AYLA TURGAY

SARS-CoV-2 VARIANTS IN NORTHERN CYPRUS

MASTER THESIS

2022

NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF MOLECULAR MEDICINE

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M.Sc. THESIS

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Approval

We certify that we have read the thesis submitted by Ayla Turgay titled "**The prevalence of SARS-CoV-2 variants in Northern Cyprus**" 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.

Ayla Turgay

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Acknowledgements

I would deeply like to declare my gratitude to my supervisor, Assoc. Prof Mahmut Ç. Ergören, who guided me throughout this project and gave me the opportunity to do this project.
I would also like to thank my friends, family and work colleagues who supported me and offered deep insight into the study.

Ayla Turgay

Abstract

The prevalence of SARS-CoV-2 variants in Northern Cyprus

MASTER THESIS IN MOLECULAR MEDICINE

AYLA TURGAY

THESIS SUPERVISOR

ASSOC. PROF. MAHMUT ÇERKEZ ERGÖREN

AIM:

The aim of the study is to investigate the prevalence of SARS-CoV-2 variants of concern causing new Coronavirus Disease (COVID-19) in Northern Cyprus *via* design *in-house* RT-qPCR kit.

BACKGROUND:

In Wuhan Province, China a novel coronavirus strain which was discovered and reported to theWorld Health Organization (WHO) that a causative factor varied for many pneumonia cases in 2019. The novel virus severe acute respiratory syndrome coronavirus 2 abbreviated for SARS-CoV-2 was named later on as the virus had presented a high genome comparison to SARS-CoV by the CSG- International Committee of the Coronavirus Study Group and the coronavirus disease 2019 or COVID-19 was assigned by WHO. However, according to the huge amount of cases spreaded worldwide the WHO, had to declare the dilemma as a pandemic on March 11th, 2020. This virus, SARS-CoV-2 acknowledge to affects and damages the lung that leads to respiratory failure and as a result of possibly leading to death. Patients who are over the age of 65 are more likely to be severely affected and have critical chronic disease or smoke are in the category of high-risk group. The symptoms of this virus is usually dry cough, tachypnea, shortness of breath and high fever. Some patients may have rare symptoms such as headache, abdominal pain, diarrhea and confusion. Other patients that are infected with this virus may be asymptomatic.

SARS-CoV-2 is transmitted from human to human directly by respiratory droplets of an infected patient or indirectly by forming any contact with contaminated surface.

As known SARS-CoV and MERS-CoV have been identified previously and they infect humans but the new coronavirus, SARS-CoV-2 has a much higher rate of transmission. Inspite the fact

that there are many efforts but no current approved vaccine oreffective treatment. It is crucial to early detect the virus and if so to isolate the patient who is infected, especially for asymptomatic patients. To prevent the spread and to keep the pandemic under control it is important to include hand hygiene and social distancing is daily life.

There are four known SARS-CoV-2 variants which are *alpha, beta, gamma* and *delta*. The first SARS-CoV-2 variant was documented in September 2020 by WHO, the *alpha* variant also known and found in the United Kingdom. The *beta* Coronavirus was first documented by WHO in May 2020 in South Africa. The Brazil variant also known as *gamma* was first documented by WHO in November 2020. The last known variant *delta* which is the most popular variant in Northern Cyprus was first documented in October 2020 and found in India.

METHODS:

Between 1st June 2021 and 30th October 2021, a total of 1408 SARS-CoV-2 RT-qPCR positive patients were detected in Near East University DESAM Research Institute COVID-19 PCR Laboratory. Samples, that were preserved in -20^{0}C or -80^{0}C, will be used for SARS-CoV-2 variant genotyping after nucleic acid isolation.An *in-house* RT-qPCR SARS-CoV-2 variant detection kit was designed to detect Alpha (B.1.1.7 or VOC-202012/01, United Kingdom), Gamma (P.1, Brazil), Beta (B.1.351, South Africa) and Delta (B.1.617.2) variants of SARS-CoV-2 by NEU DESAM Research Institute. Mutation-specific primers and probe sequences were designed for spike (S) gene N501Y, HV69-70del, K417N and T478K mutation regions. HV69-70del and K417N primers were designed for the wild-type genotype, while others targeted the mutant genotypes. The kit was optimized and standardized using multiple SARS-CoV-2 samples and commercially available variant specific SARS-CoV-2 RNA samples. Commercial *in vitro* diagnostic kits and sequencing data was used for confirmation.

RESULTS:

A total number of 1408 SARS-CoV-2 RT-qPCR positive patients were detected in Near East University DESAM Research Institute COVID-19 PCR Laboratory.The variants observed in this study were SARS-CoV-2 UK, Brazilian, Wild Type and Delta. In between the months of June and October 291 patients was tested positive for the SARS-CoV-2 UK variant, SARS-CoV-2 Brazilian variant in total of 12. The SARS-CoV-2 Wild Type variant was found of 96. There was

an increase for the SARS-CoV-2 Delta variant in the months August, September and October but in total 1009 was found.

CONCLUSION:

In the current study, our main objective was to identifySARS-CoV-2 UK, Brazilian, Wild Type and Delta variants. Also to observe the increase of the variants in the passing months.

To sum up, the results of this study displayed that in June and July the SARS-CoV-2 UK was popular and in August, September and October SARS-CoV-2 Delta variant became popular with a rapid increase.

KEYWORDS:SARS-CoV-2, *alpha, beta, gamma,delta,* RT-qPCR

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List of abbreviations

ACE2: Angiotensin-Converting Enzyme 2

cDNA: Complementary Deoxyribonucleic Acid

DAMP: Damage-Associated Molecular Pattern

DNA: Deoxyribonucleic Acid

HCoVs: Human Coronaviruses

HSC: Human Specimen Control

IgA: Immunoglobulin A

IgG: Immunoglobulin G

IgM: Immunoglobulin M

LOD: Limit OfDetection

MERS-CoV: Middle East Respiratory Syndrome Coronavirus

NRP1: Neuropilin-1

NTC: No Template Control

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-1: Severe Acute Respiratory Syndrome Coronavirus

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2

TMPRSS2: Transmembrane Protease Serine 2

VBM: Variants Being Monitored

VOC: Variants of Concern

VOI: Variants of Interest

VUM: Variants Under Monitoring

WHO: World Health Organization

CHAPTER 1: INTRODUCTION

1.1 Introduction

In 2019, a coronavirus disease reported in Wuhan, China, as a severe acute respiratory syndrome.and it is known as SARS-CoV-2, coronavirus 2 (WHO, 2022). The WHO declared a Public Health Emergency of International Concern and shortly after on the 16th of February 2020 COVID-19 was raised to very high risk. In China, the epidemic progress has become under preliminary control although in many countries worldwide are still on alarm of the spread causing major stress and health concerns. This virus caused a pandemic which was announced by WHO on March 11, 2020. (Meng Di Jiang et al, 2020).

1.2. A New Coronavirus Disease (COVID-19)

The new corona virus known as COVID-19 is a new infectious disease caused by the virus SARS-CoV-2. Most people who are diagnosed with this disease have reported to have respiratory illness from mild to moderate and some have recovered with no medical attention or treatment. Although it has been reported that some patients had become very ill and would need medical attention, especially older people. Many older people who have been infected with this disease are possible to develop the illness and had reported other medical conditions such as cancer, diabeteschronic respiratory and cardiovascular disease. People infected could become severe ill also die changing due to age (Meng Di Jiang et al, 2020).

For prevention of the disease and to slow down the transmission to others, people should be aware of the illness and how rapidly it spreads. To protect yourself and others from COVID-19 you should keep a distance up to one meter away from other people also to wear a fitted mask, and wash the hands frequently or even use alcohol based disinfectant. Also it is important to get vaccinated (WHO, 2022).

The virus could be spread by a person who is infected through their nose or mouth with tiny liquid particles as they speak, sing, breathe, cough or sneeze. The tiny liquid particles could be ranged from large respiratory droplets to small aerosols. Also it is critical to practice respiratory etiquette also to say self-isolated at home if you feel unwell or contact a person with COVID-19 (WHO, 2022).

1.2.1 Etiology

The SARS-CoV-2 virus and all other viruses' genetic material could be altered over time. The changes that are occurred in the genetic material may have no effect or a little on the characteristics of the virus diversity. Although, some mutations are formed by significant changes in the viral characteristics such as how vigorously it spreads, the response to therapeutic drugs and vaccines and the efficiency of diagnostic tools (Meng Di Jiang et al, 2020).

1.2.2 Pathogenesis

The pathogenic stages of COVID-19 are still controversial. According to past studies have suggested that SARS-CoV can include of three stages: viral replication, immune hyperactivity, and lung destruction (Navas-Martín and Weiss, 2004). The clinical phases of COVID-19 have recently been suggested: viraemic, acute, and recovery phase (Lin et al., 2020). It is commonly assumed that the infection experiences these stages: dysregulated immune response, multiple organ damage, virus invasion and replication and recovery. The virus is replicated and then it is assembled, once it is released on target cells.

In addition, this precisely causes the parenchymal cells such as alveolar epithelial cells to destruct and become damaged. Simultaneously, a huge number of DAMP molecules and PAMP are released in order to induce the infiltration of inflammatory cells, release large amounts of cytokines also chemokines, activate the innate immune response and free proteins by proteases (Quirch et al., 2020).

After the first crucial step, the inflammatory reaction is eventually dissolved, the damaged organ is finally recovered, and some of the organs that are damaged can cause chronic steps and fibrosis, for examplecatabolism syndrome, immunosuppression, persistent inflammation and chronic critical disorders (Bhaskar et al., 2020).

Several viral and host factors influence the pathogenesis of the virus. S-protein comprises two subunits which are S1 and S2. The S1 regulation starts in the virus host cell area and tropism by the receptor binding domain. Heptadic repeat sequences that are H1 and HR2 mediate the fusing of the viral membrane to its cellular host through S2. Many earlier research have demonstrated that perhaps the S1 domain can cause levels to rise of IgG and IgA antibodies to a greater capacity. It is the expression of the focus spike proteins that is implicated in many effective COVID-19 vaccines (Wiersinga et al., 2020).

1.3 SARS-CoV-2 Genomic Evolution and Survival

Mutations in viruses are rapidly done are common and are not new nor unexpected this also includes coronavirus which causes COVID-19 pandemic. All RNA viruses can be mutated over time, some viruses can be mutated more than other viruses, since it is their nature to evolve and change gradually. If a mutation has occurred in a virus's genes the variants of viruses can occur. According to some mutations they may allow the coronavirus to spread rapidly from one person to another and this may cause more severe disease (Raman et al., 2021).

The evolutionary pressures of Coronaviruses cause the host to have a genetic diversity which causes an evolution. CoV has a large genome and the size is approximately 26.4 to 31.7 kb this is caused by the genetic diversity where the CoV could inherit different traits. The mutation rate of CoV is high according to the low fidelity viral polymerase which is estimate to have 10-4 substitutions per site per year. CoV has a high recombination frequency up to 25 percent for the whole genome of Coronavirus in vivo. Due to mutations the virus could cause an antigenic drift when it is selected. The genomes of SARS-CoV-2 are still under purifying selection (Singh et al., 2022).

1.3.1 Viral Classification and Nomenclature Tools

SARS-CoV-2 are viruses which are classified according to their characteristics such as the structure of virus assuming the envelope may be present or absent or the type of the nucleic acid such as DNA or RNA in the genome of the virus. In addition, to the approach of translating the nucleic acid to code for proteins. This virus is a single-stranded positive-sense RNA virus.Coronaviridae is the family that coronavirus relate to. The Coronaviridae has four genera which are Gamma-, Delta-, Beta-, and Alpha-coronavirus. The Betacoronavirusesand Alphacoronavirusesprimarily infect all mammalians whereas Gamma-coronaviruses and Deltacoronaviruses primarily infect birds also swine. In SARS-CoV-2 primarily the Betacoronavirus promotes and causes COVID-19 (Khan and Jamal, 2021).

1.3.2 Variants of Concern (VOC)

Following the outbreak of the pandemic in January 2020, the World Health Organization (WHO) began to monitor and analyse SARS-evolution CoV-2's with the help of institutions and researchers. However, in the late 2020 Variants of Interest (VOIs) was introduced to assist the progress of the variants that emergence posing a greater threat to global public health. VOIs was

also announced to prioritize global surveillance and research of these variants so it could be ultimately, assisting a fight against the pandemic and preventing rapid spread (WHO, 2022). In the table below shows the Variants of Concern (VOC), which was carried out by WHO with the Virus Evolution Technical Advisory Group, where the mutation combinations have been formed and the associated changes in the global public health importance (WHO, 2022).

a) Deleterious change in the epidemiology or increase in contagiousness
b) a) A change in clinical appearance of disease or increase in virulence
c) Decreased effectiveness and social measures or existing diagnoses, vaccines, therapeutics

WHO name	**Pango Lineage ****	**Remarkable Spike Mutation**	**Where and Date First Detected**
Alfa*	B.1.1.7	N501Y, D614G, P681H	United Kingdom, September 2020
Beta	B.1.351	K417N, E484K, N501Y, D614G, A701V	S.Africa, May 2020
Gamma	P.1	K417T, E484K, N501Y, D614G, H655Y	Brazil, November 2020
Delta	B.1.617.2	L452R, T478K, D614G, P681R	India, October 2020
Omicron	B.1.1.529	A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211-212, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F	In many different countries, November 2021

Table 1.1 List of Variants of Concern (VOC) and Characteristics

*It has been included in the variant category of decreasing severity by WHO.

** Includes all descendants (cov-lineages.org)

1.3.3 Variants of Interest (VOI)

The variants of genetic modifications are expected and are known to cause changes in the virus's characteristics such as therapeutic,diagnostic, immune escape, disease severity and transmissibility. In this case, SARS-CoV-2 is known as SARS-CoV-2 Variants of Interest(Table 2) because the transmission caused in a community that is found in more than one country with

an increased prevalence also a high rate of cases over time. Also SARS-CoV-2 can cause epidemiological effects that occur with more than one COVID-19 disease (WHO, 2022).

WHO name	**Pango Lineage ***	**Remarkable Spike Mutation**	**Where and Date First Detected**
Lambda	C.37	L452Q, F490S, D614G	Peru, December 2020
Mu	B.1.621	R346K, E484K, N501Y, D614G, P681H	Colombia, January 2021

Table 1.2 Variants of Interest (VOI List and Characteristics)

*Includes all descendants (cov-lineages.org)

Due to epidemic intelligence variants could act differently but they are similar to the VOC because they may have some properties in common but due to a less evidence, the Evaluated Variants are included in this list instead. The variants of Decreased Severity are variants with no circulating virus, indicating they have no effect and do not have any concerning characteristics. (WHO, 2022).

1.3.4 Variants under monitoring (VUM)

Genetic alterations in SARS-CoV-2 variants can influence and change viral properties. It acts as risk to the future but there is no proof of epidemiological effect orphenotypic effect and it remains unclear, so it requires analyses and evaluations for new evidences (WHO, 2022).

The variants under monitoring may pose to future risk so the SARS-CoV-2 variant that has genetic changes and are affecting the characteristics such as phenotypic or epidemiological of the virus whereas these variants are currently unclear due to lack of evidence. So it is required to enhance more observations and analyzing. Below the table 3 shows the VUMList and Characteristics (WHO, 2022).

Pango Lineage **	Where and Date First Detected
AZ.5#	Many Countries, January-2021
C.1.2	S.Africa, May 2021
B.1.617.1§	India, October-2020
B.1.526§	USA, November-2020
B.1.525§	Many Countries, December-2020
B.1.630	Dominican Republic, March 2021
B.1.640	Republic of the Congo, September 2021

Table 1.3 Variants under monitoring (VUM) (VUM List and Characteristics)

*Includes all descendent lineages.

#formerly tracked under parent lineage B.1.1.318

§Former VOIs: Kappa: B.1.617.1; Iota: B.1.526; Eta: B.1.525

1.3.5 Variants Being Monitored (VBM)

The variants being monitored are usually VOI or VOC that have been downgraded to this category due to the sustained reduction over time globally. Also, when a variant does not act a specific risk to the public evidences have approved it (WHO, 2022).

1.4 SARS-CoV-2 Variant Pathogenesis and Host Response

The process pathogenesis of the disease that develops an infection and in SARS-CoV-2 variants causes a viral pathogenic mechanism to consists of attaching the virus to the host receptor to enter and to start viral replication and the site of entry. Then the viral will spread to the target organs which start to shed to the environment. However, viral pathogenesis could be affected by some factors which are the accessibility of the virus the tissue or cell. The cell or tissue susceptibility to virus may cause multiplication in a way the virus will develop a defense against the host, and pathogenesis may be influenced by internal factors such as its viral factors or genomics such as the of comorbidities or stress, host health, gender and age (Hakim et al., 2022).

1.5 COVID-19 Diagnostic Techniques

In the HCoVs the spike glycoproteins S will bind to the surface of the cell enzymes as receptors and mediate the membrane fusion so the viral can enter to the host cells. Other HCoVs cleave the S protein into S1 also S2, which bind to the membrane fusion, indicating that they are mediated with one or more host proteases.SARS coronavirus 1 and 2 the S proteins consist a component S1 with a receptor-binding domain that interacts to ACE2.Surface target cells exposed to SARS-CoV-2 express the receptor in several human organs.Neuropilin-1 (NRP1) is a multifunctional receptor involved in the signal transduction of many endogenous cytokines and it is expressed in pulmonary olfactory endothelial cells pulmonary cells. The transmembrane protease serine 2 (TMPRSS2) co-expresses with ACE2 and allows it to bind to furin-cleaved substrates, promoting entrance and infecting SARS-CoV-2. Here, in the TMPRSS2 it leads to a cleavage of ACE2 which activates the S protein to mediate the entry of coronavirus host cells. Pathogenicity of SARS-CoV-2 and host response dynamics are associated by cell entrance efficiency and viral load variability via adaptive and innate immunity. (Hakim et al., 2022).

1.5.1 Rapid Antigen Tests

In April 2020 the World Health Organization (WHO) did not approve to the use of antigen for patient care to detect rapid antigen diagnostic however the study of the efficiency diagnostics is highly recommended. However, there is a major weakness of antigen test because it is known that they have a low sensitivity and may cause false negative results. The antigen tests for Coronavirus are reported to have a low sensitivity but are cheap and quick compared to rRT-PCR. There are new techniques for SARS-CoV-2 for antigen tests such as using a fluorescence immunochromatographic antigen test where a study shows that there are showed 100% specificity for positive patients and 93.9% sensitivity for the patients who showed symptoms in a week (Kyosei et al., 2022).

Due to the pandemic of COVID-19 the demand for tests have increased to develop a range of kits for detecting positive SARS-CoV-2 patients based on their variants as a result of pushing many inventors and biotechnology. Serological and molecular assays are the most prevalent and well-known approaches for detecting SARS-CoV-2.rRT-PCR which is based on viral RNA is the molecular approach for detection. (Dhamad and AbdalRhida, 2022).

1.5.2 Serological Tests

The serological method is also known as antibody test which could be used to test for SARS-CoV-2 infection in patients if it has passed or present and to track the process of the disease. Serological test can expose any present antibodies such as IgA, IgG and IgM in a COVID-19 patient with a sample of the patient's serum or plasma. The aim of antibodies is to develop in the immune system to fight against COVID-19 and to defend against mechanism for SARS-CoV-2. After a few days of the infection the first step is that the IgM is produced and the infection will last for approximately two weeks and then the antibody IgG is produced that lasts longer. If the patient is detected with IgM it indicates that there is an early stage infection whereas if IgG is detected in the patient, it indicates that there is a current infection (Dhamad and AbdalRhida, 2022).

It is an advantage of detecting the virus early in order not to result in a crossover reaction between antibodies other than SARS-CoV but can detect antibodies for coronavirus family members (Dhamad and AbdalRhida, 2022).

1.5.3 Real-Time Polymerase Chain Reaction (RT-qPCR)

The gold standard and reliable technique for detecting SARS-CoV-2 is RT-PCR, which is known for its high sensitivity (positive sensitivity) and specificity (negative specificity).This approach was created to detect COVID-19 virus by generating cDNA from COVID-19 virus RNA extraction. For this virus there are specific primers which follow the genes of 2019nCoV-N1 (N1), 2019nCoV-N2 (N2) and RNAse P (RP; internal control) and other health agencies. The virus is collected from the upper respiratory system using swabs from the nasopharyngeal and oropharyngeal cavities, which are the most common specimens used to identify COVID-19 virus.As a result, the sample is considered positive if both N1 and N2 genes are positive. When a sample is determined to be positive, the presence of viral RNA in COVID-19 is confirmed but it does not reveal the virus viability. In the run of the PCR there should be three controls besides from the internal control (RP), which are 2019-nCoV Positive Control (nCoVPC), No Template Control (NTC) and Human Specimen Control (HSC) run to ensure that the outcome is correct.Despite the fact that rRT-PCR is the gold standard method, it may have some limitations and disadvantages, such as expensive, professional skills needed, time-consuming and has to be applied in the laboratory (Dhamad and AbdalRhida, 2022).

In the technique of PCR there are many advantages of the limit of detection (LOD) such as SARS-CoV-2 RNA could be copied a few times for many nucleic acids. However, there are weakness of PCR which could be false negative amplifications or false positive amplifications can be found. In the detected the designed primer could cross react with non-specific nucleic acids of other viruses or bacteria. Also, the laboratory could be contaminated if there are unskilled technicians causing false positive results. In addition, mutations that are in the target genes can cause false negative results and the primers or probes which are designed could not identify the targets. It is not easy to design primer and probe sequences. Another limitation is that the sample volume may cause problems such as the PCR can detect the target nucleic acids. PCR is time consuming, expensive and the techniques could be complicated. As a result of many limitations many researchers prefer antigen tests for the proteins in SARS-CoV-2 week (Kyosei et al., 2022).

1.6 The Work in this Thesis

This study was conducted to detect Alpha (B.1.1.7, UK), Gamma (P.1, Brazil), Beta (B.1.351, South Africa) and Delta (B.1.617.2) variants of positive SARS-CoV-2 patients.

CHAPTER 2: MATERIALS AND METHODS

2.1 Materials

2.1.1 Suppliers

HiMediaInsta Q96™ Real Time (HiMedia, Mumbai, India), Tianlong GeneRotex96 Rotary Nucleic Acid Extraction System (TIANLONG, Shaanxi, China)

2.1.2 Sample Collection

Between 1st June 2021 and 30th October 2021, a total of 1408 SARS-CoV-2 RT-qPCR positive patients were detected in Near East University DESAM Research Institute COVID-19 PCR Laboratory. Samples, that were preserved in -20^{0}C or -80^{0}C, will be used for SARS-CoV-2 variant genotyping after nucleic acid isolation.

2.1.2.2 Oligonucleotide primers

An *in-house* RT-qPCR SARS-CoV-2 variant detection kit was designed to detect Alpha (B.1.1.7, UK), Gamma (P.1, Brazil), Beta (B.1.351, South Africa) and Delta (B.1.617.2) variants of SARS-CoV-2 by NEU DESAM Research Institute. Mutation-specific primers and probe sequences were designed for spike (S) gene N501Y, HV69-70del, K417N and T478K mutation regions. HV69-70del and K417N primers were designed for the wild-type genotype, while others targeted the mutant genotypes. Multiple SARS-CoV-2 samples and commercially available variant specific SARS-CoV-2 RNA samples were used to optimize and standardize the kit. For confirmation, commercial *in vitro* diagnostic kits and sequencing data were used.

2.2 Methods

2.2.1 RNA Extraction

RNA isolation will be performed from 200 microliters of swab sample using Ianlong Viral DNA and RNA Extraction Kit (Ref. T014H, Ianlong, China) in Auromatic Nucleic Acid Extractor GeneRotex 96 (Ianlong, China) machine upon the manufacturers' instructions.

SARS-CoV-2 Variant Detection

An *in-house* RT-qPCR SARS-CoV-2 variant detection kit was designed to detect Alpha (B.1.1.7, UK), Gamma (P.1, Brazil), Beta (B.1.351, South Africa) and Delta (B.1.617.2) variants of SARS-CoV-2 by NEU DESAM Research Institute. Mutation-specific primers and probe sequences were designed for spike (S) gene N501Y, HV69-70del, K417N and T478K mutation regions. HV69-70del and K417N primers were designed for the wild-type genotype, while others targeted the mutant genotypes. Multiple SARS-CoV-2 samples and commercially available variant specific SARS-CoV-2 RNA samples were used to optimize and standardize the kit. Commercial *in vitro* diagnostic kits and sequencing data was used for confirmation.

Four RT-qPCR reactions will be set up for each sample, using different mutation mastermixes according to the manufacturers' instructions with 15 microliters of RT-qPCR mastermix and 6 microliters of RNA sample using the cycling parameters given in Table 4.

RT-qPCR Cycling Conditions		
Cycle	**Temperature**	**Duration**
1	52°C	05:00
1	95°C	00:10
40	95°C	00:05
	60°C	00:15
Read TexasRed channel.		

Table 2.1 RT-qPCR cycling conditions are given in this table.

Samples with a cycle threshold (Ct) value below 40 will be noted as + for the specific reaction and analysis will be performed according to the expected RT-qPCR reaction results and evaluations that are given in Table 5 below.

UK-	**SA-**	**Brazil-**	**India-**

	Alpha	***Beta***	***Gamma***	***Delta***
69-70 del	-	+	+	+
N501Y	+	+	+	-
K417N	+	-	+	+
T478K	-	-	-	+

Table 2.2 Expected RT-qPCR reaction results and evaluations for each variant.

Each RT-qPCR run will comprise a non-template negative control and a positive control, both (negative/positive control) given with the kits.

CHAPTER 3: RESULTS

3.1 Introduction

In the PCR Laboratory of the Near East University DESAM Research Institute, 1408 SARS-CoV-2 RT-qPCR positive patients were found. The variants observed in this study were SARS-CoV-2 UK, Brazilian, Wild Type and Delta. Between June and October, 291 patients were found to be infected with the SARS-CoV-2 UK variant and the SARS-CoV-2 Brazilian variant was in total of 12. A total of 96 SARS-CoV-2 Wild Type variants were discovered. The SARS-CoV-2 Delta variant was detected in greater numbers in the months of August, September, and October, totaling 1009.

CHAPTER 4: DISCUSSION

4.1 Introduction

Between November 2020 and October 2021, there were approximately 10 different SARS-CoV-2 variants were found in the TRNC as a result of genome sequencing and mutation determination studies done in 2067 SARS-CoV-2 RT-PCR, due to positive cases detected in the COVID-19 PCR Diagnostic Laboratory. According to findings from the SARS-CoV-2 genome research at Erasmus University, B.1.1.209 (Netherlands), B.1.1 (USA), B.1.1.82 (Wales), B.1.1.162 (Australia) and B.1 (Italy) within the country, do not cause local transmission. Three different UK origin variants were declared in mid-December (B.1.1.29, B.1.258 and B.1.1.7 (Alpha)) were effective in local transmission.

The SARS-CoV-2 Alpha variant, which was observed in 45% positive cases in the month of January and remained a dominant variant in the TRNC pandemic for a long time because of the higher transmission rate compared to other variants and the rate rapidly increased to 90% by June. The SARS-CoV-2 Gamma (Brazil, P.1) variant, which was detected for the first time in January 2020, was observed for the first time in May, while the SARS-CoV-2 Beta (South Africa, B.1.351) variant was not detected among the samples studied. However, the SARS-CoV-2 Delta (India, B.1.617.2) variant, in April it made its first appearance in India. and this variant became an effective variant with a great transmission rate than the Alpha variant in a short amount of time around the world. The Delta variant has been reported in our country at the end of June for the first time, it became dominant in a very short time when detected, and the SARS-CoV-2 RT-PCR positive cases rate increased over 90% in August-October (Table 6).

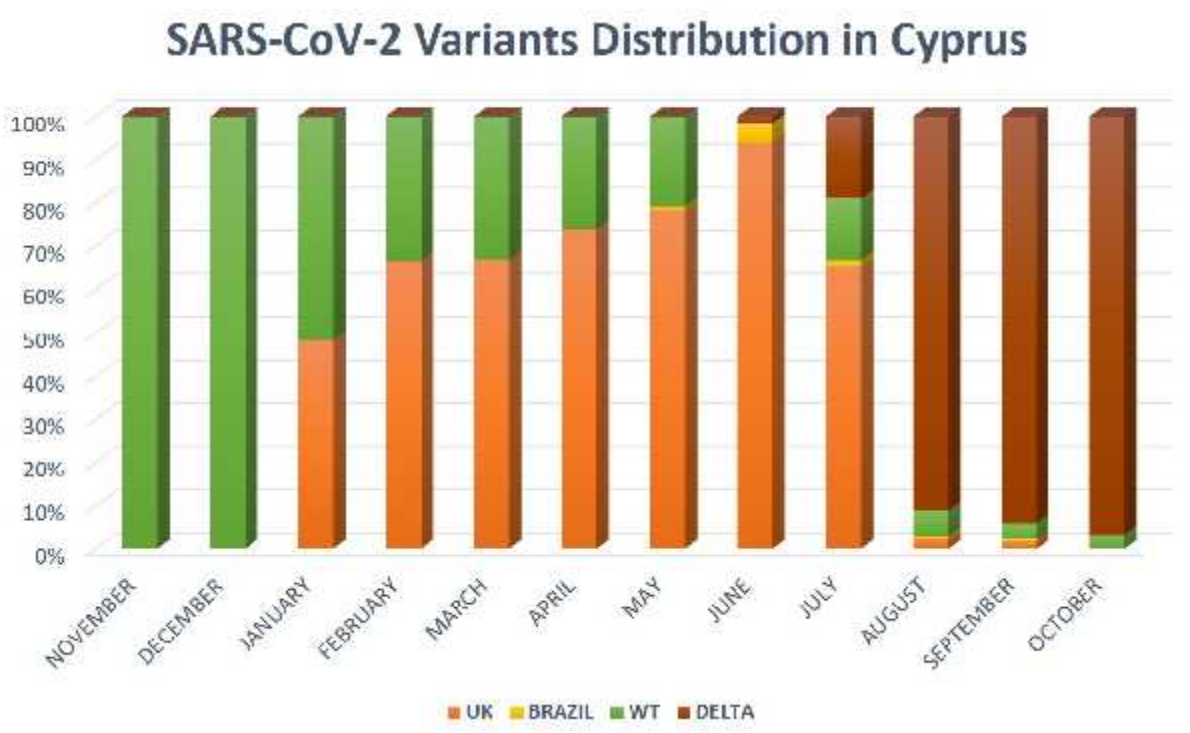

Table 3.1 NEU COVID-19 PCR Diagnostic Laboratory, SARS-CoV-2 variant distribution plot between November 2020 and October 2021

4.4 Conclusion

After the comparative analysis of our results with the TRNC Ministry of Health Infectious Diseases Supreme Committee COVID-19 Weekly Outbreak Report (10.11.2021-16.11.2021), Delta variant observed in isolated cases in June was found to be higher than other viral strains detected so far. As of mid-July, the number of SARS-CoV-2 positive patients and/or hospitalized patients detected daily increased at least three times compared to June (June positive patient/July positive patient: ~200/1250; June hospitalized/ July hospitalized inpatient: 200/1000). The "Delta variant effect" reached its highest level in August with 90% of cases, increasing the number of positive patients twelve times compared to June when the Alpha variant was dominant (positive cases in June / number of cases in August: 200/2400) and in August. In the study carried out from the end of June until the end of October, it was determined that local contamination was dominant with ~100% dominance rate. COVID-19 deaths increased by 2.7 times due to an increase in the number of patients, hospitalizations, and intensive care units, in October compared to June (June death/ October death: 35/90).

4.5 Final remarks and future work

In the current study, our main objective was to detect the SARS-CoV-2 variants (Alpha (B.1.1.7, UK), Gamma (P.1, Brazil), Beta (B.1.351, South Africa) and Delta (B.1.617.2) of concern causing new Coronavirus Disease (COVID-19) in Northern Cyprus *via* design *in-house* RT-qPCR kit.Mutation-specific primers and probe sequences were designed for spike (S) gene N501Y, HV69-70del, K417N and T478K mutation regions. HV69-70del and K417N primers were designed for the wild-type genotype, while others targeted the mutant genotypes.

To sum up, the results of this study displayed that in the month of June the UK variant appear the most in SARS-CoV-2 positive patients then in July the UK variant started to decrease because of the dominant variant Delta was mostly found followed with variant of Wild Type. In the months of August, September and October the Delta variant was so dominant the it appeared in almost 90% of SARS-CoV-2 positive patients. The earliest dominant variant was the UK, variant which later evolved into the Delta variant.

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APPENDICES

Appendix A: Ethical Approval Document

YAKIN DOĞU ÜNİVERSİTESİ
BİLİMSEL ARAŞTIRMALAR ETİK KURULU

ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi :23.12.2021
Toplantı No : 2021/98
Proje No :1443

Yakın Doğu Üniversitesi Tıp Fakültesi öğretim üyelerinden Doç. Dr. Mahmut Çerkez Ergören'in sorumlu araştırmacısı olduğu, YDU/2021/98-1443 proje numaralı ve **"The prevalence of SARS-CoV-2 variants in Northern Cyprus"** 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	✓	✓