NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF MOLECULAR MEDICINE

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SARS-CoV-2 Delta variant

BEGIMAI MAMUROVA

MASTER THESIS IN MOLECULAR MEDICINE

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Approval

We certify that we have read the thesis submitted by Begimai Mamurova titled "Association between vitamin D receptor gene polymorphisms and COVID-19 causing SARS-CoV-2 Delta variant" and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Educational Sciences.

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Declaration

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

Begimai Mamurova

22/02/2022

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ABSTRACT

Association between vitamin D receptor gene polymorphisms and COVID-19 causing SARS-CoV-2 Delta variant

AIM:

This study was conducted to investigate the allelic frequencies and genotypic distribution between *FokI* and *TaqI VDR* gene polymorphisms in COVID-19 patients who are infected by SARS-CoV-2 Delta variant.

BACKGROUND:

COVID-19 disease is a severe illness that caused millions of people's deaths worldwide and long-term health issues for those who have survived the disease. The SARS-CoV-2 can trigger a respiratory tract infection and affect the upper respiratory tract as well as the lower respiratory tract. The virus can be transmitted the same way as other coronaviruses, mainly through person-to-person direct contact. The severity of the infection can vary from mild to fatal.

COVID-19 symptoms vary, but they commonly include fever, cough, headache, fatigue, and loss of smell and taste. The majority of infected people (81%) may experience mild to moderate symptoms, while 14% may develop severe symptoms and 5% might experience severe symptoms such as respiratory failure or multi-organ dysfunction.

CoV entry into host cells is a multi-stage process involving several different domains in protein S, which facilitate viral binding to the target cell, receptor involvement, protease processing, and membrane fusion.

The SARS-CoV-2 genome has evolved over time due to random mutations, resulting in the emergence of genetic variants that are thought to be more contagious. Notably, Alpha and Delta variants were indicated to be more contagious than previously discovered virus strains.

COVID-19 pathophysiology involves a number of signaling pathways and cellular components, including vitamin D2. Vitamin D is an essential immune system regulator.

Vitamin D, in particular, has anti-infective and immunomodulatory properties, as it enhances intercellular barriers, stimulates innate immunity, and helps regulate adaptive immunity. Many studies are being conducted to analyze the influence of vitamin D specifically within the context of the COVID-19 pandemic, considering the molecular pathways and metabolism of vitamin D.

COVID-19 prevalence and mortality rates may be affected by the modulatory effect of persons' bioavailable vitamin D levels, which is influenced by genetic factors such as polymorphisms in the VDR gene.

METHODS:

A total number of 200 individuals who admitted to Near East University Hospital COVID-19 PCR Diagnosis Laboratory for routine SARS-CoV-2 RT-PCR test was used in this study. The control group consisted of individuals who were SARS-CoV-2 RT-qPCR negative. On the other hand, the case group consisted of patients who were SARS-CoV-2 RT-qPCR positive, infected with SARS-CoV-2 Delta variant.

PCR was used to amplify of target mutated regions of the *VDR* gene from isolated genomic DNA. The PCR step was done for all 200 samples using for each *FokI* and *TaqI* restriction enzymes, 400 in total. PCR reaction was carried out in a total volume of 20 µl, containing 15 µl of PCR mixture and 5 µl of DNA. Following the PCR, the gel electrophoresis was done for the yield amplified products.. Visualization of the bands (*TaqI* 500bp, *FokI* 265bp) was through an ultraviolent trans-illuminator. After visualization of the bands RFLP analysis was done for *Taq1* and *Fok1* polymorphisms by the use of mutation specific restriction enzymes. RFLP analysis was performed in a total volume of 20 µl, containing 10 µl of RFLP mixture and 10 µl of amplified product from PCR. The samples incubated at 37°C for 30 minutes for Fok1 and at 65°C for 30 minutes for Taq1 polymorphisms for the RFLP analysis.

RESULTS:

A total number of 200 individuals who admitted to Near East University Hospital COVID-19 PCR Diagnosis Laboratory for routine SARS-CoV-2 RT-PCR test was used in this study to investigate the allelic frequencies and genotypic distribution between *VDR* gene *FokI* (rs10735810) and *TaqI* (rs731236) polymorphisms in COVID-19 patients who are infected by SARS-CoV-2 Delta variant and compared them with those

who were tested negative for SARS-CoV-2 as a control group. The results indicated that there is a strong association between *TaqI* and *FokI* VDR gene polymorphisms and COVID-19 causing the SARS-CoV-2 Delta variant, and patients with *FokI* and *TaqI* gene polymorphisms may tend to be more susceptible to getting infected with the SARS-CoV-2 Delta variant.

CONCLUSION:

In the current study, our main objective was to identify if there is an association between vitamin D receptor gene polymorphisms and COVID-19 causing the SARS-CoV-2 Delta variant.

To sum up, the results of this study displayed significant differences in genotype frequencies of *FokI*-rs10735810 and *TaqI*-rs731236 variants between SARS-CoV-2 Delta variant infected patients and the control group. The results also suggest that the patients with *FokI* and *TaqI* gene polymorphisms may tend to be more susceptible to getting infected with the SARS-CoV-2 Delta variant. Limitations of this study includes lack of data about vitamin D levels of patients and involving only Delta variant of SARS-CoV-2 to investigate the allelic frequencies and genotypic distribution between vitamin D receptor gene polymorphisms in COVID-19 patients. Confirmation of these findings is in need of further study using a wider range of data.

KEYWORDS: COVID-19, Delta variant, *VDR* gene polymorphisms, *FokI, TaqI,* vitamin D

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LIST OF ABBREVIATIONS

ACE2: Angiotensin-Converting Enzyme 2

ARDS: Acute Respiratory Distress Syndrome

ASP: Allele Specific Primer

bp: Base pair

BPA: Bisphenol

cDNA: Complementary deoxyribonucleic acid

CSG: Coronavirus Study Group

CVDs: Cardiovascular Diseases

DAMP: Damage-associated molecular pattern

DHEAS: Dehydroepiandrosterone sulfate

DM: Diabetes mellitus

DMV: Double membrane vesicles

FDA: Food and drug administration

HWE: Hardy-Weinberg equilibrium

MERS-CoV: Middle East Respiratory Syndrome coronavirus

NIH: National institute of health

nM: Nanomolar

PAMP: Pathogen-associated molecular pattern

PCR: Polymerase chain reaction

qRT- PCR: Quantitative reverse transcriptase – polymerase chain reaction

RBD: Receptor binding domain

RNA: Ribonucleic acid

SARS-CoV: Severe acute respiratory syndrome coronavirus

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

SNP: Single nucleotide polymorphism

TBE: Tris borate EDTA

VDD: Vitamin D deficiency

VDR: Vitamin D receptor

WHO: World Health Organization

µl: Microliter

µM: Micromolar

CHAPTER 1: INTRODUCTION

1.1 Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was spread worldwide from Wuhan, China, at the end of 2019. Soon after, the World Health Organization (WHO) announced the SARS-CoV-2 infection, which caused a new Coronavirus disease (COVID-19), as a pandemic (Habas et al., 2019). SARS-CoV-2, like the Middle East Respiratory Syndrome coronavirus (MERS-CoV) and the Severe Acute Respiratory Syndrome coronavirus (SARS-CoV), is a beta-coronavirus (Di Maria et al., 2020).

COVID-19 disease is a severe illness that caused millions of people's deaths worldwide and permanent health problems for some who have survived the disease (Rauf et al., 2020).

The virus has been responsible for over 297 million infections worldwide, causing more than 5.4 million deaths. Among the countries the United States has recorded the greatest number of deaths (JHU Coronavirus Resource Center, 2022).
The SARS-CoV-2 virus can cause a respiratory tract infection, affecting both the upper and lower respiratory tracts. The virus spreads in the same way that other coronaviruses do, primarily through person-to-person contact. The infection's severity can vary from mild to fatal (Cevik et al., 2020).

Coronaviruses are viruses that are commonly found in animals, but some of them can infect humans. Many human coronaviruses are derived from bats, which are natural hosts for those kind of viruses. As a result, it is thought that the virus spread to humans via an intermediate host. The first SARS-CoV developed in bats and was transmitted to people via civet, whereas MERS-CoV originated in humans via camels as an intermediate host. Currently there is no clear information on transmission process of SARS-CoV-2 from animals to people (Wu et al., 2020).

The origin of SARS-CoV-2 is thought to be zoonotic, presumably through indirect infection. There have been various theories about the origin of index case, however the research has been ongoing, and no clear answer has been found yet. According to phylogenetic studies, SARS-CoV-2 first appeared in October or November of 2019. The

virus may have spread from Wuhan, according to phylogenetic algorithm analysis. There was a strong evidence that it was derived from a virus infecting wild bats and was transmitted to people via an intermediate host for wild animals. The chance of the virus accidentally escaping from a laboratory was also investigated, and no clue was found (Li et al., 2020).

1.2 A New Coronavirus Disease (COVID-19) Pandemic

Since the pandemic has started, researchers, international organizations and other institutional establishments have been putting effort to trace the origins of SARS-CoV-2. The majority of researchers believed that the virus had a zoonotic origin and ultimately came from a bat-borne virus such as other virus-related pandemics in human history (Salian et al., 2021).

The coronavirus can be passed from person to person via respiratory fluids of an infected individual who sneezes, coughs, breathes, or speaks in close distance to others. Droplets, which include aerosols, can be inhaled or become settled in the nose and mouth, as well as in the eyes. Infection may occur in some cases as a result of droplets interacting with contaminated objects. It is known that the virus can be transmitted from infected person to uninfected person two days before symptoms occur, and that symptoms can be observed in 14 days after the infection (Cevik et al., 2020).

The virus is able to survive some amount of time on hard surfaces, for instance, stainless steel. However, the number of virulent virus on surfaces decreases with time and is rarely present in large amounts to cause infection. Infection can occur when a person touches their nose, mouth, or eyes with virus-contaminated hands, or when they touch virus-contaminated surfaces indirectly (Rauf et al., 2020).

The number of cases doubled roughly every week in the initial stages of the pandemic. The disease spread to other Chinese provinces in early and mid-January 2020, primarily due to holidays and Wuhan's importance as a transport and railway center. China found approximately 140 new cases in a day in January 2020. Official data later revealed by that time, symptoms have been detected in 6,174 people, with the possibility that even more had become infected (China CDC Weekly, 2020). On January 30th, 2020 the WHO announced the coronavirus a health emergency of international concern, and pointed to human transmission, strongly encouraged safety equipment for healthcare

staff, and stated that virus detection testing was important due to its highly contagious nature (WHO, 2020).

COVID-19 symptoms differ, but they commonly involve loss of smell and taste, fever, shortness of breath, cough, headache, and fatigue. The majority of infected people (81%) might experience mild to moderate symptoms (including mild pneumonia), 14% might develop severe symptoms and 5% may experience critical symptoms such as respiratory failure or multi-organ dysfunction. Elderly people have a greater risk of having severe symptoms. A few people experienced various effects (prolonged COVID) months after recovery, and organ damage was noticed. Longitudinal studies are being conducted to continue investigating the long-term health impacts of the COVID-19 disease (Ji et al., 2021; Schoeni et al., 2021).

1.2.1 SARS-CoV-2 Characteristics

Coronaviruses refer to single positive-stranded RNA viruses with a round or oval (mostly polymorphic) envelope shape, characterized by crown-like protrusions on the periphery in the virus envelope, and are often associated with respiratory infections, a more sophisticated class of pathogens (Tsai and Wilson, 2020; Lee and Hsueh, 2020).

Complete analysis of the viral genome indicates that the virus shares 88% sequence similarity with two SARS-like coronaviruses derived from bats, but is faraway from SARS-CoV (Lai et al., 2020).

Based on phylogeny, taxonomy, and practice, the Coronavirus Study Group (CSG) of the International Committee on Taxonomy of Viruses eventually named it Severe Acute Respiratory Syndrome Coronavirus 2 on February 11th, 2020. (Lu et al., 2020). Shortly after, the WHO announced the coronavirus-caused disease as Coronavirus Disease 2019 (COVID-19) (Xu et al., 2020).

Coronavirus is a single-stranded ribonucleic acid, which was named after the appearance of the solar corona due to its spikes on the surface, which can reach up to 12 nm (Cheng et al., 2020). The coronaviral genome encodes four significant structural proteins in the envelope, including the spike protein (S), which attaches to the Angiotensin-Converting Enzyme 2 (ACE2) receptor. Spike protein facilitates following binding of the host cell membranes and envelope, allowing the virus to enter the host cell (Kanne, 2020; Pan and Guan, 2020).

Coronaviruses are zoonotic, meaning they are spread to humans through animals. Coronaviruses may go through frequent recombination as a result of the natural recombination process, leading to structural genetic variation. SARS-CoV-2, in contrast, is not a recombinant of any sarbecoviruses. Its high affinity for the ACE2 receptor suggests a common ancestor with two bat-derived SARS-like coronaviruses, bat-SL-CoVZC45 and bat-CoVZXC2. However, no actual proof exists and molecular mechanisms are unknown (Ergoren et al., 2021).

1.2.2 SARS-CoV-2 Genome and Its Proteins

SARS-CoV-2 has a + RNA genome of nearly 29.9 kb and essential genetic differences between isolates. The SARS-CoV-2 genome enters sensitive cells that express ACE2 and TMPRSS2, and functions instantly as mRNA to translate two polyproteins, which are processed into 16 non-structural proteins (nsp116) from the ORF1a and ORF1b regions, to start genome transcription and replication (Woo et al., 2012).

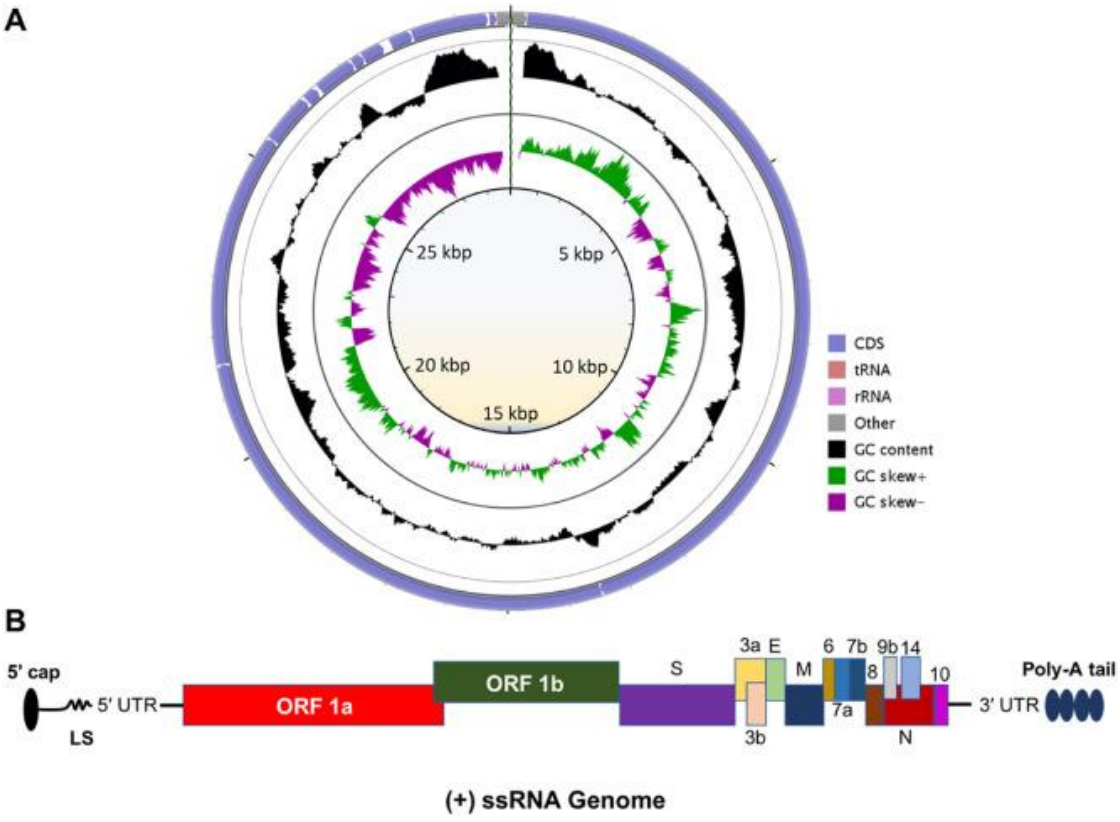

Figure 1: *Genome architecture of SARS-CoV-2.* A. Reference genome of SARS-CoV-2. B. 5′ capped mRNA (Adapted from Naqvi et al., 2020).

Viruses in the same category share some characteristics. The virus consists of four key structural proteins: envelope (E), spike (S), nucleocapsid (N), and membrane (M),

necessary for viral function and structure (She et al., 2020). S and N are the most essential proteins. N aids in the proper formation of the capsid and the structure. S aids the virus's attachment to the host cell (Siu et al., 2008; Walls et al., 2020). The S protein is composed of three distinct domains: a short intracellular tail, a large outer domain, and a single transmembrane channel anchor. Those parts are capable of attaching the host cell effectively. The outer domain of these segments includes two subdivisions, which are found in the crown structure: the S1, which contains receptor binding domain; and the S2, which regulates membrane fusion (Zumla et al., 2016). The SARS-CoV-2 genome also modulates up to six accessory proteins, although translation of these proteins demands the use of individual sub-genomic RNAs (sgRNAs). Complete viral genomic RNA (gRNA) and short RNA primer (sgRNA) are formed in double membrane vesicles (DMV) by replication-transcription complex (Brant et al., 2021).

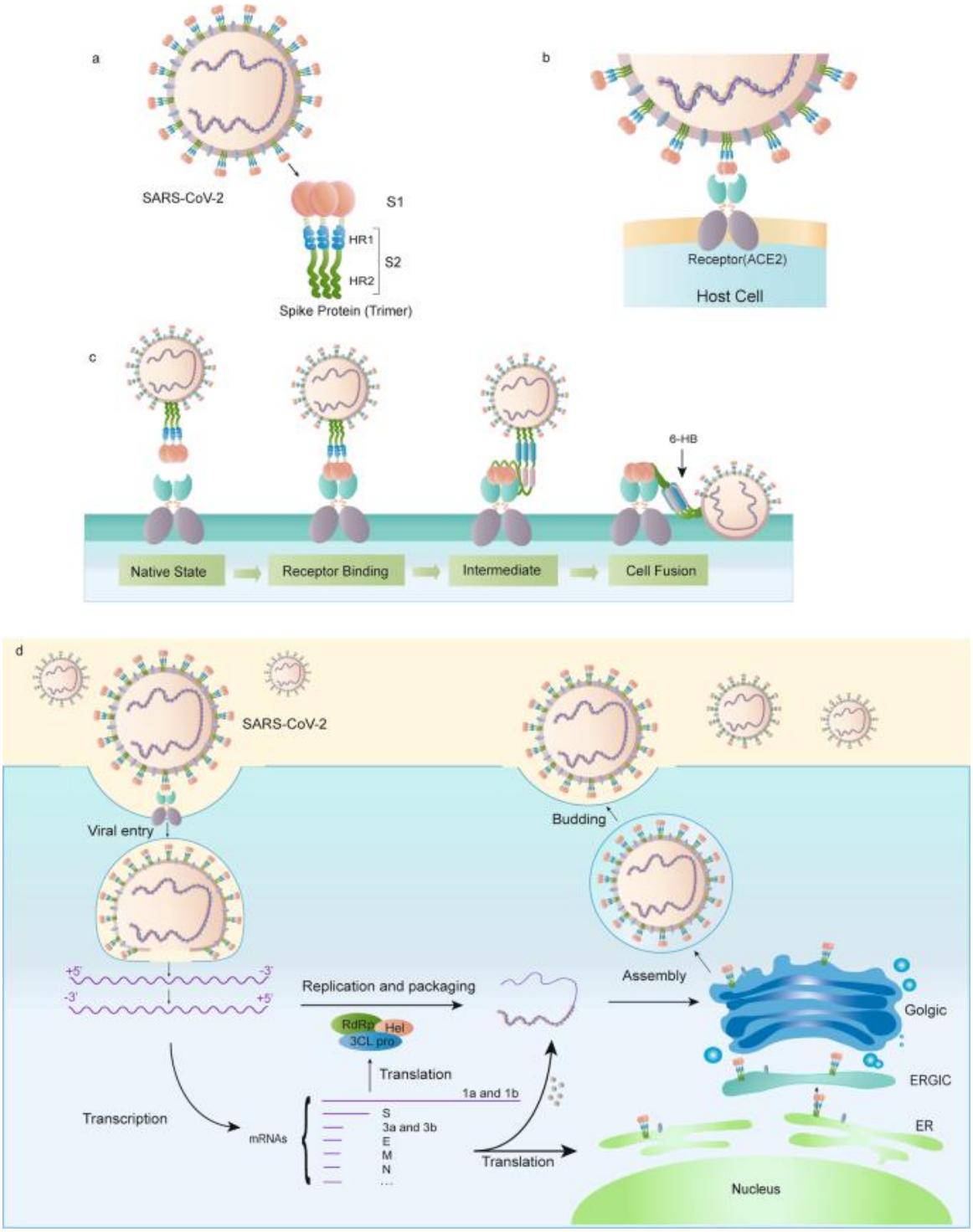

Figure 2. A) The schematic structure of the S protein. B) The binding of the S protein to the receptor ACE2. C) The binding and virus–cell fusion process mediated by the S protein. D) The life cycle of SARS-CoV-2 in host cells. (Adapted from Huang et al., 2020).

1.2.3 Comparison of SARS-CoV, MERS-CoV and SARS-CoV-2

MERS-CoV, SARS-CoV, and SARS-CoV-2 are three extremely virulent and fatal human coronaviruses that have appeared in the last twenty years. The financial forces and health risks posed by these coronaviruses are significant, and they are becoming more severe as the number of global infectious diseases and deaths related to SARS-CoV-2 and MERS-CoV rises.

Coronaviruses are classified into four genera: Alphacoronaviruses, Betacoronaviruses, Gammacoronaviruses, and Deltacoronaviruses (Woo et al., 2012 and Cui et al., 2019). SARS-CoV and MERS-CoV, which led to prior outbreaks in 2002 and 2012 respectively, and the new SARS-CoV-2, are Betacoronaviruses, the main causes of infections in mammals and people (Snijder et al., 2003; Tang et al., 2015; Chan et al., 2015). Gammacoronavirus and Deltacoronavirus, on the other hand, mainly infect fish and birds, though a few cases have been detected to cause disease in mammals (Woo et al., 2010). The existence of the Nsp1 in Alpha and Betacoronaviruses is the main distinguishing feature between the four genera. Among these two genera, Nsp1 has distinct protein size and sequence characteristics. In Gammacoronavirus and Deltacoronavirus, however, no Nsp1 counterpart has been identified (King et al., 2012).

SARS-CoV and MERS-CoV are both very pathogenic viruses with fatal consequences (Rota et al., 2003; Li, 2016). SARS-CoV-2 has a high death rate as 3.8% and a greater rate of infection than the SARS-CoV does. (Benvenuto et al., 2020; Mousavizadeh and Ghasemi, 2020; WHO, 2020). To compare, mortality rate of SARS-CoV is 10% and that of MERS-CoV is 36% (de Groot et al., 2013; Li, 2016).

The genome of SARS-CoV-2 is organized similarly to that of other hCoVs and has a 3′-poly-A tail and a 5′-cap, which allows it to be translated by the host translation machinery (Wu et al., 2020). The formation of two polypeptides is facilitated by a Orf1a and Orf1b frameshift, at the 5′ end of SARS-CoV-2. Those polypeptides are then utilized by breaking down proteins into simpler products to generate 16 non-structural proteins engaged in different process stages of the infection cycle (Mousavizadeh and Ghasemi, 2020). The structural proteins N, M, S, and E are encoded at the 3′ end (Paules et al., 2020).

It is also worth noting that the underlying mechanism of COVID-19 is related to that of SARS-CoV. According to studies, the main component RBM of the SARS-CoV-2 is an

amino acid residue (Gln493). Furthermore, Gln493 promotes viral S protein binding to virus and integration into ACE2 proteins in human cells, resulting in human respiratory infections (Yin et al., 2018). Preventing the virus entry is the most unchallenging and most efficient method to fight SARS-CoV-2 because it has been applied in preceding viruses of the same type (Walls et al., 2020). The host ACE2 protein will not change, which is the main benefit, so there is no need to be concerned about beneficial mutations that could inhibit drug discovery process (Killerby et al., 2018).

SARS-CoV and MERS-CoV have significantly increased rates of death than SARS-CoV-2. COVID-19, on the other hand, is more contagious - SARS-CoV-2 tends to spread more quickly among humans, resulting in a higher case count. Despite the lower mortality rate, the total number of deaths caused by COVID-19 far outnumbers those resulted in due to SARS-CoV or MERS-CoV.
No cases of SARS-CoV have been detected in over a decade, but MERS-CoV has been an ongoing public health problem (Maldonado et al., 2021).

1.2.4 Genomic Evolution and SARS-CoV-2 Variants

Virus mutations, such as the coronavirus that is causing the COVID-19 pandemic, are not new or unexpected. Because it is in their nature to evolve and change gradually, all RNA viruses mutate over time. Virus variants arise due to mutation in the genes of virus, which may allow the coronavirus to spread more quickly and potentially trigger more serious complications of the disease (Raman et al., 2021).

SARS-CoV-2 variants of concern identified by WHO include Alpha, Beta, Gamma, Delta, and Omicron. A variant of concern has a dramatically intensified attachment attraction in the RBD-hACE2 complex due to mutations in the spike protein receptor binding domain (RBD), enhancing the virus's transmissibility. Secondly, it is linked to the intensity of the COVID-19 disease and its spread in the human population (Shahhosseini et al., 2021). Variants of concern modulate or even enhance their ability to replicate regardless of growing immunity in the population, which can occur through infection recovery or vaccination (Hendyet al., 2021).

The fairly low percentage of infections at the start of the pandemic compared to recent period of the pandemic led to decreased chances to mutate the viral genome and thus lower chances for distinct variants to develop (Tregoning et al., 2021). Because variants

were less common, S-protein mutations in the RBD, which interacts with ACE2, were also rare (Piplani et al., 2021).

The SARS-CoV-2 genome has changed over time due to random mutations, resulting in the emergence of genetic variants that are believed to be highly infectious. It is worth noting that Alpha and Delta variants were discovered to be more contagious than formerly observed virus strains (Gallagher J, 2021).

The Alpha variant was discovered in the UK in October 2020, in a sample collected in Kent. Between October and December 2020, its prevalence doubled nearly every week. Many countries are known to report lower cases of the Alpha variant because the most often used tests do not differentiate this SARS-CoV-2 variant from others. RNA sequencing is needed to detect this variant, though an RT-PCR test for certain variants can be used as a supplementing first-screening test for Alpha before performing whole-genome sequencing.

Later in December 2020 Beta variant was discovered in South Africa. This variant was distinguished by the fact that it was prevalent in younger population without any accompanying medical conditions. However, in contrast to other variants, it caused more life threatening illness. The variant can easily attach to human cells due to three mutations in the RBD in the virus's spike glycoprotein, which are N501Y, K417N, and E484K (Abdool Karim and Salim, 2020).

The Gamma variant has 17 specific amino acid mutations and was discovered by the National Institute of Infectious Diseases (NIID) on January 6th, 2021 in Tokyo. Ten of seventeen amino acid mutations of this variant are in its spike protein (Faria et al., 2021). According to a study, Gamma infections can result in approximately ten times the viral ton of people infected by other lineages reported in Brazil. Gamma also demonstrated 2.2 times greater risk of transmission with similar pathogenicity for both adults and elderly people, indicating that this variant is more effective at infecting young people regardless of gender (Nascimento and Souza, 2021).

According to a study, in which samples taken in Manaus were investigated, the Gamma variant is 1.4–2.2 times more likely to be transmitted and can prevent 25–61% of inherited immunity from earlier coronavirus diseases, increasing the chances of reinfection after recovery. In terms of mortality ratio, Gamma infections were observed to be 10–80% more lethal (Zimmer, 2021).

Initial information from two research studies show that the Oxford–AstraZeneca vaccine and CoronaVac are successful in the fight opposed to the Gamma variant (Gaier, 2021).

The Delta variant was discovered in India and has spread to at least 185 countries (WHO, 2021). Public Health England reported on June 3rd, 2021, that 12 of the 42 deaths from the Delta variant in England were among the fully vaccinated, and that it was almost two times more contagious than Alpha variant (Pearson et al., 2021).

In June 2021, cases of a Delta variant with the K417N mutation, which brought up concerns about the impact of vaccine and antibody treatment ineffectiveness and a higher likelihood of reinfection, started occurring (Acharya, 2021).

The Omicron variant was discovered in November 2021 in South Africa and Botswana (WHO, 2021). The variant contains numerous mutations, most of which are extremely concerning. The number of Omicron cases is growing throughout South Africa. According to some findings, this variant is more likely to result in reinfection. There are currently studies being conducted to determine the exact effect on disease transmission, morbidity, and other aspects (BBC News, 2021).

There are also variants of interest declared by WHO, such as Lambda and Mu. In comparison to other strains, there is insufficient information on outbreak and vaccine resistance. Furthermore, there are "variants of interest" ('VUI') which seem to satisfy COVID-19 pandemic criteria (WHO, 2021).

At the beginning of the current study Delta variant of the SARS-CoV-2 was considered the latest and the most spread variant of concern, which resulted in sample collection of this variant.

1.2.5 Pathogenesis

COVID-19 pathogenic stages remain controversial. Previous studies have reported that SARS-CoV infection can be divided into three stages: viral replication, immune hyperactivity, and lung destruction (Navas-Martn and Weiss, 2004). COVID-19 has been classified into three clinical phases: viraemic, acute, and recovery (Lin et al., 2020). Virus intervention and reproduction, poorly regulated immune reaction, organ failures, and recovery are all frequently reported during the infection process. It multiplies, gets together, and is released on target cells, leading to parenchymal cell

damage and disruption. Simultaneously, a vast amount of damage-associated molecular pattern (DAMP) and pathogen-associated molecular pattern (PAMP) molecules are emitted to induce the innate immune response, initiate inflammatory cell invasion, and maintain significant amounts of cytokines, chemokines, and proteases free proteins (Quirch et al., 2020).

Following this critical stage, the inflammatory reaction progressively dissipates, the damaged organ gradually recovers, and pathology and chronic steps, for example, chronic disorders, prolonged inflammation, lowering body's immune response, and catabolism syndrome, occur in some of the malfunctioning organs (Bhaskar et al., 2020).

The pathogenesis of the virus is influenced by several factors. Thus, S1 specifies the virus's host cell area and tropism through the RBD, and S2 modulates virus membrane binding to its cellular host via H1 and HR2. According to research, the S1 domain significantly increases the levels of IgG and IgA antibodies. A lot of essential COVID-19 vaccines are based on the expression of focus spike proteins (Wiersinga et al., 2020).

1.2.5.1 Host Cell Invasion

CoV entrance into host cells is a multi-stage procedure that includes numerous different domains in protein S, which facilitate viral binding to the target cell, receptor interaction, protease exposing, and membrane attachment. The viral genome is then emitted into the cytoplasm, where it multiplies inside of host cells (Letko et al., 2020). Three CoVs in particular (human CoVNL63, SARS-CoV, and SARS-CoV-2) that are bound to the same ACE2 result in different disease intensities, highlighting the differences between these three coronaviruses (Davidson et al., 2020).

Coronavirus spike proteins play critical roles in virus binding and entrance into target cells. ACE2, an enzyme that helps regulate blood pressure, is the receptor for SARS-CoV and SARS-CoV-2. SARS-CoV cell entry is unaffected by ACE2 catalytic activity (Wan et al., 2020).

The entry contains two spike protein subunits that perform distinct functions. The S1 modulates ACE2 binding via the RBD. The binding of the virus and host cell membranes is driven by the S2 subunit, which contains the fusion peptide and transmembrane domains. The peak protein must be spared to two locations directly in

the cell membrane, endosomes, or both in order to be stimulated for the fusion (Davidson et al., 2020).

1.2.5.2 Host Response to SARS-CoV-2

The innate immunologic reaction is a significant obstacle to viral infection. It recognizes and reacts to viruses using different pattern recognition receptors (PRRs) (Iwasaki and Pillai, 2014). The severity of the host's immune and inflammatory responses is determined by the kind of virus, amount of virus in the blood, and the host's immunity condition and age. To remove the invading viruses, the host innate immune cells are encouraged to generate cytokines and chemokines effective against viruses (Asehnoune et al., 2016).

Pathogenic RNAs are released after CoVs enter human host cells, where they start to act as PAMPs, which can be identified by PRRs such as retinoic acid-inducible type I receptors and toll-like receptors (Takeuchi and Akira, 2010). Stimulation of the mentioned cell receptors causes transportation of nuclear transcription factors to the cell nucleus via the cytosol, gene transcription and expression of acute inflammatory response proteins, cytokines and chemokines, which can cause inflammation (Huang et al., 2020).

1.2.5.2.1 Cytokine response

Physical and chemical barriers mediated by antimicrobial peptides and free radicals, soluble agents, and innate immune cells comprise the innate immune system (Williams, 2011; Koenderman et al., 2014).

Innate immune system has several functions. Firstly, the primary objective is to avoid pathogen entry via physical and chemical barriers. Secondly, infection spread via the complement system and other humoral factors should be prevented. Thirdly, it is important to eliminate pathogens via phagocytosis and cytotoxicity mechanisms. Last but not the least, the adaptive immune system should initiate via the production of some cytokines (Williams, 2011; Tosi, 2005).

Different cell types generate and release cytokines, which form a molecular network and exert paracrine and endocrine effects via receptors representing the target cell. These molecules are generated as a result detection of particular pathogen structures via their receptors (Prieto and Cotman, 2017).

At first, cytokines were classified according to the activity they accomplished, which included regulation of the immune system and acting as an effector on cells. Those impacts were observed at the local level, as well as at the tissue or system level. Cytokines play a significant role in regulating the organism's homeostasis. However, this homeostasis can be interrupted if synthesis or signaling pathway of cytokines in the cell is disrupted, which can lead to pathology (Gadina et al., 2017). Cytokines are divided into five categories starting from type I cytokines till type V cytokines. The IL-17 family may enhance systemic levels during a pathological condition and influence by attaching to their receptors, where signal translation occurs, contributing to gene expression and, ultimately, regulating the target cell role (Kany et al., 2019). The cell's cytokine pattern is mainly determined by the characteristics of the antigenic stimulus and stimulated cell type. Cytokines impair white blood cells' ability to react to a microbial stimulus by enhancing the discharge of free radicals and nitrogen species, and cytokine release modulating arachidonic acid derivatives. Moreover, cytokines can induce cell death by binding to death domain-containing receptors, such as TNF receptor 1 (R1) (Kakar, 2017).

The inflammation intensity can be associated with cytokine storm (Soy et al., 2020). As it is a main reason of complications and deaths in COVID-19 disease, treatment is suggested to fight the cytokine storm (Quirch et al., 2020).

A cytokine storm is a result of an acute hyper-inflammatory reaction that leads to medical problems in many diseases. In COVID-19, it is linked to a poor outcome of disease and greater death rate. The cytokine storm is deliberately resulted when cytokines such as IL-1, IL-2, IL-6, TNF-alpha, and interferon-gamma, which are major elements of immune responses, are produced. The central nervous system is often affected by cytokine storm and its cells, which are also engaged in the exemption of cytokines, causing inflammation, that also have an impact on the nervous system (Bhaskar et al., 2020).

1.2.5.2.2 Non-cytokine mediators

Chemokines are small molecules that primarily aim to promote the movement of leukocytes. They are also known as chemotactic cytokines and belong to a family of peptides that are structurally similar to cytokines, and produced following certain signals, such as proinflammatory cytokines, and are engaged in recruiting monocytes,

lymphocytes, neutrophils (Proudfoot et al., 2015). According to the systematic nomenclature, those molecules are characterized by the appearance of four preserved cysteine residues and are categorized into four groups depending on the amount of amino acids between the first two cysteines (Deshmane et al., 2009).

1.2.5.2.3 Hormones

A disrupted and excessive innate immune response is related to severe COVID-19 outcomes. Patients die not only as a result of viral replication, but also due to the cytokine storm caused by the infection. I immune cells invade organ tissues in order to keep the body safe, causing monocytes and macrophages to increase the concentration of the primary pro-inflammatory cytokine, IL6, as well as IL1beta and TNF-alpha (Shimabukuro-Vornhagen et al., 2018).

Jarvis et al. (2020) proposed that estrogen and its receptors are important in minimizing cytokine storm. I is also suggested that estrogen receptors can be found on all immune cells, and estrogen treatment diminishes the innate immune response, lowers pro-inflammatory cytokines, and increases anti-inflammatory cytokines.

Another immunomodulating sex hormone that can be used in the fight against COVID-19 is progesterone. When progesterone is present, CD4+ T-helper cells shift from Th-1 to Th-2 in the development of anti-inflammatory cytokines, particularly IL4 and IL10. All of this is related to the shift in innate and adaptive immune responses seen during pregnancy, which aims at minimizing pro-inflammatory responses to prevent fetal rejection and promoting passive transfer of maternal antibodies (Mauvais-Jarvis et al., 2020).

1.2.5.2.4 Cellular immune response

There are no particular drugs against viruses, so the body's immune reaction is a significant aspect influencing disease development and prognosis. As a result, a greater knowledge of the cellular immune reaction process from mild illness to lethal disease is required for the development of diagnostic markers and plan of action for COVID-19 therapy.

The progression of the antiviral immunologic reaction in SARS-CoV-2 also include a structured cellular and molecular cascade, which modulate virus removal and immune destruction. Several innate immune recognition mechanisms protect from viruses

throughout infection process (Stetson and Medzhitov, 2006). After a few hours, the innate immune system initiates a rapid antiviral reaction via cytokines and chemokines to prevent virus replication. Afterwards, the adaptive immune system starts to operate. T lymphocytes are important in virus discharge after infection, they instantly diffuse and kill infected cells to completely remove viruses, and they release cytokines to boost the immune reaction of T lymphocytes and other immunocompromised cells. The body then inhibits the activity of innate immunity in order to protect the host from damage (Kim et al., 2007). Innate immune cells and adaptive regulatory cell types help to resolve inflammation after pathogens are removed (Dorward et al., 2020).

1.2.6 Identification of SARS-CoV-2 and Therapeutics

The initial course of action in COVID-19 management is fast and precise diagnosis of SARS-CoV-2, which is accomplished by real-time reverse transcription–polymerase chain reaction (RT–PCR), detecting nucleic acids of virus in nasopharyngeal fluids (Liu et al., 2020). Testing is used to avoid infectious transmission among individuals and groupings, which may involve infected people without symptoms, whose viral discharge may unintentionally spread the infection to the older adults and people with chronic conditions (Wang et al., 2020). Accurate viral detection is the first step toward comprising the COVID-19 pandemic (Loeffelholz et al., 2020).

SARS-CoV-2 pandemic diagnostic tests rely on discovering antibody, protein, and nucleic acid. However, detection of nucleic acid by RT–PCR considered as the gold standard (Kevadiya et al., 2021). In comparison to the currently available serological tests, nucleic acid tests have increased efficiency for virus detection. SARS-CoV-2 detection is dependent on RT–PCR as an accurate method. Although the test is accurate, the results still have not aided viral infection containment (Okba et al., 2020).

Chest CT scans, in addition to laboratory tests, can help in the diagnosis of COVID-19 in individuals who are suspected of infection.

Rapid antigen test detects specific proteins in the virus. A long nasal swab is used for this test to get a fluid sample, and its advantage is that the result can be ready in a few minutes. However, it is not as accurate as RT-PCR test, meaning that the chance of false-negative results are higher (Winichakoon, 2020).

1.2.6.1 Clinical Features and Risk Factors

COVID-19 symptoms differ in severity, varying from minor to severe illness (CDC, 2021). Muscle soreness, loss of smell and taste, cough, diarrhea, and fever are all considered common symptoms of COVID-19 (Grant et al., 2020). People suffering from the same infection may experience various symptoms, which may keep on evolving. There are three groupings of symptoms: one respiratory symptom group involving cough, fever, and shortness of breath; another one is musculoskeletal symptom group including muscle soreness; and the third one is digestive symptom group involving vomiting and diarrhea. COVID-19 is associated with loss of taste and loss of smell in people who have never had ear, nose, or throat problems.

Not less than one-third of those infected with the virus show no symptoms at all. These asymptomatic carriers are less likely to be tested and thus contribute to the disease's spread. Other infected individuals experience symptoms afterwards and may transmit the virus to others (Gao et al., 2020).

The majority of patients get better after the disease's acute phase. However, some people keep on suffering from a variety of complications, even months after recovery. In this condition long-term organ damage has been reported. Longitudinal researches are being conducted to continue discovering the disease's long-term impact. COVID-19 can affect anyone, but some people might have severe symptoms due to other factors called co-morbidity. These are commonly called “risk factors” for example; advanced age, obesity, cancer and other medical conditions (Struyf et al., 2021).

1.3 Human Genetic Variations

The genetic variation in humans create diversity within and between populations. There can be multiple variants (alleles) in one locus called polymorphism.

A polymorphism is a genetic variant occurring in not less than 1% of the population. These mutations can generally occur in non-coding regions called introns. They do not have any impact on protein translation, however they might play role in gene expression when they are located in the regulatory region of the genome (Valdivielso et al., 2006).

1.3.1 Host Polymorphisms and COVID-19

Genetic factors can affect the emergence and spread of infectious diseases, according to evidence (Hill, 2012). Several genetic variants, primarily single nucleotide

polymorphisms (SNPs), are linked to sensitivity to viral respiratory infections. Till recently, the particular role of genetic make-up in COVID-19 disease has been understudied, despite the fact that proinflammatory-related pathways have been implicated (Ye et al., 2020).

Acknowledging the host genetic factors engaged in SARS-CoV might lead to the development of novel treatment methods to prevent control this disease using an individualized medical perspective (Ramos-Lopez et al., 2017).

1.4 The Role of Vitamin D in the Human body

Vitamin D is a fat-soluble vitamin which plays a vital role in bones strengthening, muscles, and general well-being of an individual. Two main forms of vitamin D are D2, also known as ergocalciferol, and D3, also called cholecalciferol. The primary biological function of vitamin D is to keep average calcium and phosphorus blood levels (Krawiec and Dominiak, 2018). Vitamin D rich diet may also reduce an individual's danger of developing osteoporosis, hypertension, cancer, and numerous autoimmune diseases. Studies report that it triggers the body's immune cells to generate antibodies; for this reason, vitamin D supports a general increase in the strength of the immune system. To date, greater than 500 research help the function of vitamin D in the health of the immune system. Results from some research report that vitamin D assists withinside the renovation of joint and muscle comfort, in addition to the renovation of a healthful mood, and helps breast, colon, and prostate health (Krawiec and Dominiak, 2018). Vitamin D is known to aid the body's absorption and retention of calcium and phosphorus, both of which are crucial for bone formation. Furthermore, studies reported that vitamin D can inhibit cancer cell growth, aid in infection control, and reduce inflammation. Vitamin D is related to a number of diseases, such as cardiovascular disease, diabetes, cancer, autoimmune disease, infectious disease, and others (Charoenngam, Holick, 2020).

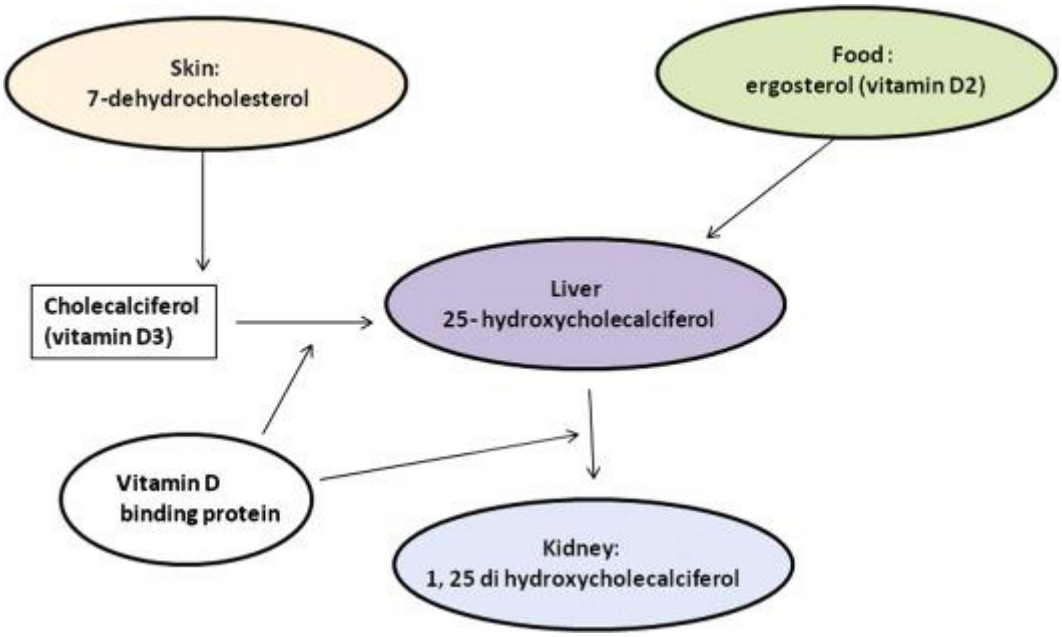

Figure 3. *Vitamin D metabolism.* (Adapted from Galliera and Romanelli, 2020).

Vitamin D also has anti-inflammatory functions especially in viral infections, and can act as an immune modulator (Charoenngam, Holick, 2020).

Although many people normally achieve good enough quantities of vitamin D through daylight exposure and nutritional sources, a few people can be at a more threat for deficiencies, mainly at some point of the winter season (Holick, 2004).

Patients who can be at excessive danger for vitamin D deficiencies consist of the elderly, overweight individuals, only breastfed infants, and people who have constrained solar exposure. In addition, people who have fats malabsorption syndromes, like cystic fibrosis or inflammatory bowel ailment, are vulnerable to have vitamin D deficiency. The use of specific medicines consisting of phenytoin and carbamazepine might also additionally grow the metabolism of vitamin D. These medications increase vitamin D metabolism in the liver to inactive compounds while lowering calcium absorption (Norman, 2008).

1.4.1 Vitamin D and Disease Associations

Vitamin D is essential for calcium homeostasis and metabolism. It was discovered while looking for a missing nutritional substance in children with rickets, an infantile form of osteomalacia (Wolf, 2004). Vitamin D supplements are administered to patients to cure

or reduce the risk of developing osteomalacia and rickets. Other medical impacts of vitamin D supplements in people taking vitamin D are contradictory. The influence of vitamin D supplementation on deaths is unclear, with one meta-analysis finding a small drop in death rates in the older adults and another concluding that there is no clear objectives for recommending supplementation and that no additional research of this nature is required (Bjelakovic et al., 2014).

Multiple studies have found that vitamin D has a wide range of effects on the biological processes modulating calcium and phosphorus metabolism, effects on cell growth, differentiation, cell death, immunomodulation and genome stability. Recent research has also identified a correlation between vitamin D and cardiovascular disease, diabetes, cancer, autoimmune diseases, infectious diseases, and others (Wang et al., 2017).

Vitamin D deficiency (VDD) is related to a broad range of diseases, involving skeletal issues, immune system deficiencies, depression, and cardiovascular disease. Limited sun exposure, old age, and sedentary lifestyle are known as factors linked to a greater risk of VDD along with VDR gene mutations (Kandemis et al., 2021).
Obviously there is many unknown information about COVID-19 disease, since it has newly emerged. However there are some studies revealing association between COVID-19 and vitamin D. Vitamin D level has been found to be low in COVID-19 patients in many studies (Carpagnano et al., 2021), and on the contrary increased vitamin D levels have been suggested to help in avoiding COVID-19 (Weir et al., 2020).
In recent years, it has been discovered that a large proportion of the Northern Cypriot population is vitamin D deficient. Considering the fact that Cyprus receives constant sunlight throughout the year and that the Mediterranean diet is rich in vitamin D containing foods, it is reasonable to conclude that factors other than exposure to sun and diet are influencing vitamin D levels. Kandemis et al., (2021) investigated relationships between vitamin D levels and common VDR polymorphisms in 131 Turkish Cypriots, and larger numbers of young patients suffering from the consequences of low vitamin D levels were identified due to a significant change in the population's lifestyle.

A new study from Trinity College and the University of Edinburgh looked at the relationship between vitamin D and COVID-19. Nearly 500,000 people in the UK participated in this research, and each participant's exposure to UVB radiation prior to COVID-19 infection was evaluated individually. The study's findings indicate that

ultraviolet B (UVB) radiation which is essential for vitamin D production within the skin, was very protective against severe disease and death at an individual's accommodation in the weeks prior to COVID-19 infection. This suggests that vitamin D may also protect against excessive COVID-19 disease and death (Martineau et al., 2017).

1.4.2 Vitamin D Receptor Gene Polymorphisms and COVID-19

Thus, vitamin D has a lot of functions maintaining a good health of human, but its biologically active form can only start acting after attaching to its concrete vitamin D receptor encoded by VDR gene. The VDR gene codes for a vitamin D receptor (VDR), a protein enabling the body to react to vitamin D. (Martineau et al., 2017).

COVID-19 prevalence and mortality rates may be affected by the modulatory effect of individuals' biologically available vitamin D levels, which is defined by genetic factors such as polymorphisms in the VDR gene (Abdollahzadeh et al., 2021).

The *VDR* gene is found on chromosome 12 and its most common polymorphisms are the *TaqI*-rs731236 T>C, *ApaI*-rs7975232 A>C, *BsmI*-rs1544410 G>A and *FokI*-rs10735810 C>T. These genetic variants have been related to a higher risk of chronic diseases like autoimmune diseases, type 2 diabetes, and cancer (Li et al., 2013 and Lee et al., 2011).

However, since several genetic association research findings come to contradictory conclusions, Usategui-Martn et al. (2022) conducted a study to assess the reaction to vitamin D supplementation based on the *FokI, TaqI, BsmI,* and *ApaI* polymorphisms in the VDR gene. The FF genotype of the *FokI* variant and the variant allele of the *TaqI* were linked to a greater reaction to vitamin D supplementation, according to the findings, whereas *BsmI a*nd *ApaI* were not.

Peralta et al. (2021) discovered a relationship between the likelihood of acquiring COVID-19 and the genotypes of the *TaqI* in Cuban patients. Polymorphisms in the gene coding for the formation of VDR have been linked to D hypovitaminosis (Santos et al., 2012). Exon nine codon 352 is polymorphic, and it can be ATC or ATT. *TaqI* polymorphism has been characterized as a factor associated with infectious disease resistance (Bellamy et al., 2021); thus, it could be linked to COVID-19.

The F allele of the *FokI* polymorphism was linked to the production of a more active protein (Arai et al., 1997). VDR activity may be correlated with improved reaction to supplements of vitamin D.

To summarize, the *TaqI* and *FokI* polymorphisms may contribute to modulating the response to vitamin D supplementation because they are related to improved reaction to supplementation as well as a predisposition to chronic diseases, which is why we chose these polymorphisms for this study.

1.5 The Work in this Thesis

This study was conducted to investigate the allelic frequencies and genotyping distribution between *FokI* and *TaqI VDR* polymorphisms in COVID-19 patients infected by SARS-CoV-2 Delta variant to indicate the putative impact of *VDR* genetic variations on COVID-severity.

CHAPTER 2: MATERIALS AND METHODS

2.1 Materials

2.1.1 Suppliers

HiMedia Insta Q96™ Real Time (HiMedia, Mumbai, India), Tianlong GeneRotex96 Rotary Nucleic Acid Extraction System (TIANLONG, Shaanxi, China), Bio-Rad MyCycler™ Thermal Cycler System (Bio-Rad, California, USA), Applied Biosystems Veriti Thermal Cycler (Applied Biosystems, Waltham, Massachusetts, USA), DNR Bio Imaging Systems MınıBIS Pro (DNR Bio Imaging Systems, Neve Yamin, Israel), Cleaver Scientific gel electrophoresis instrument and power supply (Cleaver Scientific, Rugby, UK).

2.1.2 Sample Collection

A total number of 200 individuals who admitted to Near East University Hospital COVID-19 PCR Diagnosis Laboratory for routine SARS-CoV-2 RT-PCR test was used in this study. The control group consisted of individuals who were SARS-CoV-2 RT-qPCR negative. On the other hand, the case group consisted of patients who were SARS-CoV-2 RT-qPCR positive, infected with SARS-CoV-2 Delta variant.

2.1.3 Chemical Reagents

2.1.3.1 Molecular Weight Markers

GelPilot 50 bp DNA ladder (QIAGEN, Hilden, Germany) catalogue no. 239025) and GeneRuler 50 bp DNA ladder (Thermo Scientific™, Pittsburg, USA, catalogue no. SM0371) were used as a molecular weight marker.

2.1.3.2 Oligonucleotide Primers

The primers pairs which were designed for FokI (SNP: rs10735810 C>T) and TaqI (SNP: rs731236 T>C), polymorphisms were synthesized from Oligomer company (Table 1).

Primers	**Sequence**
FokI (rs10735810) Forward	5’-AGC TGG CCC TGG CAC TGA CTC TGC TCT-3’

Reverse	5’-ATG GAA ACA CCT TGC TTC TTC TCC CTC-3’
TaqI (rs731236)	
Forward	5’-CAG AGC ATC GAC AGG GAG CAA-3’
Reverse	5’-CAC TTC GAG CAC AAG GGG CGT TAGC-3’

Table 1: The sequence of *FokI* and *TaqI* forward and reverse primers for multiplex PCR

2.1.3.3 Enzymes

The *TaqI* enzyme recognizing T^CGA sites (Thermo Fisher Scientific, Waltham, Massachusetts, USA, catalogue no. K1991) and the *FokI* enzyme recognizing GGATG (9/13) ^ site (Thermo Fisher Scientific, catalogue no. FD2144) were used to digest of PCR products following DNA amplification.

2.1.3.4 Standard Solutions

A 10x stock of Tris-Borate/ EDTA (TBE) which is an electrophoresis buffer (108 gr Tris and 55 gr Boric acid and 40 ml 0.5 M EDTA pH 8.0) was dissolved in 1 ml water. 10X TBE buffer was diluted to 1X (100 ml from 10X TBE + 900 ml of Distilled water).

2X PCR Master Mix (Thermo Fisher Scientific, catalogue no. K0172), which is the solution includes Taq DNA Polymerase, dNTPs, and all the components required for the PCR, was used for amplification of DNA.

2.1.3.5 Other Chemical Agents

Agarose powder (Sigma-Aldrich, catalogue no. 11388983001) was used to gel electrophoresis showing PCR-RFLP products of samples. Ethidium bromide (EtBr) (Sigma-Aldrich, catalogue no. E1385) which is a fluorescent dye was used for making agarose gel visible.

2.1.3.6 Computers

GelCapture Software packages were used to view and analyze the gel images and store the imaging data. Statistical analysis of data has been done using Statistical Package for the Social Sciences (SPSS).

2.2 Methods

2.2.1 The Detection of SARS-CoV-2 from VNAT Solution by Real-Time Polymerase Chain Reaction

SARS-CoV-2 detection was obtained with UNIPLEX SARS-CoV-2 RT-qPCR diagnosis kit (Near East University, Nicosia, Cyprus) from VNAT Solution by 2X PCR Master Mix (Thermo Fisher Scientific, Waltham, Massachusetts, USA, catalogue no. K0172), Real-Time Polymerase Chain Reaction according to manufactory instructions.

2.2.2 Nucleic Acid Extraction

The viral RNA from the VNAT solution was extracted by viral DNA RNA extraction kit (Tianlong, China, catalogue no. T014H).

Human DNA was isolated from VNAT solutions which diagnosis were done for COVID-19 using DNA extraction kit (Tianlong, China, catalogue no. T191H).

2.2.3 SARS-CoV-2 Mutation Typing and variant identifying using TaqMan Allele Specific Primers (ASPs)

The SARS-CoV-2 positive patients were included to variant identifying analysis by using TaqMan allele specific primers for mutations of SARS-CoV-2 Delta variant. The variant identifying analysis was done by use of Multiplex SARS-CoV-2 VOC RT-qPCR identifying kit (Near East University, Nicosia, Cyprus) according to manufactory instructions.

2.2.4 Amplifying the *VDR* Gene Target Regions by PCR

PCR was used to amplify of target mutated regions of the *VDR* gene from isolated genomic DNA. The PCR step was done for all 200 samples using for each *FokI* and *TaqI* restriction enzymes, 400 in total. PCR reaction was performed in a total volume of 20 µl, containing 15 µl of PCR mixture and 5 µl of DNA and the PCR reaction mixture composition was presented in Table 2. Thermal cycling conditions to amplify of target mutated regions of the *VDR* gene including the temperature regimes and the durations of each step were presented in Table 3.

Component	1X
PCR Master mix	10 µl
FokI/TaqI Forward primer (10µM)	0,75 µl
FokI/TaqI Reverse primer (10µM)	0,75 µl
PCR grade Distilled water	3,5 µl

Table 2. Master Mixture composition for FokI and TaqI PCR

Stage	Temperature	Time	Cycles
Initial denaturation	94 ℃	5 minute	1 cycle
Denaturation	94 ℃	30 seconds	35 cycles
Annealing	63 ℃	30 seconds	
Extension	72 ℃	1 minute	
Termination	72 ℃	7 minutes	1 cycle

Table 3. Thermal cycling conditions for *FokI* and *TaqI* PCR.

Following the PCR, the gel electrophoresis was done for the yield amplified products. A 2% concentrated agarose gel was prepared (4 grams of agarose were combined with 200 ml of TBE buffer). After dissolving agarose, the compound was cooled down and 10µL EtBr was added. 10 µl of each PCR product with 2 µl of loading dye and 5µl 50bp ladder were loaded into the agarose gel. The Bio-Rad electrophoresis device was used to run the samples at 90-110 volts. The entire procedure lasted approximately 90 minutes. The bands (*TaqI* 500bp, *FokI* 265bp) were visualized using an ultraviolent trans-illuminator.

2.2.5 Genotyping the *VDR* Gene Polymorphisms by Restriction Fragment Length Polymorphisms (RFLPs)

After visualization of the bands RFLP analysis was done for *Taq1* and *Fok1* polymorphisms by the use of mutation specific restriction enzymes. RFLP analysis was carried out in a total volume of 20 µl, containing 10 µl of RFLP mixture and 10 µl of amplified product from PCR. The exact RFLP mixture composition was presented in Table 4. The samples stayed at 37℃ for 30 minutes for Fok1 and at 65℃ for 30 minutes for Taq1 polymorphisms for the RFLP analysis.

Component	1X

Distilled water	4 µl
Digest Green Buffer	5 µl
FokI/Taq1 restriction enzyme	1 µl
PCR product	10 µl

Table 4. RFLP mixture for FokI and Taq1 polymorphisms

A 3% concentrated agarose gel, containing ethidium bromide was prepared (6 grams of agarose were combined with 200 ml of TBE buffer) to separate the RFLP products. The 10 µl of RFLP products with 2 µl of loading dye and 5 ml 50 bp ladder were loaded into agarose gel. The Bio-Rad electrophoresis device was used to run the samples at 90-110 volts. The entire procedure lasted approximately 90 minute. The bands were visualized using an ultraviolent trans-illuminator.

In the presence of mutation, for *Fok1* restriction endonuclease recognizes the sequence and cut the PCR product into 196 and 69 bp fragments and the uncut 265 bp fragment indicates the Wild type genotype (Figure 1). In case of *TaqI* polymorphism, the Taq1 restriction endonuclease recognizes the sequence and digest yielded PCR product into 295 and 205 bp fragments and the uncut 500 bp fragment indicates the Wild type genotype, the electropherogram of *Taq1* polymorphism analysis is presented in Figure 2.

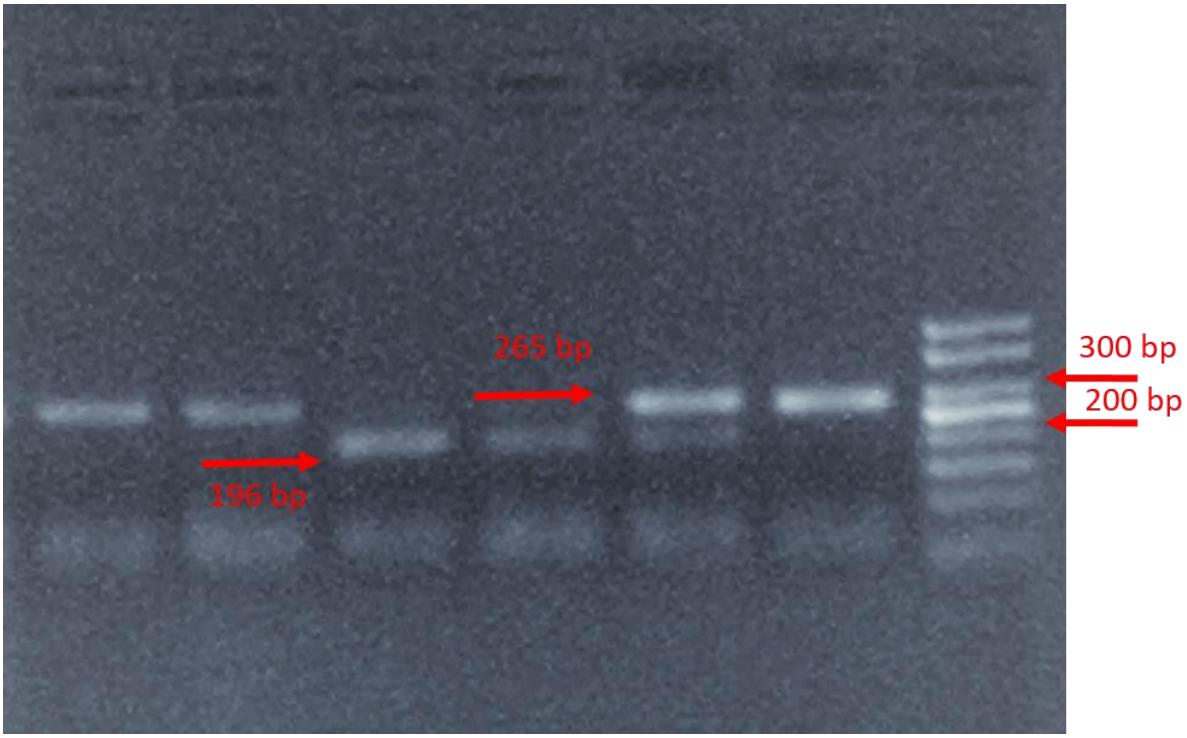

Figure 4: The electropherogram of *FokI* rs10735810 polymorphism PCR-RFLP analysis. Line 1, 2 and 6: Wild type 265 bp fragment, Line 3 and 4: Homozygote 196, 69 bp, Line 5: Heterozygote 265, 196 and 69 bp and Line 7: GelPilot 50 bp DNA ladder (QIAGEN, catalogue no. 239025). The DNA ladders were used for approximate sizing of DNA fragments

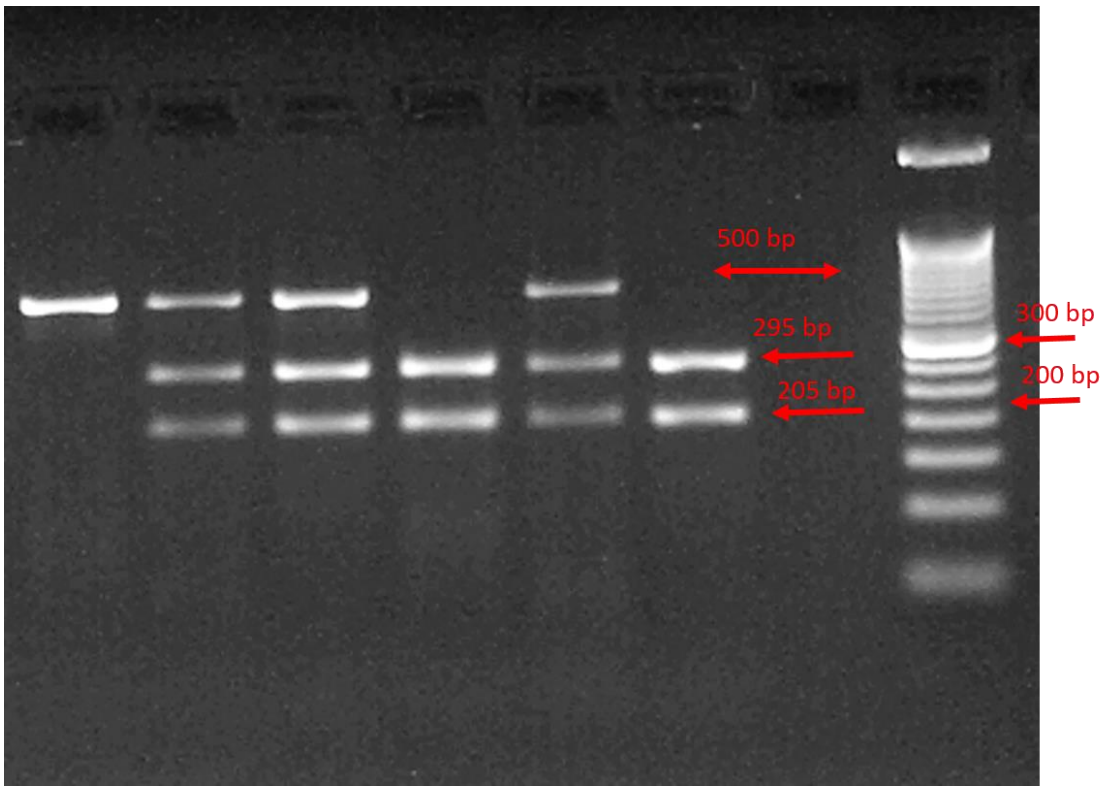

Figure 5: The electropherogram of *TaqI* rs731236 polymorphism PCR-RFLP analysis

Line 1: Wild type 500 bp fragment, Line 2, 3 and 5: Heterozygote 500, 295 and 205 bp, Line 4 and 6: Homozygote 295 and 205 bp and Line 7: GeneRuler 50 bp DNA ladder (Thermo Scientific™, catalogue no. SM0371). The DNA ladders were used for approximate sizing of DNA fragments

2.2.6 Statistical Analysis

SPSS software (Statistical Package for the Social Sciences, SPSS Inc, Chicago, IL, USA, and version 25) was used to perform statistical analysis of the data. Descriptive data and genotype data of the study group were demonstrated as mean ± standard deviation (SD)

or number and frequency, where applicable. The Student's t-test and Mann–Whitney U test were used to make comparisons between normal and non-normally distributed quantitative variables. Chi square (x2) was used to compare the genotype and allelic frequency distributions of *FokI* and *TaqI* polymorphisms between study groups. When the conditions for using the chi-square test were not accomplished, Pearson's chi-square test or Fisher's exact test were used to confirm the association of categorical variables between study groups. Hardy-Weinberg equilibrium (HWE) was evaluated by Fischer's exact test. The OR and 95% CI were calculated using binary logistic regression analysis with codominant, dominant, additive, and recessive inheritance models. Akaike's information criterion (ACI) was used to select the inheritance model that is the most suitable for the data. To assess group differences, the data were log transformed to satisfy ANOVA criteria before being subjected to one-way ANOVA with Tukey's posthoc analysis. *TaqI* and *FokI* polymorphisms were assessed for their relative risks in COVID-19 Delta variant patients by estimating odds ratios (ORs) and 95% confidence intervals (CIs), which were considered separate outcomes. In all cases differences were found important at $p < 0.05$.

CHAPTER 3: RESULTS

3.1 Introduction

At the onset of the pandemic, researchers noted overlaps between populations with greater chance of developing severe form of COVID-19 and those who are thought to be vitamin D deficient, particularly among obese people, the elderly, and those with darker skin, because they may not make enough vitamin D from sunlight. In addition, patients with COVID-19 disease may have vitamin D deficiency due to spending most of the time indoors because of the COVID-19 pandemic (Ricci et al., 2021).

Vitamin D supplementation decreases the chances of respiratory infection, controls cytokine production, and may reduce the risk of other viruses like influenza. In people with COVID-19, a respiratory infection can set off cytokine storms, a vicious cycle in which inflammatory cells harm organs across the whole body and increase death rates. Increased numbers of COVID-19 deaths in the elderly and people with chronic illnesses imply that a compromised immune system plays a significant role in poor results.

Adequate vitamin D levels may provide modest protection to vulnerable populations (D'Avolio et al., 2020).

There are several observational studies showing that low vitamin D levels are linked to a higher chances of becoming seriously ill with COVID-19 (Martineau and Forouhi, 2020).

In a cohort study by Meltzer et al., 2020 with a total number of 489 patients the chances of testing positive for COVID-19 were 1.77 times higher for patients with vitamin D deficiency (VDD) in contrast to those with adequate amount (Meltzer et al., 2020).

Another research found that 82.2% of 216 COVID-19 hospitalized patients in Spain have vitamin D deficiency. Although VDD in patients hospitalized for COVID-19 had a high frequency, no link was found between plasma concentrations of 25OHD and the intensity of SARS-CoV-2 (Hernández et al., 2021).

In this study, we investigated the allelic frequencies and genotypic distribution between vitamin D receptor (VDR) gene polymorphisms, such as *FokI* rs10735810 and *TaqI* rs731236 in COVID-19 patients infected by the SARS-CoV-2 Delta variant and compared them with those who were tested negative for SARS-CoV-2 as a control group.

3.2 General characteristics of the study group

The study group includes 100 COVID-19 patients infected by the SARS-CoV-2 Delta variant and 100 non-infected patients as a control group. The mean age of COVID-19 Delta variant patient's ± SD was 35.64±15.84. The gender distribution of the patients' group is 38% female and 62% male.

3.3 Allelic and genotypic distribution frequency of VDR *FokI* and *TaqI* polymorphisms in COVID-19 patients.

The genotypic and allelic frequency distributions of *FokI* rs10735810 and *TaqI* rs731236 SNPs in patients infected by the SARS-CoV-2 Delta variant and control group are presented in Table 5. There was found an essential contrast in genotype frequencies of rs10735810 and rs731236 variants between SARS-CoV-2 Delta variant infected patients and control group ($p<0.05$) (Table 5).

Furthermore, the risk alleles, rs10735810 allele and rs731236 allele, were found to be statistically significant (OR=2.00, 95% CI=1.30-3.06, OR=1.92, 95% CI=1.27-2.91, respectively) in SARS-CoV-2 Delta variant infected patients compared to controls.

SNP	Genotypic Frequencies n (%)		P-Value	Allelic Frequencies			X^2	OR/CI(95%)	P-Value
Genotype	Cases (n=100)	Control (n=100)		Allele	Cases (n=100)	Control (n=100)			
FokI-rs10735810									
TT	40(40)	59(59)							
TC	38(38)	32(32)	*0.008*	T/C	0.60/0.40	0.75/0.25	10.26	2.00/1.30-3.06	*0.001*
CC	22(22)	9(9)							
TaqI-rs731236									
TT	33(33)	53(53)							
TC	46(46)	36(36)	*0.011*	T/C	0.56/0.44	0.71/0.29	9.71	1.92/1.27-2.91	*0.001*
CC	21(21)	11(11)							

Table 5: The genotypic and allelic frequency distributions of Fok1 and Taq1 SNPs in the study group

The study group includes 100 COVID-19 patients infected by the SARS-CoV-2 Delta variant and 100 non-infected patients as a control group. OR: Odds Ratio, CI: Confidence Interval * Chi-square and HWE tests were used to draw a comparison between the genotypic and allelic frequency distributions of polymorphisms. Differences were found to be important at $p< 0.05$ in all cases.

3.4. Analysis of rs10735810 and rs731236 SNPs based on the four genetic inheritance models

The statistical power to recognize disease sensitivity loci in genetic association studies was reliant on the genetic models tested. As a result, the genotype frequencies were examined utilizing four genetic models: additive, co-dominant, dominant, and recessive. A strong relationship between rs10735810 and a greater risk of SARS-CoV-2 Delta variant was observed in all four models, co-dominant genotype (TT) vs (CC) (OR=3.21, 95% CI=1.35-7.92, p<0.006); co-dominant genotype (CC) vs (TT) (OR=0.30, 95% CI=0.12-0.73, p<0.006); dominant (OR=0.38, 95% CI=0.16-0.77, p<0.008); recessive (OR=0.9, 95% CI=0.12-1.67, p<0.024); additive (OR=0.89, 95% CI=0.11-1.66, p<0.022). Furthermore, strong positive correlations between rs731236 and risk of

SARS-CoV-2 Delta variant were also identified in co-dominant genotype (TT) vs (CC) (OR=3.06, 95% CI=1.31-7.16, p<0.008); co-dominant genotype (CC) vs (TT) (OR=0.32, 95% CI=0.14-0.76, p<0.008); dominant (OR=0.35, 95% CI=0.18-0.72, p<0.005); recessive (OR=0.65, 95% CI=0.83-1.38, p<0.052); additive (OR=0.64, 95% CI=0.08-1.37, p<0.01).

SNP	Model of Inheritance	OR (95 % CI)	p-Value	AIC[a]
FokI-rs10735810	**Co-dominant**			
	TT vs CC	3.21 (1.35-7.92)	***0.006***	-
	CC vs TT	0.30 (0.12-0.73)	***0.006***	-
	Dominant TT vs TC+CC	0.38 (0.16-0.77)	***0.008***	14.05
	Recessive CC vs TT+TC	0.9 (0.12-1.67)	***0.024***	13.29
	Additive TT vs TC vs CC	0.89 (0.11-1.66)	***0.022***	19.42
TaqI-rs731236	**Co-dominant**			
	TT vs CC	3.06 (1.31-7.16)	***0.008***	-
	CC vs TT	0.32 (0.14-0.76)	***0.008***	-
	Dominant TT vs TC+CC	0.35 (0.18-0.72)	***0.005***	14.01
	Recessive CC vs TT+TC	0.65 (0.83-1.38)	0.052	13.40
	Additive TT vs TC vs CC	0.64 (0.08-1.37)	***0.01***	19.53

Table 6: Analysis of SNPs based on the four genetic inheritance models.

The AIC: The inheritance model with the lowest AIC value is recommended.

OR: Odds ratio, CI: Confidence interval, AIC: Akaike's information criterion.

[d] p-value≤0.05 considered statistically significant. P-values in bold remained significant after the Bonferroni correction.

3.5 Association between SNPs and their distribution among the study group

Mutation analysis of studied polymorphisms of *VDR* showed that 40% of SARS-CoV-2 Delta variant infected patients (case group) were the wild type for *FokI* rs10735810, while 60% of patients carried at least one mutant allele (homozygous or heterozygous). The control group has consisted of 59% wild type, and 41% of individuals in the control group carried mutation at least one allele. The differences in the distribution of the *FokI* rs10735810 polymorphism between the two groups were statistically significant (OR=0.46, 95% CI=0.26-0.81, p<0.005) (Figure 6).

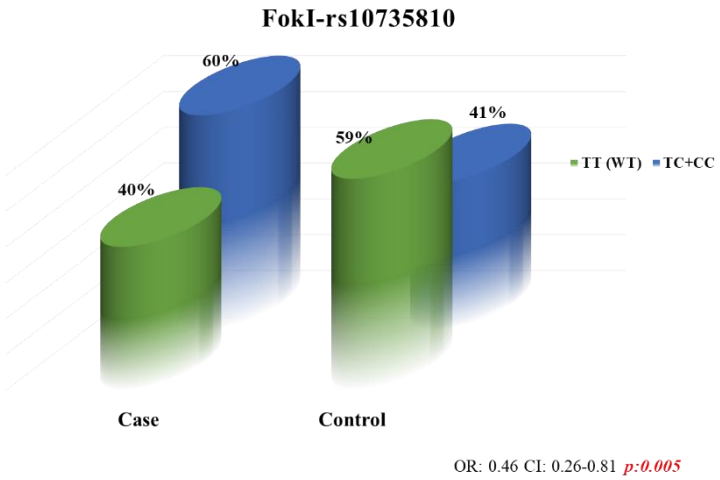

Figure 6: *FokI* polymorphism genotype distribution *FokI* TT (Wild Type), *FokI* TC (Heterozygote), *FokI* CC (Homozygote) compared with a control group

Furthermore, the same analysis was done for *TaqI* rs731236 and the analysis showed that 33% of SARS-CoV-2 Delta variant infected patients (case group) had the wild type genotype, while 67% of the patients carried at least one mutant allele (homozygous or heterozygous). However, the wild type genotype was more prevalent in the control group (53%), and the frequency of the mutant genotypes (homozygous or heterozygous) was lower (47%) in comparison to the case group (OR=0.44, 95% CI=0.24-0.77, p<0.003) (Figure 7).

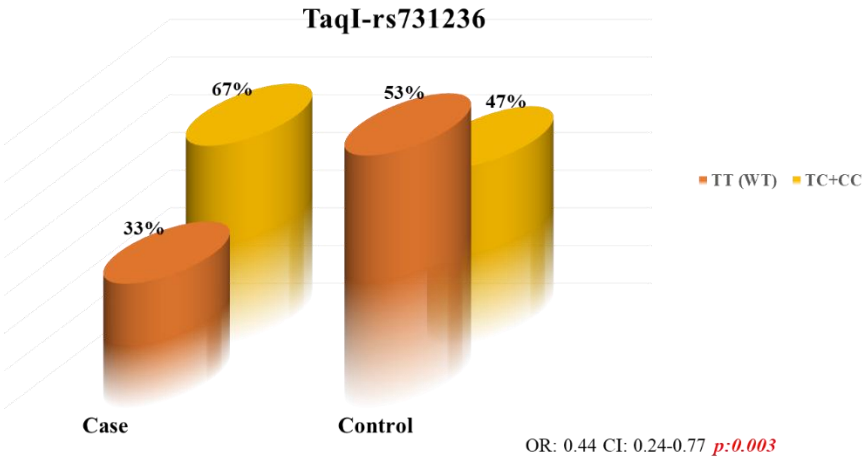

Figure 7: *TaqI* polymorphism genotype distribution *TaqI* TT (Wild Type), *TaqI* TC (Heterozygote), *TaqI* CC (Homozygote) compared with a control group.

We also investigated the distribution genotypes of *FokI* (rs10735810) and *TaqI* (rs731236) polymorphisms together among the study group. The genotypes were grouped as follows; Group1: *FokI* TT (Wild Type) + *TaqI* TT (Wild Type), Group2: *FokI* TC (Heterozygote) + *TaqI* TC (Heterozygote), Group3: *FokI* CC (Homozygote) + *TaqI* CC (Homozygote).

Thus, the highest number of patients with group 1 (wild-type genotype) was found in the control group, which is 72.7% compared with 39.4% in the case group (OR=0.25, 95% CI=0.10-0.57, p<0.001). On the contrary, there were not found any patients with group 3 (homozygous genotype) in the control group, while the number of patients with group 3 in the case group was 15.1%. Finally, most of the SARS-CoV-2 Delta variant infected patients had group 2 (heterozygous genotype), reaching 45.5%, while 27.7% of the control group patients had group 2 (Figure 8).

These results suggest that patients with *FokI* and *TaqI* gene polymorphisms may tend to be more vulnerable to getting infected with the SARS-CoV-2 Delta variant.

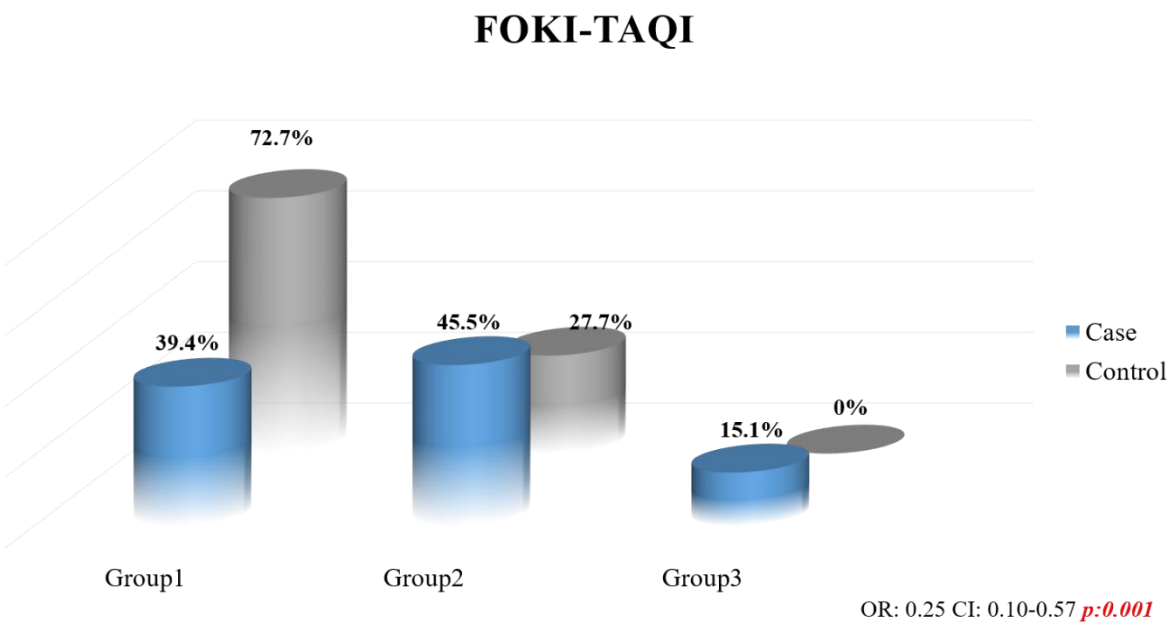

Figure 8: Comparison of *FokI* (rs10735810) and *TaqI* (rs731236) polymorphisms genotypes distributions among the study group. Group1: *FokI* TT (Wild Type) + *TaqI* TT (Wild Type), Group2: *FokI* TC (Heterozygote) + *TaqI* TC (Heterozygote), Group3: *FokI* CC (Homozygote) + *TaqI* CC (Homozygote)

3.6 Conclusion

A total number of 200 individuals who admitted to Near East University Hospital COVID-19 PCR Diagnosis Laboratory for routine SARS-CoV-2 RT-PCR test was used in this study to investigate the allelic frequencies and genotypic distribution between *VDR* gene *FokI* (rs10735810) and *TaqI* (rs731236) polymorphisms in COVID-19 patients, infected by SARS-CoV-2 Delta variant and compared them with those who were tested negative for SARS-CoV-2 as a control group. The results indicated that there is a strong relationship between *TaqI* and *FokI* VDR gene polymorphisms and COVID-19 causing the SARS-CoV-2 Delta variant, and patients with *FokI* and *TaqI* gene polymorphisms may tend to be more vulnerable to getting infected with the SARS-CoV-2 Delta variant.

CHAPTER 4: DISCUSSION

4.1 Introduction

COVID-19 is a rapidly evolving pandemic disease, which created a significant concern to the entire world as of 2019. The virus is highly transmissible, primarily through droplets and breath, as well as through direct contact. The disease's clinical features vary from entirely asymptomatic forms to severe symptoms like ARDS, commonly related to multi-organ failure (Hu et al., 2021).

The immune defenses of each patient are critical in limiting the chances of getting SARS-CoV-2. The pathophysiology of this viral disease is influenced by the patient's immunoinflammatory state. COVID-19 pathophysiology involves a number of signaling pathways and cellular components, including vitamin D2 (Raoult et al., 2020). Vitamin D is an essential immune system modulator, and has anti-infective and immunomodulatory effect by enhancing intercellular barriers, stimulating innate immunity, and regulating adaptive immunity (Aranow, 2011).

Deficiency and insufficiency of vitamin D are related to numerous diseases, such as metabolic diseases, autoimmune diseases, cardiovascular diseases, diabetes, infections, and have been extensively studied by scientists and physicians (Holick, 2017). Several studies, in particular, have looked into the link between VDD and the chances of getting respiratory infections.

To give an example, Mamani et al. discovered a link between pneumonia and low serum levels of 25-hydroxyvitamin D [25(OH) D]. The results indicated unfavorable consequences in ARDS patients with VDD (Mamani et al., 2017).

Furthermore, Martineau et al. came to the conclusion in a meta-analysis that vitamin D supplementation minimizes the chances of acute respiratory infections, particularly in individuals with the lowest levels of 25(OH) D (Martineau et al., 2019).

Another study of 76 patients in Spain hospitalized with COVID-19 discovered that treatment with big doses of vitamin D decreased the chances of admission into intensive care unit. However, broader investigations are needed to find a precise conclusion (Entrenas Castillo et al., 2020).

4.2 The association between *VDR* gene polymorphisms and COVID-19 diseases

With the discovery of the VDR in roughly all cells and the more recent discovery of thousands of VDR binding sites throughout the genome, importance of vitamin D and its effect on multiple biological processes has risen.

Numerous studies have found that vitamin D has a wide range of effects on the biological pathways that control calcium and phosphorus metabolism, as well as effects on cell growth, differentiation, cell death, immunomodulation (Wang et al., 2017).

Vitamin D promotes the body's absorption and maintenance of calcium and phosphorus, both of which are necessary for bone formation. Furthermore, studies show that vitamin D can inhibit cancer cell growth, aid in infection control, and reduce inflammation.

Thus, vitamin D serves many functions in maintaining human health, but its biologically active form can only begin acting after attaching to its specific vitamin D receptor, which is encrypted by the VDR gene.

The VDR gene contains over 200 SNPs and codes for VDR, which enables the body to react to vitamin D. (Martineau et al., 2017).

Several genetic variations have been discovered in the *VDR*. The most common polymorphisms of the *VDR* gene are the *Taq*I-rs731236, *Apa*I-rs7975232, *Bsm*I-rs1544410 and *Fok*I-rs10735810, which is why we focused on *TaqI* and *FokI* SNPs in the current study. *TaqI* is found on the 9th exon of the 3′ terminal, and *FokI*, found on exon 2, is located on the promoter of the 5′ terminal (Whitfield et al., 2001; Nejentsev et al., 2004; Uitterlinden et al., 2004).

COVID-19 prevalence and mortality rates may be affected by the modulatory effect of peoples' bioavailable vitamin D levels, which is influenced by genetic factors such as polymorphisms in the VDR gene (Abdollahzadeh et al., 2021).

A recent study looked at the genotypes of the *TaqI* polymorphism in the VDR gene and the medical features of COVID-19 in 104 Cuban patients. The researchers concluded that there is evidence of a link between the likelihood of having COVID-19 and the genotypes of the *TaqI* polymorphism of the VDR gene in the Cuban patients who were under investigation (Peralta et al., 2021).

Another study was designed to identify is a link between polymorphisms in vitamin D-related genes and vitamin D concentrations, as well as a relationship between those polymorphisms and COVID-19 intensity. Secondly, the prevalence of the genetic variants under evaluation was compared to the frequency seen in the European population. The genetic variants in vitamin D-related genes and vitamin D concentrations in hospitalized patients were evaluated, as well as the relationship between these data and COVID-19 severity. The percentage of patients with VDD in the group that died was 76%, thus being greater compared to the group with moderate symptoms, which is 59% and the group with severe symptoms, which is 64% (Freitas et al., 2021).

Both of these studies support the results of our study that a strong correlation between *TaqI* and *FokI* VDR gene polymorphisms and COVID-19 causing the SARS-CoV-2 virus is observed.

A polymorphism at locus rs2228570 was linked to virus infection in a meta-analysis by Laplana et al. The TT genotype and T allele have been linked to a greater risk of infection with enveloped viruses such as Respiratory Syncytial Virus. Several studies have found that taking vitamin D supplements can lower the possibility of serious infection and death from influenza and COVID-19. However, none of the studies have found that vitamin D receptor polymorphisms affect COVID-19 disease outcomes (Laplana et al., 2018).

In the current study, we intended to look into the allelic frequencies and genotypic distribution between VDR gene polymorphisms in patients infected by SARS-CoV-2 Delta variant and compared them with those who were tested negative for SARS-CoV-2 as a control group. The results indicated that there is a strong relationship between *TaqI* and *FokI* VDR gene polymorphisms and COVID-19 causing the SARS-CoV-2 Delta variant. These findings imply that the patients with *FokI* and *TaqI* gene polymorphisms may tend to be more sensitive to the SARS-CoV-2 Delta variant (Figure 3).

However, there is limited amount of research examining a linkage between *TaqI* and *FokI* VDR gene polymorphisms and the SARS-CoV-2 Delta variant, so more studies need to be conducted in this topic to make these findings more significant.

4.3 The impact of Vitamin D on SARS-CoV-2 infected patients causing COVID-19 disease

At the present time, prevention remains the best method to fight the COVID-19 pandemic because there is no proven therapeutic treatment. One of the methods of prevention is believed to be vitamin D supplementation (Ebadi and Montano-Loza, 2020).

This study is intended to look into the possibility of a link between vitamin D receptor gene polymorphisms and COVID-19, which causes the SARS-CoV-2 Delta variant.

Overall, vitamin D decreases the chance of microbial infection by regulating the innate and adaptive immune systems and having antiviral and anti-inflammatory properties. In addition, vitamin D has a fundamental impact on increasing the expression of ACE2, a key receptor involved in the SARS-CoV-2 pathogenesis. Vitamin D may also improve the expression of genes engaged in oxidation, regulate adaptive immunity, and promote cellular immunity by reducing the cytokine storm (Cristian et al., 2020).

Vitamin D3 production in the skin is not an enzymatic process. D3 (cholecalciferol) is synthesized from 7-dehydrocholesterol (7DHC) in a two-step process in which the B-ring is broken by ultraviolet light radiation from the sun, yielding preD3, which is then converted into D3. The rate of D3 formation is affected by both the intensity of UVB rays and the level of skin pigmentation (Holick et al., 1980). Melanin in the skin, clothing, and sunscreen blocks UVB rays from approaching 7DHC, reducing D3 production. Vitamin D can also be received through healthy diet, especially from eggs, fatty fish, and cheese.

Thus, vitamin D deficiency can develop as a result of insufficient sunlight exposure and vitamin D dietary intake, genetic defects of vitamin D receptors, and severe liver or kidney diseases (Armas et al., 2004).

Numerous experiments have been performed to study the impact of vitamin D, specifically in the scope of the COVID-19, considering the molecular pathways of vitamin D.

A recent study, for example, found that hospitalized COVID-19 patients who have sufficient vitamin D levels had a lower risk of complications and death (Maghbooli et al., 2020).

Another study, which included 77 older patients in France, found that taking vitamin D supplements on a regular basis for a year prior to a COVID-19 infection was related to less severe condition and a higher chance of survival than if no vitamin D was taken (Annweiler et al., 2020).

The findings of these studies show that there can be a direct association between VDD and COVID-19 intensity, making the current study's findings even more significant.

According to an article published in the Journal of the National Medical Association, VDD may be an important contributor to overall number of COVID-19 cases and deaths among US Black and Latino populations (Campbell et al., 2021). The findings of this study, however, cannot be adapted to everyone because the Black and Latino populations are vitamin D deficient due to the color of their skin.

Furthermore, a recent study based on information obtained from Israel's first two coronavirus waves prior to vaccines usage, discovered that there is a higher chance that vitamin D deficient people have severe case of COVID-19. Researchers looked at vitamin D levels in about 250 patients with a positive COVID-19 test and found that the chances of having a serious case of COVID-19 for vitamin D deficient patients was 14 times higher, and the mortality rate for patients with VDD was 25.6%, compared to 2.3% for those with sufficient levels. The researchers emphasized that vitamin supplements are not a replacement for vaccinations, but can help to strengthen the immune system (Dror et al., 2022).

4.4 Conclusion

COVID-19 disease is still an important public health concern worldwide. This pandemic has severely harmed healthcare systems' ability to continue providing quality health care. As healthcare systems around the world struggle to meet the rising demand for COVID-19 care, sustaining preventive and therapeutic services is critical, especially for the most vulnerable populations such as children, the elderly, people with chronic illnesses, minorities, and people with disabilities.

It is very crucial to maintain preventive measures to avoid getting infected or minimize the severity of the disease by maintaining a safe distance from others, eating a well-balanced diet in order to be healthier with a stronger immune system.

4.5 Final remarks and future work

In the current study, our main objective was to identify if there is a relationship between vitamin D receptor gene polymorphisms and COVID-19 causing the SARS-CoV-2 Delta variant.

To sum up, the results of this study displayed important differences in genotype frequencies of *FokI*-rs10735810 and *TaqI*-rs731236 variants between SARS-CoV-2 Delta variant infected patients and the control group. The findings also imply that the patients with *FokI* and *TaqI* gene polymorphisms may tend to be more sensitive and susceptible to getting infected with the SARS-CoV-2 Delta variant.

Limitations of this study includes lack of data about vitamin D levels of patients and involving only Delta variant of SARS-CoV-2 to investigate the allelic frequencies and genotypic distribution between VDR gene polymorphisms in COVID-19 patients. Further research with a broader range of data is required to confirm these findings.

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Appendix A: Ethical Approval Document

YAKIN DOĞU ÜNİVERSİTESİ
BİLİMSEL ARAŞTIRMALAR ETİK KURULU

ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi :27.01.2022
Toplantı No :2022/99
Proje No :1451

Yakın Doğu Üniversitesi Tıp Fakültesi öğretim üyelerinden Doç. Dr. Mahmut Çerkez Ergören'in sorumlu araştırmacısı olduğu, YDU/2022/99-1451 proje numaralı ve **"Association between vitamin D receptor gene polymorphisms and COVID-19 causing SARS-CoV-2 Delta variant"** başlıklı proje önerisi kurulumuzca değerlendirilmiş olup, etik olarak uygun bulunmuştur.

Prof. Dr. Şanda Çalı
Yakın Doğu Üniversitesi
Bilimsel Araştırmalar Etik Kurulu Başkanı

Kurul Üyesi	**Toplantıya Katılım**	**Karar**
	Katıldı(✓)/ Katılmadı(X)	*Onay(✓)/ Ret(X)*
Prof. Dr. Tamer Yılmaz	✓	✓
Prof. Dr. Şahan Saygı	✓	✓
Prof. Dr. Nurhan Bayraktar	✓	✓
Prof. Dr. Mehmet Özmenoğlu	*X*	*X*
Prof. Dr. İlker Etikan	✓	✓
Doç. Dr. Mehtap Tınazlı	✓	✓
Doç. Dr. Nilüfer Galip Çelik	✓	✓
Doç. Dr. Emil Mammadov	✓	✓
Doç. Dr. Ali Cenk Özay	*X*	*X*

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