	±10.9	±14.3	±10.5	
VLDL	Mean	36.4	45.6	63	44.1	0.01
	S.D	±12.1	±27.1	±60.3	±30.8	
LDL	Mean	80.7	88.7	74.2	82.9	0.2
	S.D	±17.7	±38.3	±24.7	±28.7	
BUN	Mean	23.6	24.2	27.1	24.4	0.815
	S.D	±19.1	±16.4	±22.8	±18.6	
CRP	Mean	48.4	51.5	27.1	46.4	0.331
	S.D	±53.3	±62.1	±38.6	±55.2	

Ferritin	Mean	1163.6	451.4	334	757.3	0.611
	S.D	±5469.5	±689.8	±227.2	±3709.9	

As shown in Table 4.4, this study endeavors to explore the correlation between the polymorphism of the MTHFR gene and various clinical parameters in individuals afflicted with COVID-19. Employing the Statistical Package for the Social Sciences (SPSS) software, the mean and standard deviation were calculated for clinical parameters across distinct genotypes, namely CT, CC, and TT. Subsequent to this, a one-way analysis of variance (ANOVA) was applied to ascertain the presence of statistically significant differences among the aforementioned genotypes. Among the numerous clinical parameters subjected to scrutiny, only triglyceride (TG) and very-low-density lipoprotein (VLDL) exhibited noteworthy variances ($p = 0.01$) across the genotypes. Conversely, the remaining clinical parameters failed to demonstrate statistically significant distinctions.

Table 4.5: Pairwise Comparison between individuals and laboratory parameters with a p-value.

	control-mild	control-Intensive care	control-Inpatient	mild-Intensive care	mild-Inpatient	Intensive care-Inpatient
Glucose	0.999	0.001	0.999	0.001	0.736	0.048
ALT	0.999	0.020	0.999	0.009	0.999	0.865
AST	0.999	0.007	0.999	0.006	0.999	0.718
HDL	0.086	0.001	0.738	0.882	0.999	0.999
VLDL	0.020	0.999	0.999	0.035	0.999	0.999
LDL	0.018	0.001	0.149	0.005	0.999	0.149
TG	0.173	0.999	0.999	0.288	0.999	0.999
BUN	0.999	0.001	0.500	0.001	0.125	0.001
CRP	0.999	0.001	0.018	0.001	0.047	0.001
Ferritin	0.999	0.196	0.999	0.22	0.999	0.999

In accordance with the delineations presented in Table 4.5, the classification of clinical severity encompasses six distinct groups, namely Control-Mild, Control-Intensive Care, Control-Inpatient, Mild-Intensive Care, Mild-Inpatient, and Intensive Care-Inpatient. An exhaustive scrutiny of each group was conducted with regard to ten discrete clinical laboratory parameters. Subsequently, p-values were computed to ascertain the statistical significance of the observed outcomes, with the conventional

threshold of $p < 0.05$ demarcating significance. Notably, among the diverse clinical laboratory outcomes under consideration, 31.6% were determined to be statistically significant, while the remaining 68.4% did not attain statistical significance.

The analysis reveals noteworthy statistical distinctions within various clinical parameters across distinct severity groups. Specifically, within the Control-Mild group, discernible statistical disparities manifest in the VLDL and LDL levels, with p-values of 0.020 and 0.018, respectively. These outcomes underscore a substantial and statistically significant divergence in VLDL and LDL concentrations within the Control-Mild cohort. Furthermore, in the Control-Intensive Care group, all parameters, with the exception of VLDL, TG, and Ferritin, exhibit statistically significant differences, denoted by p-values ranging from 0.001 to 0.02. This implies that, barring the specified parameters, the Control-Intensive Care cohort displays notable variations in clinical measures. Conversely, within the Control-Inpatient group, sole statistical significance is observed in the parameter of CRP, with a p-value of 0.018, indicating a distinct elevation in CRP levels among individuals within this cohort.

In the subsequent exploration of the Mild-Intensive Care group, statistically significant differences emerge across parameters, excluding HDL, TG, and Ferritin, with p-values ranging from 0.001 to 0.036. Analogously, the Mild-Inpatient group exclusively exhibits statistical significance in the CRP parameter, recording a p-value of 0.047.

Finally, within the Intensive Care-Inpatient group, statistical significance is confined to specific parameters, namely Glucose, BUN, and CRP, each possessing p-values of 0.048, 0.001, and 0.001, respectively. These findings underscore the nuanced nature of clinical variations among distinct severity cohorts, elucidating the significance of these differences in a comprehensive manner.

5. DISCUSSION AND CONCLUSION

The infectious disease known as Coronavirus Disease 2019 (COVID-19) is caused by a newly identified strain of coronavirus, as described by the World Health Organization (WHO, 2020). Coronaviruses, including this recently discovered variant, carry significant importance as pathogens that impact both humans and animals. In the latter part of 2019, a previously unrecognized coronavirus was identified as the causative agent of a cluster of pneumonia cases concentrated in Wuhan, located in the Hubei Province of China. This pathogen rapidly spread, resulting in an epidemic within China and subsequently leading to an increasing number of cases in various countries worldwide. In February 2020, the World Health Organization formally designated the condition as COVID-19, denoting coronavirus disease in the year 2019. The particular virus accountable for COVID-19 is identified as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), formerly referred to as 2019-nCoV (Zhu et al., 2020).

The genetic locus denoted as MTHFR C677T represents a prevalent gene variant characterized by a deviation in the DNA sequence from the anticipated norm. Specifically situated at the 677 position within the MTHFR gene, the canonical DNA base "C" is supplanted by the gene variant "T" This genetic variation pertains to the encoding of an enzyme pivotal to the enzymatic conversion of folate and homocysteine, essential compounds intricately involved in diverse biological processes (Goyette et al., 1998).

The (MTHFR) gene, a crucial determinant governing folate metabolism and homocysteine levels, has exhibited associations with 44 various health conditions, particularly through its distinctive genetic variants, notably the C677T mutation. Amidst the ongoing COVID-19 pandemic, substantial scientific inquiry has been directed towards investigating potential correlations between MTHFR gene polymorphisms and their implications for susceptibility to, or the severity of, COVID-19. The conceivable rationale underlying the association between MTHFR gene mutations and COVID-19 outcomes is rooted in the intricate interplay among the MTHFR gene, the immune system, and the impact of hyperhomocysteinemia on vascular health (Brustolin et al., 2010).

In a scholarly Investigation undertaken by Tekcan et al. (2023), an in-depth investigation was conducted on a cohort of 100 patients diagnosed with COVID-19 in

Turkey, systematically categorized into three distinct cohorts: intensive care, inpatient service, and outpatient treatment groups. The study identified a heightened prevalence of the MTHFR gene C677T CT genotype and T allele among individuals in the intensive care cohort. Additionally, individuals with positive polymerase chain reaction (PCR) results exhibited an increased incidence of the MTHFR C677T CC genotype and C allele. Notably, those testing positive for the CT genotype manifested an elevated frequency of the MTHFR gene CT genotype. These observations suggest the potential existence of a genetic predisposition that may contribute to the morbidity and mortality associated with COVID-19 within the Turkish population (Tekcan et al., 2023).

While in another distinctive investigation examining the interrelation between the prevalence of the MTHFR C677-T gene polymorphism and the global occurrence of COVID-19, coupled with its associated mortality, as conducted by Ponti et al. (2021), a discernable correlation was identified, particularly with regard to the C677T variant and the mortality associated with coronavirus infection. The study postulates that the genetic polymorphism of MTHFR C677T harbors the potential to impact both the frequency and severity of COVID-19 pandemic infections. A conspicuous global pattern is evident, underscoring an association between the widespread prevalence of the MTHFR 677T allele and the incidence and mortality rates linked to COVID-19. Of particular note is the observation that the Latino population, characterized by an elevated prevalence of the MTHFR 677T allele, encounters a correspondingly heightened incidence and mortality in relation to COVID-19 compared to other global populations. Statistical analysis further reinforces the assertion of a relatively robust correlation between the MTHFR C677T gene polymorphism and fatalities stemming from coronavirus infection (Ponti et al., 2021).

In a research investigation conducted at Ain-Shams University in Egypt, a cohort comprising 33 individuals diagnosed with COVID-19 was examined, comprising 19 males and 14 females, with an average age of 45.42 ± 10.56 years. Additionally, a control group consisting of 13 subjects, including 6 males and 7 females, with an average age of 34.85 ± 13.13 years, was included in the study. This pilot study represents the first systematic exploration of the potential implications of the MTHFR gene mutation in the context of COVID-19 within the Egyptian population. The outcomes of this investigation revealed that the incidence of the MTHFR gene mutation

among COVID-19 patients was 30.3%. Notably, these findings suggest a plausible correlation between the presence of an inherited MTHFR gene mutation and the manifestation of severe forms of COVID-19, as well as an increased propensity for thromboembolic events and mortality. The identification of such a connection underscores the imperative for further comprehensive research into the underlying biological mechanisms and clinical ramifications associated with inherited MTHFR gene mutations in the context of COVID-19. Furthermore, it accentuates the need for the development of optimal strategies for anticoagulation management tailored specifically for individuals harboring MTHFR gene mutations in order to enhance clinical outcomes in this population (Abdelfattah et al., 2023).

In a separate investigation involving an Uzbek population comprising 80 COVID-19 patients, the research findings indicate a noteworthy elevation in homocysteine levels within the blood among individuals harboring the non-wild allele of the MTHFR gene, specifically the 677 C>T polymorphisms. This disparity was observed in comparison to both the control cohort and patients exhibiting the wild homozygous genotype. Moreover, a correlative analysis unveiled a substantial prevalence of moderate and severe disease manifestations among patients carrying the mutant T and C alleles of the aforementioned polymorphisms. Beyond the hyperergic inflammatory and hypercoagulopathic responses elicited by COVID-19, the concomitant exposure to hyperhomocysteinemia significantly amplifies the susceptibility to severe illness when juxtaposed with patients possessing the homozygous wild genotype (Khidoyatovna et al., 2022).

In another investigation encompassing a cohort of 613 individuals, comprising 373 individuals diagnosed with hypertension and 240 healthy counterparts, as conducted by Xu et al., (2023), it was discerned that the CT and TT genotypes as well as the T allele pertaining to the MTHFR C677T locus exhibited statistically significant associations with an elevated predisposition to hypertension within the Bai ethnic group domiciled in the Yunnan province of China. Within the hypertensive cohort, the MTHFR C677T polymorphism manifested noteworthy correlations with diverse biochemical indicators, including levels of fasting blood glucose, fructosamine, apolipoprotein A1, homocysteine, superoxide dismutase, and malondialdehyde. The findings of the study substantiate the proposition that genetic variants within the

MTHFR C677T loci bear substantial relevance to the susceptibility profile for hypertension prevalent among the Bai populace in Yunnan, China (Xu et al., 2023).

A comprehensive study was conducted within the Mexican population, involving 243 individuals diagnosed with acute lymphoblastic leukemia (ALL), comprising 136 cases in pediatric patients and 107 cases in adults, alongside a control group of 379 unaffected individuals. Analysis of the data revealed the distribution rates of the T allele within the MTHFR C677T polymorphism, indicating frequencies of 43% among pediatric cases, 52% among adult cases, and 46% among the control cohort. Notably, statistical analysis did not yield a significant correlation between the presence of the T allele and susceptibility to acute lymphoblastic leukemia (ALL) among pediatric patients. Conversely, among adults, a notable increase in the risk of ALL was observed in association with the presence of the T allele. The study underscores an association between the MTHFR C677T polymorphism and an augmented susceptibility to ALL particularly within the adult segment of the Mexican population, whereas such an association was not evident among pediatric patients. Further elucidation of the underlying mechanistic pathways and validation of these findings necessitate additional research endeavors involving larger and more demographically diverse cohorts (Pablo and Espinoza, 2022).

However, in a distinct study conducted by Lak et al., (2021) on the Iranian population, encompassing a sample of 200 subjects, consisting of 100 patients afflicted with colorectal cancer and 100 unaffected controls, a significant correlation was identified between the C677T polymorphism within the MTHFR gene and an increased predisposition to colorectal cancer within the Iranian cohort (Lak et al., 2021).

In our investigation, encompassing a cohort of 140 participants, comprising 99 individuals diagnosed with COVID-19 and 41 individuals in the control group, our findings indicate that the genetic variations observed in the majority of instances are not correlated with the manifestation of the disease. As delineated in the tables presented, these associations do not achieve statistical significance. However, our analysis suggests a marginal association between MTHFR gene polymorphism and the severity of COVID-19.

The comprehensive exploration of social demographic characteristics among individuals with varying genotypes is essential for a thorough comprehension of

MTHFR gene polymorphism in individuals affected by COVID-19. This encompasses variables such as gender, age, ethnicity, smoking habits, alcohol consumption, and hypertension. The scrutiny of the distribution of these attributes serves to recognize potential confounding elements, thereby facilitating a more nuanced insight into the correlations between MTHFR gene polymorphism and COVID-19.

Upon comparing our study with other investigations, it is evident that a discernible correlation in MTHFR C677T gene polymorphism has been identified, such as in studies conducted by ponti et al., Abdelfattah et al., Khidoyatovna et al., Pablo and Espinoza, and Tekcan et al., also Xu et al., found that there is an association between MTHFR gene and susceptibility of hypertension and Lak et al., found an association between MTHFR gene and Colorectal cancer. Also our study has revealed a subtle association between the MTHFR C677T gene polymorphism and the severity of Covid-19 in Turkish population.

The current study, carried out on the Turkish population, reveals a modest association between MTHFR C677T gene polymorphism (rs1801133) with the severity of Covid-19 in patients. Notably, 42.86% of the findings exhibited significance in genotypes comparison between Control with patient, mild and inpatient with a p-value of 0.0154, 0.0222 and 0.0204 respectively which is less than 0.05 in the comparisons between genotypes and groups, and 31.6% in the associations between laboratory parameters and groups. It is crucial to acknowledge the restricted sample size, and expanding the cohort can enhance the dependability and applicability of the study's outcomes.

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