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Detection of Severe and Acute Respiratory Syndrome Corona- Virus -2(SARS-CoV-2) and Measurement of Serum Troponin I Among Heart Disease Patients in Thi- Qar Province

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By

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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صَدَقَ اللَّهُ الْعَلِيُّ الْعَظِيمُ

Certification

certify that this thesis (**Molecular Detection of severe acute Respiratory Syndrome and Measurement of Serum Troponin I among Heart Disease Patients in Thi-Qar Province**) Was prepared under our supervision at the Department of Microbiology, Faculty of Veterinary Medicine, University of Thi-Qar, as a partial requirement for Master degree in Veterinary Medicine / Microbiology.

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Dedication

To those whose love and sacrifices were the invisible wings that carried me through my journey, whose wisdom and guidance were my guiding stars (dear to my heart, my father and mother).

To the unsung heroes of my life story. Your continuous encouragement and belief in me has been the light that guides me (my sisters and brother).

To the martyrs of Iraq who sacrificed their lives in order for the flag of Iraq to remain high

To the victims of this disease have mercy, and may those who recover from it
have eternal health

TABAREK

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TABAREK

Summary

Summary

The novel coronavirus (SARS-CoV-2), responsible for the ongoing pandemic, continues to record a significant number of cases and fatalities. This virus causes a severe respiratory illness that poses a life-threatening risk. This study aimed to investigate the impact of SARS-CoV-2 on heart disease patients using molecular tests (PCR) to detect the presence of three genes (N, RdRp, and E) for confirmation of infection, alongside the analysis of Troponin I as a serological marker.

The study included 240 random blood samples and nasopharyngeal swabs: 120 from the target group, and a control group (60 positive control samples and 60 negative control samples) from heart disease patients infected with SARS-CoV-2 at Al-Nasiriya General Hospital, Al-Hussein Teaching Hospital, and the Heart Hospital in Dhi Qar Governorate, southern Iraq, during the period from September 2023 to January 2024. RT-qPCR diagnostic results indicated that (79.17%) (95\120) of samples were positive, while (20.83%) (25\120) were negative. The results showed significant differences in infection rates across the months, with the highest infection rate recorded in December at (27.37%).

Regarding demographic characteristics, the results indicated that the infection rate in Thi-Qar Governorate was (61.05%) among males compared to (38.95%) among females, with no significant statistical differences in the virus prevalence between genders ($P > 0.05$). In terms of residence, the highest infection rate was recorded in Nasiriya City at (67.37%), followed by Al-Nasr District at (10.53%), while the lowest rates were observed in Al-Shatra and Al-Gharraf Districts at (4.21%). Statistically significant differences were observed based on residence ($P < 0.05$).

In terms of age groups, the highest infection rate was observed in the age group of 55-63 years at (24.21%), followed by the 46-54 year age group at

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(17.89%). The lowest infection rates were in the 91-98 year age group at (1.05%) and the 82-90 year age group at (2.11%). Significant statistical differences were found in infection rates based on age groups ($P<0.05$).

According to the analysis of pathological markers associated with COVID-19 infection, the highest infection rate was found among patients with angina at (32.63%), followed by tachycardia at (15.79%). The lowest infection rates were recorded among myocardial infarction patients at (14.74%) and patients with ventricular septal defects at (2.11%). The results indicated significant statistical differences in infection rates based on associated heart conditions ($P<0.05$).

When comparing study samples with control samples (the positive control group consisting of heart disease patients and the negative control group consisting of heart disease patients not infected with COVID-19), the study revealed that (37.5%) of heart disease patients infected with COVID-19 had positive Troponin I test results. Among the positive control group of heart disease patients, (56.67%) had positive Troponin I results, while in the negative control group, the positivity rate was (35.5%). Significant statistical differences were observed in Troponin I levels among the study groups ($P<0.05$), indicating that COVID-19 patients are more susceptible to cardiac complications.

Regarding the analysis of Troponin I test results by age within the study samples, the highest positivity rate was observed in the 46-54 year age group at (24.44%), while the lowest positivity rate was in the 82-90 year age group at 2.22%. Significant statistical differences were noted between age groups for Troponin I test results ($P<0.05$).

In the negative control group (heart disease patients), the highest positivity rate for Troponin I was observed in the 37-45 and 55-63 year age groups at (19.05%), while the lowest positivity rate was found in the 91-98 year age group

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at 4.76%, with significant statistical differences noted in Troponin I levels ($P < 0.05$).

Regarding Troponin levels, the highest positivity rate was recorded within the range of 0.010-1.070 ng/L at (78.95%), with significant statistical differences observed between the different Troponin I level.

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List of Abbreviations

Symbol	Description
ACE2	Angiotensin-converting enzyme 2
CK - MB	Creatine Phosphokinase-cardiac muscle
COVID-19	Coronavirus disease 2019
CTnI	Cardiac troponin I
CVDs	Cardiovascular
E Gene	Envelope gene
EDTA	Ethylene diamine tetra acetic acid
HF	Heart failure
ICT	International committee on taxonomy
ICU	International Care Unit
MERS	Middle East respiratory syndrome
MI	Myocardial infarction
N Gene	Nucleocapsid gene
Nsp	Non-structural proteins
ORFs	Open reading frames
PCR	Polymerase Chain Reaction
RBD	Receptor-binding domain
RdRp	RNA-depend RNA polymerase
RNA	Ribonuclease Acid
RNase	Ribonuclease
RT	Reverse Transcription
RTC	Replicas transcriptase complex
SARS-CoV-2	Severe acute respiratory syndrome
TMPRSS2	Transmembrane protease serine 2
TMS2	Transmembrane domain 2
VSD	Ventricular Septal Defect
VTM	Viral Transportation Medium

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Global COVID-19 virus pandemic a comprehensive review and analysis of the current state and future perspectives (chen *et al.*,2023). While respiratory failure is the most commonly known complication of COVID-19, It can also lead to dysfunction in various organ systems, including the cardiovascular system, these cardiovascular complications can range from mild myocardial injury to more severe conditions such as heart failure (HF), myocardial infarction, and cardiogenic shock, which may ultimately result in cardiovascular-related deaths (Bashir *et al.*, 2023).

COVID-19 virus infection has been associated with the development of heart failure (HF) through different mechanisms. Such, as myocardial infarction, myocarditis, microthrombi, and stress cardiomyopathy (Italia *et al.*, 2021). These conditions can occur as a result of direct viral effects or immune-mediated responses caused by SARS-CoV-2. Patients with COVID-19 and myocardial infarction are particularly at a higher risk of experiencing cardiogenic shock, While the direct impact of COVID-19 virus on the cardiovascular system has garnered significant attention, the pandemic has substantially impacted the healthcare systems addressing HF (Gupta *et al.*, 2020).

Cardiovascular diseases (CVDs) are a leading cause of death globally, accounting for nearly one-third of all deaths. Unfortunately, insufficient attention has been given to patients with CVD who also contract COVID-19, Existing studies examining the clinical aspects of COVID-19 have not sufficiently categorized CVDs, yet these conditions these may significantly impact the prognosis of COVID-19 (Sharma., 2020).

COVID-19 enhances morbidity in patients with cardiovascular diseases (CVDs) via interacting with the cardiovascular system on multiple levels, thereby worsening the diseased conditions and leading to myocardial injury and

dysfunction, SARS-CoV2 should be eradicated worldwide due to its high mortality and morbidity and widespread distribution. (Clerkin *et al.*, 2020).

The lungs is the organ that commonly affected by COVID-19, leading to several pulmonary complications. Such as, pulmonary vascular endothelialitis, thrombosis, angiogenesis, interstitial pneumonitis, and severe acute respiratory distress syndrome. Additionally, the cardiovascular system is also frequently involved, resulting in various cardiac complications, including cardiovascular disease, arrhythmia (such as ventricular tachyarrhythmia, atrial fibrillation, and ventricular fibrillation), hypertension, cardiac injury, heart failure, and fulminant myocarditis. These cardiac complications are identified as significant factors influencing the mortality of patients with COVID-19,(Clerkin *et al.*, 2020, Sharma, 2020). COVID-19 patients exhibit a higher incidence of cardiovascular diseases (CVDs), with myocardial injury occurring in 7% of cases (rising to 22% in critically ill patients), the prognosis of COVID-19 may vary depending on the specific CVDs present, such as ischemic heart disease, heart failure, and cardiac arrhythmia. Notably, fatigue and dyspnea, which are cardinal symptoms of heart failure, are frequently observed in COVID-19 patients, particularly in severe cases, (Huang *et al.*, 2020). Therefore, COVID-19 diagnosis in patients with chronic heart failure is a sever issue. Moreover, both heart failure and COVID-19 lead to hypoxemia, that leads to death ,Nevertheless, proper attention has not been given to CVD patients having COVID-19(Chen *et al.*, 2020).

In this research, we are investigating the potential impact of COVID-19 on patients with heart failure. Specifically, The aim of this study investigate whether heart failure patients are at a higher risk of contracting the virus and experiencing severe symptoms compared to patients without heart disease.

1.2 Aim of study

The aim of this study is to detect SARS-CoV-2 in heart disease patients as well as the effect of the diseases as predisposing factors for infection of this virus. This aim was performed by the following objectives:

1. Using RT-PCR to detection SARS-CoV-2 in the patients.
2. Measurement of the troponin I level in the blood of those patients by serological method.

CHAPTER TWO

LITERATURES REVIEWS

2.1 Historical of Coronaviruses

In the mid of 1930s, a severe respiratory infection in chickens was identified as the earliest known disease caused by coronaviruses, Now, it is considered avian infectious bronchitis, caused by the avian infectious bronchitis virus (IBV) (Jaiswal *et al.*, 2020).

After less than a century, in 2003 SARS outbreak, another highly pathogenic illness, known as Middle East respiratory syndrome in 2012 (MERS-CoV) emerged and predominantly affected affluent urban areas in Middle Eastern countries (Zaki *et al.*, 2012). Both SARS-CoV and MERS-CoV were transmitted to humans from specific animal sources, with SARS-CoV originating from feline animals (civet) and MERS-CoV from dromedary camels (Guan *et al.*, 2003; Hemida *et al.*, 2013, Alagaili *et al.*, 2014). Extensive research on human coronaviruses has greatly enhanced our understanding of their biological characteristics and distinct behaviors. In late December 2019, several patients in Wuhan, Hubei Province, China, presented with unexplained fever and lower respiratory tract symptoms. Initially classified as pneumonia of unknown etiology, the cause of this respiratory infection remained unidentified. Subsequent investigations by the Chinese Center for Disease Control and Prevention led to the identification of the pathogen, initially named 2019 novel coronavirus (2019-nCoV). On February 11, 2020, the WHO Director-General, Dr. Tedros Adhanom Ghebreyesus, officially named the disease COVID-19, denoting "coronavirus disease 2019." In February 2020, the WHO escalated the threat level of this epidemic to "very high." Organizations worldwide are now working diligently to develop countermeasures to prevent the potentially devastating effects of this new human coronavirus, The renewed interest in this group of viruses has led to the discovery of numerous coronaviruses and their ability to cross over between different animal species over time (Jaiswal *et al.*, 2020).

2.2 Classification

The International Committee on Taxonomy (ICT) of viruses has placed coronaviruses within the family Coronaviridae and the order Nidovirales. The two representative genera within the family Coronaviridae are Toroviruses and coronaviruses, further classified in the subfamily Coronavirinae. Through analysis of genetic trees and partial nucleotide sequences of RNA-dependent RNA polymerase, the subfamily Coronavirinae was divided into four genera—alpha (α) coronavirus, beta (β) coronavirus, gamma (γ) coronavirus, and delta (δ) coronavirus (Woo *et al.*, 2009). The α coronaviruses and β coronaviruses infect only warm-blooded animals. The γ coronaviruses and δ coronaviruses infect birds, but some can also infect mammals. Phylogenetic relationship of various human and animal coronaviruses and the list of known coronaviruses are shown in, Figure 2-1 (Malik *et al.*, 2020).

In humans, infection with α and β coronaviruses leads to respiratory disorders, while in animals it causes gastrointestinal disorders (Su *et al.*, 2016; Forni *et al.* 2017). Extensive research study on the source of infection of SARS-CoV and MERS-CoV has found bats as a reservoir, and it has led to a better understanding of coronavirus microbiology. At present, seven species of α coronavirus and four species of β coronavirus have been identified only in bats (Forni *et al.*, 2017; Wang, *et al.*, 2018 ;Jaiswal *et al.*, 2020).

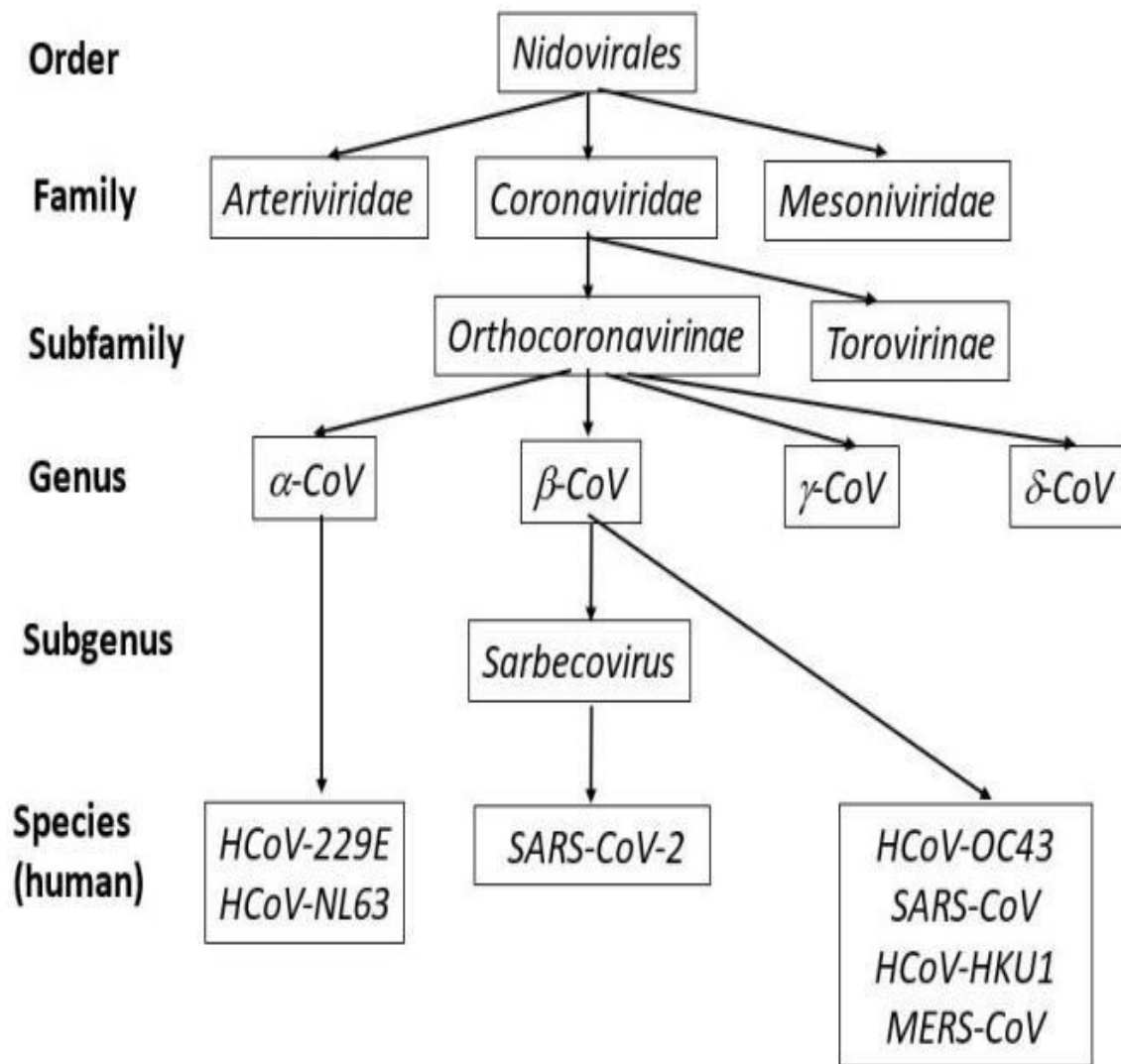


Figure 2-1: Classification of Human Coronaviruses (Malik et al., 2020).

2.3 SARS-CoV-2 Structure

The SARS-CoV-2 viruses have a spherical enveloped structure and contain a single strand of positive-sense RNA, similar to the host mRNA, spanning approximately 26 to 32 kb. Their distinctive morphological characteristics include club-shaped projections from the viral envelope that resemble a crown or a solar corona. These projections comprise a highly glycosylated protein known as the spike protein. Additionally, the viruses have three other structural proteins: the envelope, membrane, and nucleocapsid proteins, (Bergmann and Silverman, 2020) . (Figure 2-2)

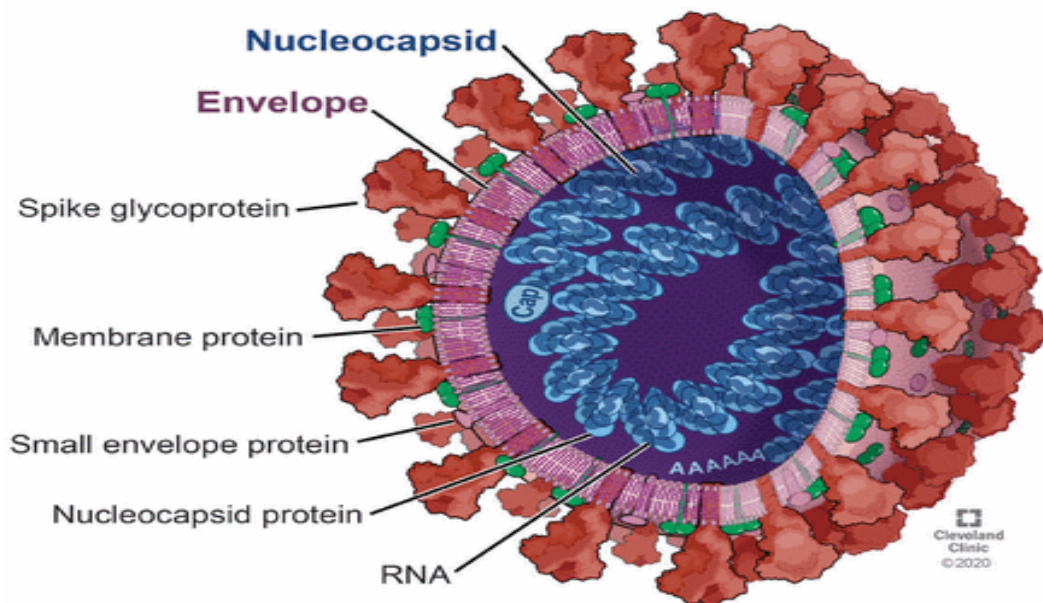


Figure 2-2: Structure of coronaviruses (Bergmann and Silverman 2020)

The initial two-thirds of the genome features two extensive overlapping open reading frames (ORFs) responsible for encoding 16 nonstructural proteins, such as proteases, RNA-dependent RNA polymerase (RdRp), RNA helicase, primase, and others. These proteins collectively form the viral replicase complex, as a foundation for generating viral mRNAs. All these nonstructural proteins are presented the potential targets for therapies that could theoretically be effective against all coronaviruses (Bergmann and Silverman, 2020; Rastogi *et al.*, 2020)

It has 5' untranslated region (UTR), replication complex (ORF1a and ORF1b), Spike (S) gene, Envelope (E) gene, Membrane (M) gene, Nucleocapsid (N) gene, 3' UTR, several unidentified non-structural ORFs and a poly (A) tail (Zhu *et al.*, 2020). The ORF1a gene, located at the 5' UTR, encodes for polyprotein pp1a, containing 10 nsps. Adjacent to ORF1a, the ORF1b gene encodes for polyprotein pp1ab, which contains 16 nsps. The pp1ab and pp1a proteins undergo autoproteolytic cleavage to form the viral replication complex (Rastogi *et al.*, 2020; Wu *et al.*, 2020).

The 3' UTR contains four structural genes and eight accessory genes, with the accessory genes distributed among the structural genes, and their function is mostly unknown. The SARS-CoV-2 is a non-segmented enveloped virus with a diameter of 50–200 nm (Kim *et al.*, 2020). Structurally, it has a double-layered lipid envelope,

including Spike glycoprotein (S), Envelope protein (E), Membrane glycoprotein (M), and Nucleocapsid protein (N) (Chen *et al.*, 2020; Rastogi *et al.*, 2020). The viral genome, which has a receptor-binding domain (RBD) for interaction with host cell receptors, is covered by the Spike glycoprotein (Wan *et al.*, 2020; McBride *et al.*, 2014; Rastogi *et al.*, 2020).

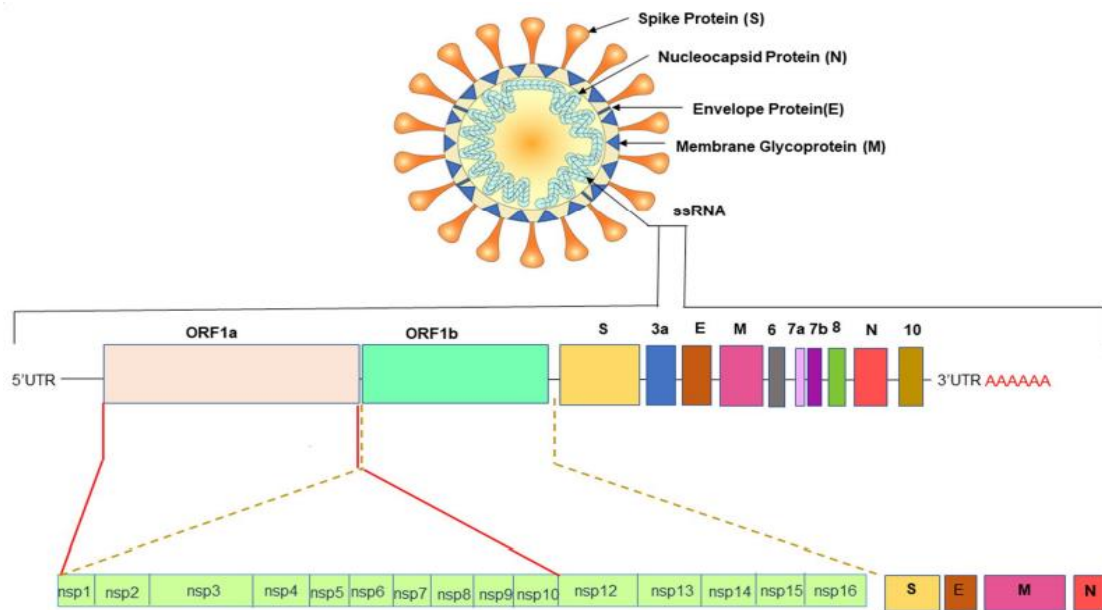


Figure 2-3: Schematic presentation of the SARS-CoV-2 genome Structure (Rastogi *et al.*

SARS-CoV-2 has sixteen non-structural proteins (nsp1–16) with key roles in viral replication and immune evasion (Wang *et al.*, 2020). Nsp1 supports RNA processing, nsp2 regulates host survival pathways, and nsp3 aids in protein separation. Nsp4 alters ER membranes, while nsp5 processes polyproteins. Nsp6 is likely a transmembrane protein. Nsp7 and nsp8 enhance nsp12 (RdRp) function, crucial for replication. Nsp9 binds ssRNA, nsp10 aids mRNA cap methylation, and nsp13 supports replication. Nsp14 acts as a proofreading exonuclease, nsp15 has endoribonuclease activity, and nsp16 is a methyltransferase (Naqvi *et al.*, 2020). These NSPs can also disrupt host defenses (Banerjee *et al.*, 2020).

2.4 Coronaveridae life cycle

The transmission, replication, and pathogenesis of coronaviruses are influenced by both the viral genome and the human host (Figure 2-4)(Bergmann and Silverman, 2020). The coronavirus spike proteins are key determinants for virus attachment and entry into target cells. The receptor for both SARS-CoV and SARS-CoV-2 is angiotensin-converting enzyme 2 (ACE2), a cell-surface enzyme involved in blood pressure regulation (Fehr *et al.*, 2015; Bergmann and Silverman, 2020).

The entry process involves two spike protein subunits with distinct functions. The S1 subunit mediates ACE2 attachment through the receptor-binding domain, while the S2 subunit, containing the fusion peptide and transmembrane domains, drives the fusion of viral and host cell membranes. Activation for fusion requires cleavage at two sites directly at the cell membrane, through endosomes, or both. The sequence of the cleavage sites determines cleavage efficiency and provides substrates for various cellular proteases. The route of infection depends on the available proteases in different cell types and the protease cleavage sites. This is illustrated by the involvement of cellular serine protease TMPRSS2, (Transmembran protease serine2), furin, and endosomal cathepsins B and L(Hoffmann *et al.*, 2020; Lan *et al.*, 2020).

During SARS-CoV-2 entry, TMPRSS2 activity also affects in viral spread and pathogenesis in SARS-CoV. Host proteases that cleave the S protein are potential targets for antiviral drugs. A higher rate of SARS-CoV-2 infections than SARS-CoV infections may be partially explained by a higher affinity of the spike protein for ACE2,(Bergmann and Silverman, 2020; V'kovski *et al.*, 2021). After the viral RNA is released into the cytoplasm and uncoated, the translation of ORF1a and ORF1ab produces polyproteins pp1a and pp1ab. These polyproteins are then processed by viral proteases encoded by ORF1a, forming 16 nonstructural proteins. The rough endoplasmic reticulum (ER)derived membranes are utilized for the formation of the RNA replicase-

transcriptase complex (RTC), which is responsible for the synthesis of (–)RNAs. These (–)RNA copies serve as templates for the production of full-length (+)RNA genomes. Additionally, transcription generates a subset of subgenomic RNAs, including those that encode structural and accessory proteins. The ER-Golgi intermediate compartment is crucial for the assembly of viral structural proteins and genomic RNA into the nucleocapsid and envelope, which are then released through exocytosis. Potential therapeutic strategies include targeting specific proteases or entry pathways, such as using anti-TMPRSS2 or chloroquine in experimental models. These treatments help assess impacts on both innate and adaptive immunity. Virus replication can be inhibited at various stages through single or combined approaches. For example, blocking virus entry can be achieved by using anti-spike antibodies from vaccines or protease inhibitors to prevent fusion (Hoffmann *et al.*, 2020; Bergmann and Silverman, 2020).

Virus protease inhibitors or nucleoside analogues (GS-5734 or EIDD-1931) can be used to target RTC formation and transcription-replication events (De Wit *et al.*, 2016; Sheahan *et al.*, 2020).

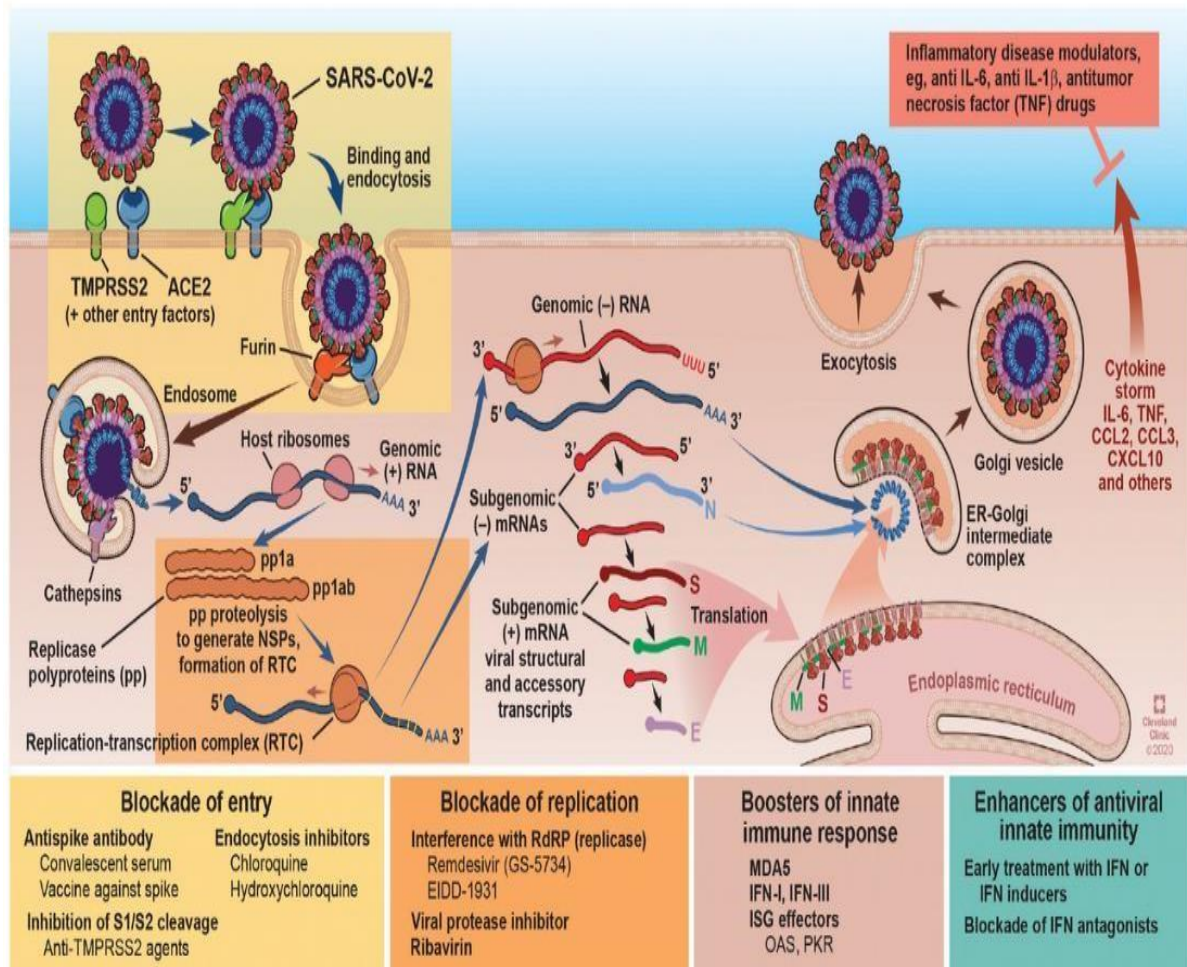


Figure 2-4: Overview of SARS-CoV-2 replication, and therapeutic targets (Bergmann and Silverman, 2020).

2.5 Coronaviruses variants

Variants are viruses with mutated genomes that may differ in severity from the original virus. When these mutations lead to distinct properties, they become strains; all strains are variants, but not all variants are strains (Lauring and Hodcroft, 2021; Gupta *et al.*, 2023). During COVID-19, 2,920 SARS-CoV-2 genomes were sampled globally from December 2019 to April 2022 (Gong *et al.*, 2023). While most mutations have minimal impact, some cause significant changes. In late 2020, variants were classified as "Variants of Interest" (VOIs) or "Variants of Concern" (VOCs) for targeted surveillance (Control and Prevention, 2021). VOCs and VOIs show higher transmissibility, immune evasion, and reduced treatment response, including Alpha, Beta, Gamma, Delta, Omicron, Mu, and Lambda (Gong *et al.*, 2023).

2.5.1 Alpha Variant (B.1.1.7)

Some significant variants include the Alpha Variant (B.1.1.7), the first SARS-CoV-2 variant designated as a VOC. It was initially identified in the United Kingdom in October 2020 and stood out due to having 23 more mutations than the original Wuhan strain, with eight mutations occurring in the S protein. The variant was responsible for a surge in cases in the United Kingdom it was considered to have an increased transmission rate (40-80%), and The mortality rate has escalated to 2.62% partly attributed to specific mutations such as N501Y, P681H, and 69/70 deletions (Vasireddy *et al.*, 2021; Gupta *et al.*, 2023; Liu ., 2021)

2.5.2 Beta Variant (B.1.351)

Another important variant is the Beta Variant (B.1.351), first detected in Nelson Mandela Bay in South Africa in October 2020. Also known as 501Y.V2, it carries 23 mutations with 17 amino acid changes. Notable mutations in the S protein include E484K, N501Y, and K417N. This variant is characterized by the presence of escape mutations under E484K and K417N, increased transmissibility, and a higher likelihood of infecting young immunocompetent individuals, leading to evasion of antibody neutralization and reduced vaccine effectiveness COVID "The mortality rate has escalated to 4.19% (Planas *et al.*, 2021; Gupta *et al.*, 2023; Manathunga *et al.*, 2023).

2.5.3 Gamma Variant (B.1.1.28.1 or P.1)

The Gamma Variant (B.1.1.28.1 or P.1) was first identified in Manaus in the Amazonas state of North Brazil. It has 17 amino acid substitutions, with 10 occurring in the S proteins, and critical mutations, including N501Y, E484K, and K417T in the receptor binding domain (RBD), which enhance its affinity to the human ACE-2 receptor ,the mortality rate has escalated to 3.6% (Planas *et al.*, 2021).

2.5.4 Kappa Variant (B.1.617.1)

B.1.617.1 was first detected in India in December 2020. This variant is one of the three sub lineages of B.1.617. Public Health England designated it as a variant under investigation (VUI-21APR-01). The important mutations noted in this variant were L452R, E484Q, and P681R (Bian *et al.*, 2021; Saville *et al.*, 2022).

2.5.5 Delta Variant (B.1.617.2)

The B.1.617.2 variant was first detected in Maharashtra, India, in late 2020 or early 2021 and has since spread to over 135 countries worldwide. Notably, the delta variant is more transmissible (1.1-1.4 times) than the alpha and kappa variants, with prominent mutations in the S protein such as T478K, P681R, and L452R. This variant has been found to impact transmissibility and antibody neutralization and is associated with increased mortality rates 2.01%. (Bian *et al.*, 2021; Saville *et al.*, 2022).

2.5.6 Omicron Variant

The Omicron variant was initially reported in South Africa in November 2021 and is considered the most mutated variant, with approximately 50 mutations in its genome, including 26-32 mutations in the S protein. Notably, 15 of these mutations are located in the receptor binding domain (RBD), contributing to increased transmissibility and immune evasion from hybrid, natural, and vaccine-induced immunity and the death rate is 0.7% (Chowdhury *et al.*, 2022; Khandia *et al.*, 2022; Rabiul Islam *et al.*, 2022).

2.6 Epidemiology

2.6.1 Coronavirus in the world

Epidemiology is vital in managing infectious diseases. During the COVID-19 outbreak in Wuhan, China, epidemiologists traced the virus's origin, implemented controls, and monitored disease trends. Studies confirmed that COVID-19 affects

all ages and spreads via symptomatic and asymptomatic carriers, both with similar viral loads (Singhal, 2020; Zou *et al.*, 2020). SARS-CoV-2 droplets travel up to 2 meters and persist on surfaces for days, with disinfectants like sodium hypochlorite and hydrogen peroxide proving effective (Kampf *et al.*, 2020). Susceptibility varies by age and health, with adults aged 35-55 most affected. High-risk groups include older adults (60+), those with chronic conditions, immunocompromised individuals, and smokers, with higher mortality observed in older populations (Adhikari *et al.*, 2020; Lai *et al.*, 2020).

2.6.2 Coronavirus in Iraq

The first case of the coronavirus in Iraq was confirmed on February 24, 2020 in Najaf. Following this, the Ministry of Health in Iraq reported more cases of COVID-19. Measures such as suspending schools and universities, restricting movement between regions, closing public places, and banning travel from certain countries were implemented to contain the virus. By March 27, cases had been confirmed in all Eighteen Iraqi governorates. Various websites provide real-time data on COVID-19 globally, including the World Health Organization, Microsoft's Bing search engine, and Worldometer. The spatial distribution of COVID-19 cases and associated deaths in Iraq has been analyzed using geographic heat maps. As of January 14, 2024, the Worldometer website recorded 2,465,545 cases and 25,375 deaths in Iraq (Al-Jumaili and Hamed, 2020; Al-Imam *et al.*, 2021).

2.7 COVID-19 Vaccines

Treatment options for individuals with COVID-19 are tailored to their specific symptoms, as there is currently no definitive cure for the disease. Ongoing efforts by researchers and healthcare professionals are focused on developing effective treatments for COVID-19 patients (Zumla *et al.*, 2020). Various potential therapies, including antiviral medications, immunosuppressants, monoclonal antibodies, and vaccines, are being

rigorously tested (Salahshoori *et al.*, 2021). Vaccine candidates against COVID-19 have diverse compositions, from traditional whole-pathogen vaccines to various new-generation vaccines. The World Health Organization reports that over 50 COVID-19 vaccine candidates are currently undergoing trials (Shahcheraghi *et al.*, 2021).

2.7.1 Killed vaccine

In live vaccines, reverse genetic techniques have been effectively applied to inactivate the exonuclease effects of protein 14 (nsp14), a nonstructural peptide, and remove the envelope protein in the disease-causing virus, similar to the Covivax vaccin (yang *et al.*, 2020).

2.7.2 DNA vaccine

A synthetic DNA vaccine has been created to produce the coronavirus spike (S) protein, which is the primary surface antigen of the virus and is currently being used in clinical trials. This vaccine, known as INO-4800, is designed based on previous knowledge and has been engineered to result in robust expression of the S protein *in vitro*. It has been found to generate effective antibodies that can neutralize SARSCoV-2 infection by preventing the S protein from binding to the ACE2 receptor (Dhama *et al.*, 2020; Smith *et al.*, 2020).

2.7.3 RNA vaccine

Preclinical studies have shown that the S protein is essential as a viral antigen in COVID-19 vaccine development. The lipid nanoparticle–mRNA vaccine, mRNA-1273, encoding the SARS-CoV-2 S protein, began its Phase I trial in the USA in March 2020. The introduction of mRNA-based vaccines marks a significant advancement in vaccine technology. Despite this progress, the rapid and effective testing and development of COVID-19 vaccines, which requires global collaboration, remains challenging. A Phase I safety trial is underway for mRNA-1273. Innovations in RNA delivery and self-amplifying

RNA technology have improved the efficacy of mRNA vaccines (Dhama *et al.*, 2020; Triggles *et al.*, 2020; Wang *et al.*, 2020). Data on safety, immune response, and tolerance from a study (Walsh *et al.*, 2020) involving participants aged 18–55 who received two doses (10, 30, or 100 µg) of BNT162b1, an mRNA vaccine with the SARS-CoV-2 S protein receptor-binding domain (RBD), have been released. Nonetheless, questions about mRNA vaccines remain. The Pfizer vaccine, along with vaccines endorsed by the (WHO), represents a significant component of the global COVID-19 vaccination efforts. (Mulligan *et al.*, 2020).

2.7.4 Subunit vaccine.

One of the challenges in using protein subunit vaccines has been achieving the antigen's most stable and effective conformation. Virus-like particles (VLPs) consist of multiple protein molecules that can self-assemble into nanostructures, enclosing the capsid proteins within themselves through recombinant expression. (Shahcheraghi *et al.*, 2021). Additionally, since VLPs do not have their own genome, they provide a level of stability similar to nonreplicating vectors, thereby confirming the safety of subunit vaccines and the efficacy of live vaccines. (Syomin and Ilyin, 2019; Shahcheraghi *et al.*, 2021).

Other subunit vaccines have been successful in several studies, including those based on MERS-CoV and SARS-CoV recombinant S and S1 proteins. Novavax subunit coronavirus vaccine that uses the spike protein to stimulate the immune response (Shahcheraghi *et al.*, 2021 ;Tai *et al.*, 2020;Wang *et al.*, 2020; Zhang *et al.*, 2020).

2.7.5 Vector-based vaccines

Viral vectors are frequently used in combination with viral vaccines, where the genetic material of one virus is employed to deliver the antigen of another virus, enabling the development of a platform system for virus creation. These tools are available for large-scale vaccine production. However, drawbacks of these types of vaccines include the requirement for various purification

methods, the need for precise purity checks, and potential viral activity (Choi *et al.*, 2013).

A COVID-19 vaccine utilizing artificial antigen-presenting cells (aAPCs) was developed by modifying aAPCs with genetically altered lentivirus containing immune regulatory genes and minigenes from SARS-CoV-2 to serve as antigenpresenting cells for the virus. A Phase I clinical trial involving approximately 100 participants commenced on 15 February 2020, and it is expected to conclude on 31 December 2024 (Helena *et al.* ,2020 ;Lythgoe and Middleton, 2020; Pooladanda *et al.*, 2020; Shahcheraghi *et al.*, 2021).

2.7.6 Inactivated covid-19 vaccine

Studies have shown that an inactivated COVID-19 vaccine candidate, BBIBP-CorV, is capable of inducing antibody production in various animal models, including rats, mice, rabbits, and primates such as rhesus macaques and cynomolgus monkeys, providing protection against SARS-CoV-2. Additionally, BBIBPCorV exhibits high efficacy and genetic stability, vaccine developed by the Chinese company Sinopharm (Wang *et al.*, 2020). Figure 2-5 shows the most important vaccine types being developed against COVID-19.

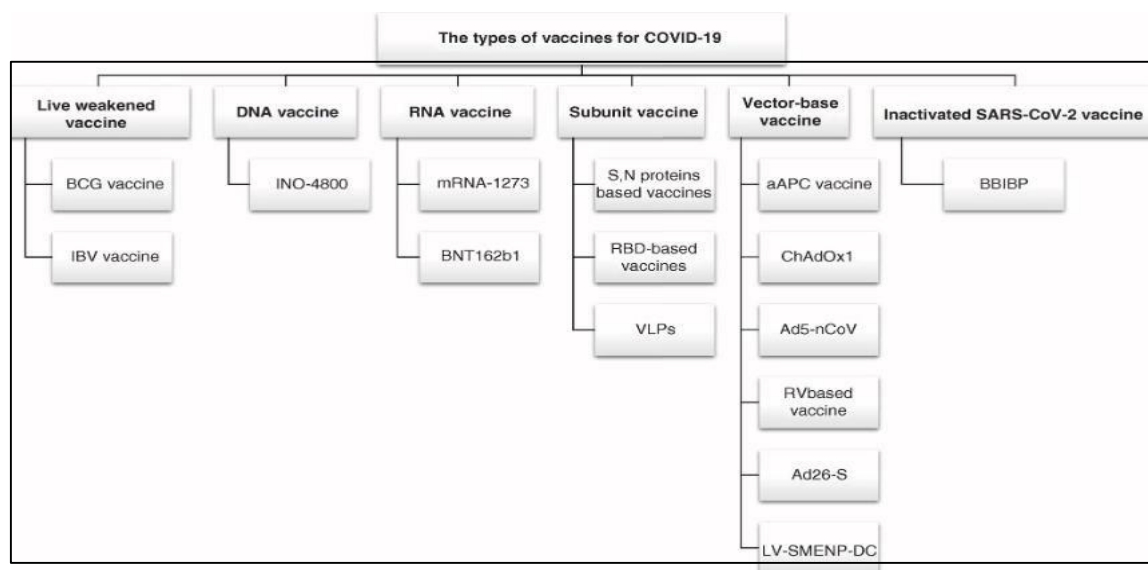


Figure 2-5: Summary of strategies for COVID-19 vaccine development compared with classical approaches (Shahcheraghi *et al.*,2021)

To expedite vaccine development and ensure equitable global distribution, the WHO is collaborating with scientists, businesses, and global health organizations through the COVAX initiative (led by WHO, GAVI CEPI). The BNT162b1 vaccine (Pfizer-BioNTech), developed by BioNTech and manufactured by Pfizer in collaboration with Fosun Pharmaceutical, is the first COVID-19 vaccine to receive emergency use authorization (EUA). Clinical trials involving 44,000 individuals have shown it to be over 95% effective (Pfizer *et al.*, 2020). The AZD1222 vaccine developed by Oxford (Voysey *et al.*, 2021) has also recently received emergency approval and has demonstrated over 70% effectiveness against COVID-19 Acute Respiratory Syndrome following clinical testing on 65,000 individuals (AstraZeneca, 2020). The mechanisms and processes of approved BNT162b1, Sinopharm, and Oxford/AstraZeneca vaccines are illustrated in figure 2-6

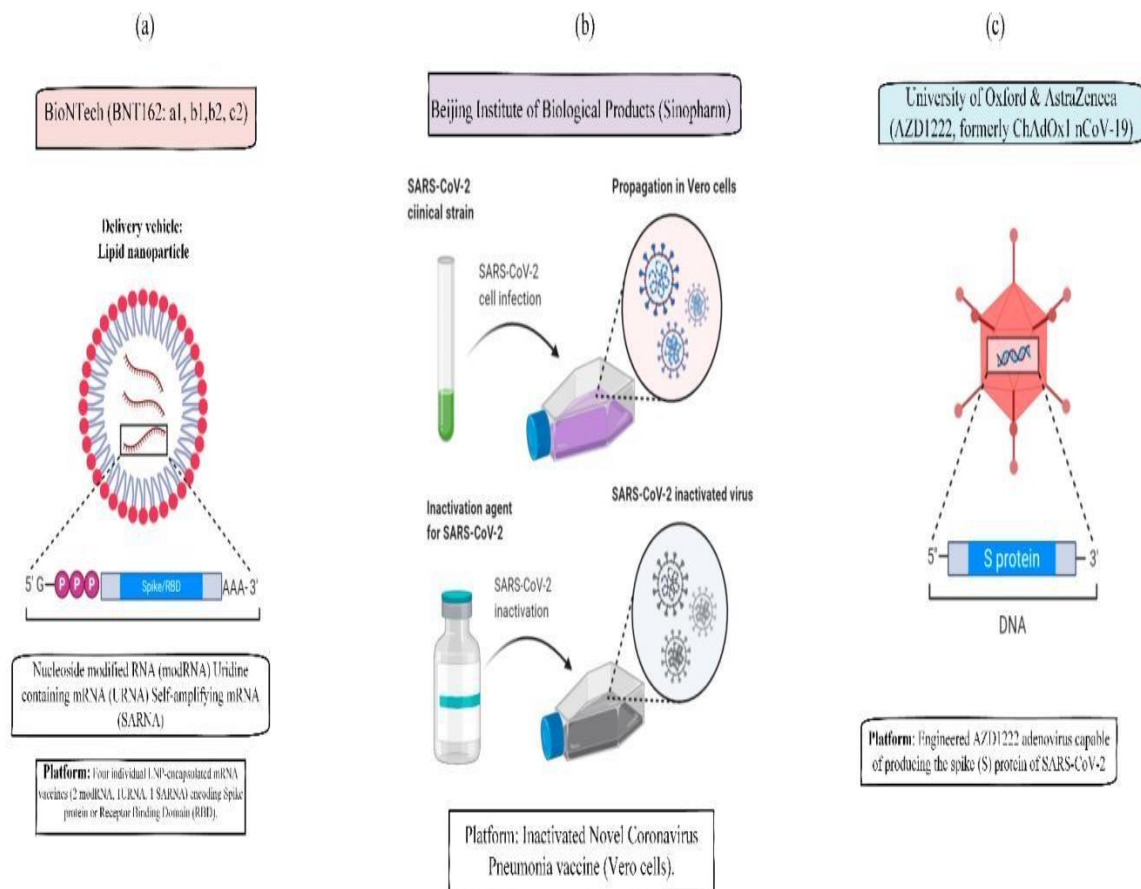


Figure 2-6: The mechanism and type of process of the approved (a) BNT162b2, (b) Sinopharm and (c) Oxford/AstraZeneca vaccine

2.8 The cardiovascular system and COVID-19

Cardiovascular disease (CVD) is a common comorbidity in patients infected with SARS and MERS, with prevalence rates of 10% and 30%, respectively (Clerkin *et al.*, 2020; Driggin *et al.*, 2020; Madjid *et al.*, 2020; Nishiga *et al.*, 2020). Early reports from China indicated that CVD, along with risk factors such as hypertension and diabetes mellitus, frequently coexists with COVID-19 (Wang *et al.*, 2020). An initial study from Wuhan, analyzing 41 hospitalized COVID-19 patients as of January 2, 2020, found that 32% had at least one comorbidity, with diabetes (20%), hypertension (15%), and other cardiovascular diseases (15%) being the most common. Subsequent research confirmed these findings and highlighted the significant prevalence of these conditions among COVID-19 patients (Guan *et al.*, 2020; Wang *et al.*, 2020; Wu and McGoogan, 2020; Zhou *et al.*, 2020).

2.9 Cardiac Enzymes in COVID-19

Enzymes play crucial roles in numerous biochemical reactions within the human body and are integral to both health and disease (Alves *et al.*, 2022). In the context of COVID-19, interactions between the virus and host enzymes lead to various physiological alterations. Severe COVID-19 patients exhibit notable biochemical abnormalities, including elevated levels of C-reactive protein (CRP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lipase, and thrombin, along with decreased lymphocyte counts (lymphopenia). Cardiac enzymes, such as thrombin, troponin, and creatine kinase, are elevated in the circulation, often indicating myocardial necrosis, as observed in myocardial infarction (Alves *et al.*, 2022).

2.9.1 Creatine Phosphokinase-MB

COVID-19 primarily affects the respiratory system, but its impact on individuals with pre-existing cardiovascular conditions is critical, as they face an elevated risk of mortality. The severity of COVID-19 is closely linked to

cardiovascular symptoms, highlighting the importance of understanding the relationship between the virus and cardiovascular complications. Cardiac myosin heavy chains (MYH6 and MYH7) are crucial for heart muscle function, and mutations in these proteins are associated with heart muscle diseases. Recent studies indicate that SARS-CoV-2 infection leads to the cleavage of MYH6/7 by the viral papain-like protease (PLpro), resulting in heart muscle damage. Secondary myocardial injury in COVID-19 patients is associated with a mortality rate exceeding 50%, underscoring the importance of monitoring cardiac biomarkers. Elevated levels of cardiac biomarkers such as troponin I, NT-pro BNP, CK-MB, and MYO have been linked to severe disease progression, reduced survival rates, and an increased incidence of heart failure, sepsis, and multiorgan failure (Qin, Cheng., *et al.*, 2020; Shafi ., *et al.*, 2020; Alves ., *et al.*, 2022).

2.9.2 Myoglobin

Myoglobin (Mb) is a dark red cytoplasmic hemoprotein that is exclusively present in cardiac myocytes and oxidative skeletal muscle fibers. It is a member of the super globin protein family and consists of a single polypeptide chain with 154 amino acids and a porphyrin ring containing a central ferrous iron molecule (Gondal *et al.*, 2023). Like hemoglobin, myoglobin has the ability to bind oxygen reversibly, forming oxymyoglobin, carboxy myoglobin, or metmyoglobin. However, unlike hemoglobin, myoglobin has just one oxygen binding site with a very high affinity. This allows myoglobin to receive oxygen from hemoglobin at the tissue level through the Bohr effect and either store it or transport it to muscle cells during periods of hypoxia, anoxia, or increased metabolic activity (Aydin *et al.*, 2019; Gondal *et al.*, 2023)

Studies have shown that severe COVID-19 patients often experience muscle wasting and dysfunction, potentially contributing to increased Mb release into the bloodstream in response to inflammation. Oxidized Mb has also been proposed as a marker of myocardial inflammation. Inflammatory

responses, including lymphopenia and cytokine storm, are closely linked to severe COVID-19 and high mortality rates (Ma *et al.*, 2021).

2.9.3 Troponin

Cardiac troponin T (cTnT) and troponin I (cTnI) are proteins that regulate the interaction between actin and myosin mediated by calcium in the heart, When it comes to identifying cardiac muscle damage, measuring serum cTnI and cTnT is more sensitive and specific than measuring cardiac muscle enzymes. Elevated levels of cardiac troponins are now widely recognized as the standard biochemical indicator for diagnosing myocardial infarction (Sharma *et al.*, 2004).

An elevation in cTn levels can signal myocardial injury, which may stem from various factors such as hypoxia, systemic inflammation, thrombosis, embolism, or sepsis. Increased cTn levels are frequently observed in severe cases of COVID-19 and in critically ill patients unrelated to COVID-19, (Kim and Aronow ,2021). In research involving patients with elevated cTnI levels, indicating cardiac injury, there was a clear association between cardiac injury and a lower survival rate, underscoring the link between myocardial health and the severity of COVID-19. However, cTnI has been found to have the highest predictive value for patient risk, making it the most promising marker for identifying patients at risk of developing a severe systemic inflammatory response to SARS-CoV-2 infection and subsequent potentially fatal cardiac complications (Arcari *et al.*, 2020; Franks *et al.*, 2020; Kim and Aronow , 2021).

2.10 Pathological signs of COVID-19 virus in cardiovascular disorders

Although the predominant clinical manifestation of COVID-19 is viral pneumonia, COVID-19 can also cause cardiovascular disorders such as myocardial injury, arrhythmias, ACS(Acute coronary syndrome), and thromboembolism (Figure 2-7), Some patients who present without the typical

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symptoms of fever or cough have cardiac symptoms as the first clinical manifestation of COVID-19 (Wang *et al.*, 2020; Zhou *et al.*, 2020).

ACE inhibitors and angiotensin II receptor blockers (ARBs) are medications commonly prescribed to lower the risk of cardiovascular diseases. These drugs have various effects that could potentially impact the susceptibility to and severity of COVID-19. (Shi *et al.*, 2020; Riphagen *et al.*, 2020).

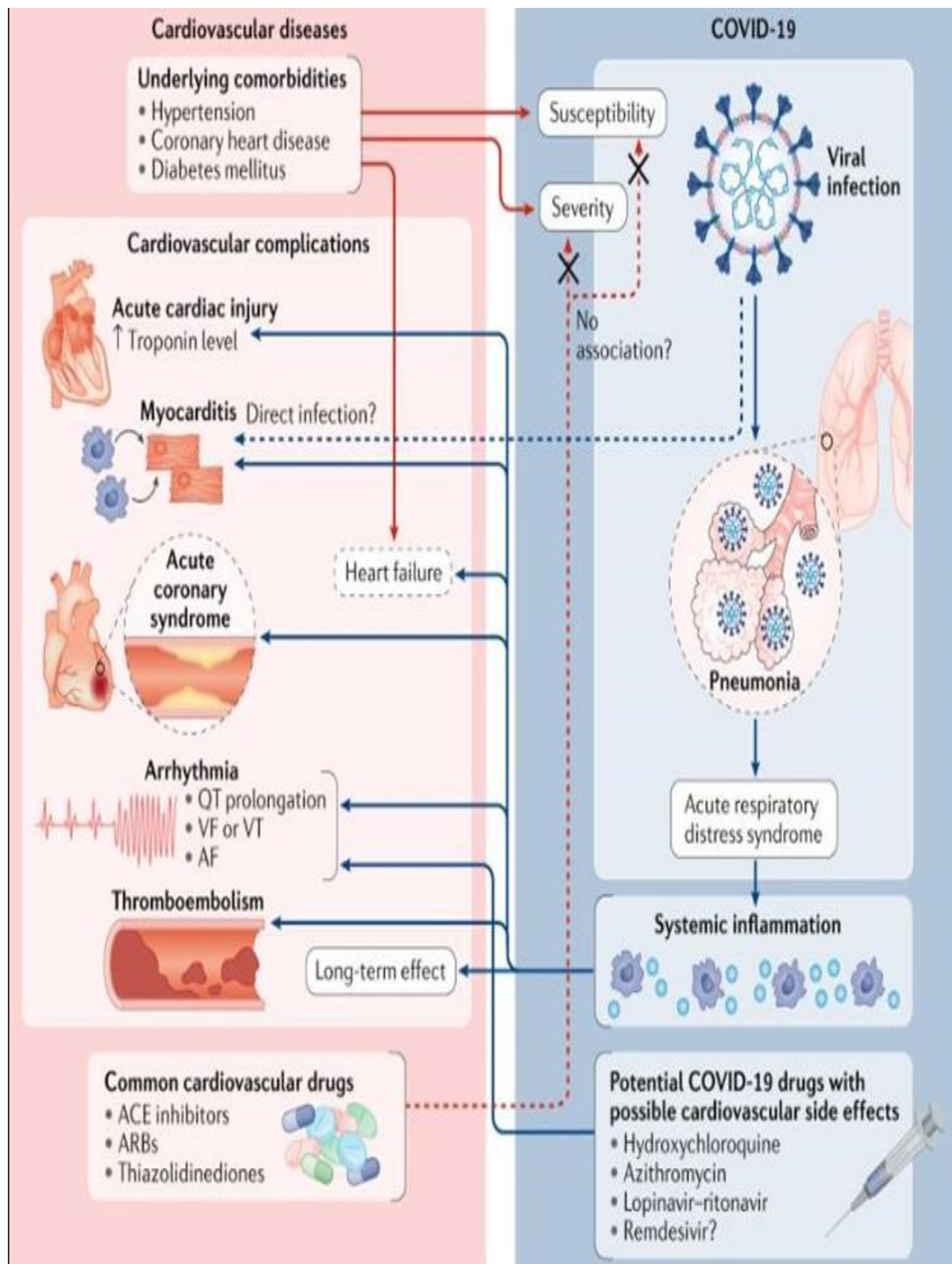


Figure 2-7: Cardiovascular comorbidities such as hypertension and coronary artery disease are associated with high mortality in patients with COVID-19. AF, atrial fibrillation; VF, ventricular fibrillation; VT, ventricular tachycardia (Wang et al., 2020).

### 2.10.1 Myocardial injury and myocarditis

In initial studies from China, it was found that a notable proportion of COVID-19 patients experienced acute myocardial injury, as indicated by elevated cardiac biomarkers or electrocardiographic abnormalities, with an incidence ranging from 7% to 20% (Clerkin *et al.*, 2020; Wang *et al.*, 2020; Zhou *et al.*, 2020). Following initial infection, SARS-CoV-2 replicates in nasal and pulmonary epithelial cells and subsequently spreads throughout the body, infecting cells that express ACE2 and other key entry proteins, such as proteases and integrin co-receptors. Cardiac myocytes, fibroblasts, and pericytes also express ACE2, facilitating viral entry into these cells (Boulos *et al.*, 2023; Sankrityayan *et al.*, 2020).

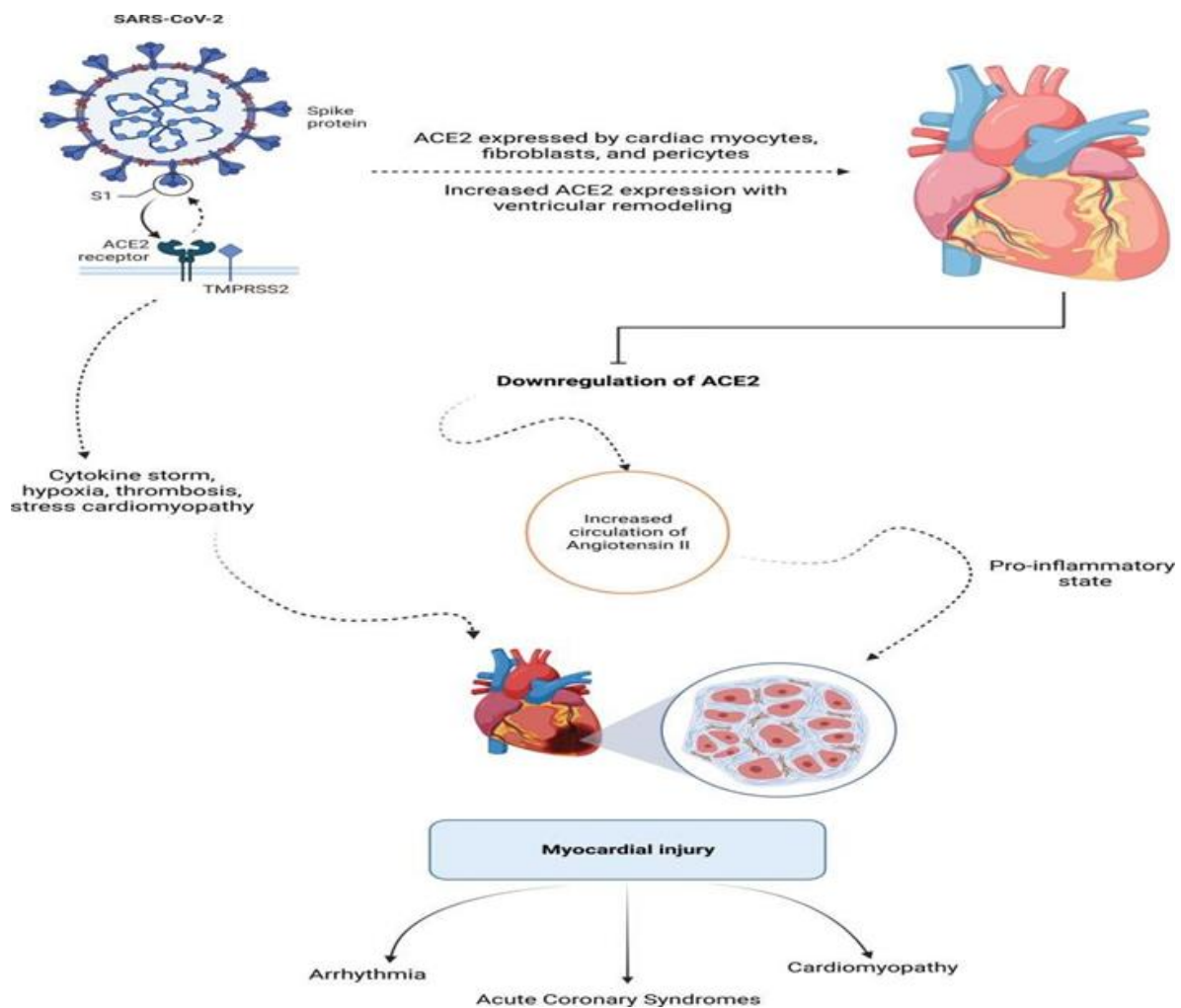


Figure 2-8: Proposed mechanisms of myocardial injury from COVID-19 (Boulos *et al.*, 2023)

### 2.10.2 Arrhythmia

Arrhythmia, a condition characterized by irregular heart rhythms, is a prevalent cardiac manifestation of COVID-19. It has been observed in 17% of patients hospitalized due to the virus and in up to 44% of patients admitted to the intensive care unit (ICU), as reported from Wuhan in the early stages of the pandemic. It is worth noting that the occurrence of arrhythmia as a complication of COVID-19 was found to be second only to the development of acute respiratory distress syndrome in an early study (Wang *et al.*, 2020). *Atrial fibrillation* is the prevailing arrhythmia observed in patients who experience arrhythmia. Nevertheless, the precise impact of COVID-19 on cardiac arrhythmias remains uncertain because arrhythmias, including atrial and ventricular tachycardia and fibrillation, can be instigated by myocardial injury or other systemic factors such as fever, sepsis, hypoxia, and electrolyte imbalances (Gopinathannair *et al.*, 2020; Saha *et al.*, 2022).

### 2.10.3 Heart Failure

In an early study from Wuhan involving 799 patients, heart failure was one of the most commonly observed complications of COVID-19, with a reported incidence of 24% in all patients and 49% in patients who died. Elevated levels of amino-terminal pro-B-type natriuretic peptide were identified in 49% of all patients (85% of those who died). Similarly, in another study of 191 patients in Wuhan, heart failure was identified in 23% of all patients and in 52% of patients who died (Chen *et al.*, 2020; Zhou *et al.*, 2020). The etiology of acute or decompensated heart failure in individuals with COVID-19 has not been thoroughly investigated. However, it is essential to consider that COVID-19 patients are often older and have pre-existing comorbidities such as coronary artery disease, hypertension, and diabetes. It is possible that heart failure in these patients could be a result of the worsening of these pre-existing conditions, whether they were previously diagnosed or unknown, or the revelation of subclinical cardiac dysfunction. Specifically, elderly patients with impaired

diastolic function may develop heart failure with preserved ejection fraction during the course of COVID-19 (Dewey *et al.*, 2020; Manka *et al.*, 2020, Mehra and Ruschitzka., 2020).

#### 2.10.4 Coagulation Abnormalities and Thrombosis

COVID-19 is associated with coagulation abnormalities, which can result in thromboembolic events(De Rosa *et al.*, 2020). Patients with COVID-19 often have elevated levels of D-dimer, and slightly prolonged prothrombin time. In an early study of 1,099 patients with COVID-19 from China, elevated levels of D-dimer ( $>0.5$  mg/l) were observed in 46% of all patients (60% of those with severe illness) (Guan *et al.*, 2020).

The occurrence of heightened thromboembolic events in individuals with COVID19 based on clinical observations indicates the presence of an increased tendency for blood clot formation. Venous thromboembolism, encompassing deep vein thrombosis and pulmonary embolism, is a frequently encountered complication in critically ill COVID-19 patients. The precise mechanisms responsible for these coagulation abnormalities, particularly the state of hypercoagulation, in the context of COVID-19 remain uncertain. One hypothesis proposes that the intense inflammatory response and damage to the endothelial cells caused by COVID-19, in conjunction with pre-existing comorbidities, may render patients more susceptible to developing a hypercoagulable state (Bikdeli *et al.*, 2020; Feng *et al.*, 2020). Of note, certain antiviral medications and investigational therapies given to these patients might promote thrombosis or bleeding events through drug–drug interactions with antiplatelet agents and anticoagulants (Sanders *et al.*, 2020).

#### 2.10.5 Angina and Atherosclerosis

Atherosclerosis, a chronic inflammatory and degenerative disease of large- and medium-sized arteries characterized by focal intimal thickening, is a leading cause of cardiovascular diseases (CVDs), the primary contributor to global

mortality. Angina pectoris, resulting from reduced blood and oxygen supply to the heart, is often linked to coronary atherosclerosis caused by arterial narrowing or blood clots (Welen Schef *et al.*, 2022; Vilaplana-Carnerero *et al.*, 2023).

There is a significant connection between inflammatory responses, COVID-19, atherosclerosis, and acute coronary syndromes. Evidence suggests a bidirectional relationship between COVID-19 and atherosclerosis, where SARS-CoV-2, like influenza, is associated with increased myocardial injury, acute coronary syndromes, thromboembolism, and other cardiovascular complications (Wang *et al.*, 2020; Ahmed *et al.*, 2022; Chaves *et al.*, 2023; Sagris *et al.*, 2021). This link may be due to viral-induced endothelial dysfunction, where SARS-CoV-2 targets endothelial cells and cardiac pericytes expressing angiotensin-converting enzyme 2 (ACE2), using transmembrane serine protease 2 and cathepsin L for entry. The virus disrupts immune responses, alters the renin-angiotensin-aldosterone system, induces vasoconstriction, and creates a prothrombotic environment by activating coagulation and inhibiting fibrinolysis, affecting cardiovascular, pulmonary, and immune functions (Liu *et al.*, 2020; Hasan *et al.*, 2022; Vilaplana-Carnerero *et al.*, 2023).

### 2.10.6 Ventricular Septal Defect

Ventricular septal defect (VSD) is the most common congenital heart defect in children and the second most common in adults. It results from an abnormal connection between the right and left ventricles, leading to a shunt that can cause complications like pulmonary arterial hypertension (PAH), ventricular dysfunction, and arrhythmias. While some VSDs close spontaneously, larger defects pose greater risks (Spicer *et al.*, 2014; Mavroudis *et al.*, 2023). VSDs can be isolated or occur with other congenital conditions like Down syndrome, where congenital heart disease is a leading cause of mortality in children under two years old (Spicer *et al.*, 2014). VSDs arise from disruptions during heart development,

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affecting around 30-60% of newborns with congenital heart defects, with an incidence of 2-6 per 1,000 births. Although many VSDs close naturally, their connection to COVID-19 in children remains under-researched (Spicer et al., 2014; Ghosh et al., 2018; Mavroudis et al., 2023).

2.11 Blood Viscosity in Patients with COVID-19

Blood viscosity, which refers to the resistance of blood flow, is determined by factors such as red blood cell deformability, hematocrit, red cell aggregation, and plasma viscosity. Elevated blood viscosity is often linked to inflammatory conditions, including viral infections, and can result in hyperviscosity syndrome (Al-Kuraishy *et al.*, 2022). In COVID-19 patients, SARS-CoV-2 infection can reduce erythrocyte deformability and increase aggregation, alongside elevated fibrinogen levels, leading to increased blood viscosity and the potential for hyperviscosity syndrome. Contributing factors include endothelial dysfunction, hypoxia, exaggerated immune responses, and coagulation abnormalities. Fever, dehydration, and severe infection further worsen viscosity (Venter *et al.*, 2020; Renoux *et al.*, 2021).

COVID-19 virus patients often experience microcirculatory failure due to changes in erythrocyte deformability and aggregation, contributing to stasis and increased viscosity. Additionally, hyperviscosity in these patients triggers the release of arginine vasopressin, which promotes pro-inflammatory cytokine secretion via NF- κ B activation, worsening the condition. Elevated NF- κ B and NLRP3 inflammasome activity also alter erythrocyte membrane properties, reducing deformability (Nader *et al.*, 2020; Varga *et al.*, 2020; Al-Kuraishy *et al.*, 2022).

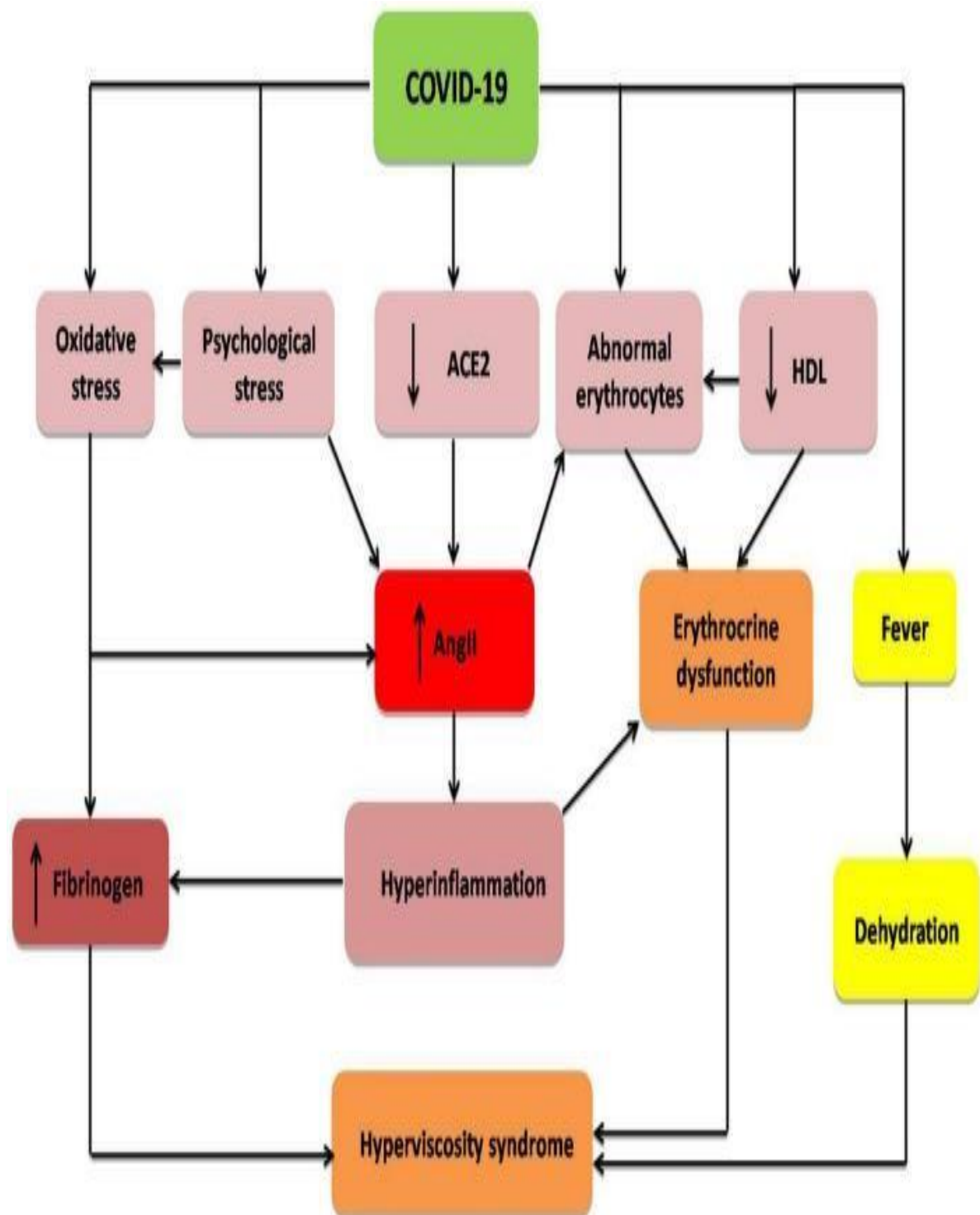


Figure 2-9: Mechanism of hyper viscosity syndrome in COVID-19: COVID-19 through down-regulation of angiotensin converting enzyme 2 (ACE2), psychological stress, hyper inflammation, oxidative stress, abnormal morphology of erythrocytes, and reduction of high density lipoprotein (HDL). These changes increase fibrinogen level and angiotensin II (AngII), with induction of erythrocyte dysfunction and subsequent development of hyperviscosity syndrome (Al-Kuraishy *et al.*, 2022).

CHAPTER THREE

MATERIALS AND

METHODS

3.1 Materials

3.1.1 Instruments and Equipments.

The instruments and equipment used in this work are listed below in table (3-1).

Table 3-1: Equipments and Instruments with their remarks standard

NO.	Tools	Company	Origin
1	96-well PCR Plate	Bioneer	Korea
2	Autoclave	HIRAYAMA	Japan
3	Centrifuge tubes (Sterile)-1.5ml	Bio basic	Canada Inc
4	Cold centrifuge	Nuve	Turky
5	Cool sample box	HEPO	Turkey
6	EDTA tube	Jiangsu Xinkang	China
7	Eppendorf tube	Sigma	England
8	Immunoassay Radiometer	Radiometer/AQT90 Flex	Denmark
9	Incubator	Heraeus	Germany
10	Micropipette 1.0-10ul	NEXTY-10	Japan
11	Micropipette 10 – 100ul	Slamed	Japan
12	Micropipette 100-1000ul	Slamed	Japan
13	Micropipette 50 – 200ul	Slamed	Japan
14	NanoDrop™ 8000 Spectrophotometer	Thermo Fisher Scientific	USA
15	PCR Tubes flat cup (Sterile) 0.2ml	Biobasic	Canada Inc
16	Roter-Gene Q	QIAGEN	USA
17	Shaker	Lab Tech	Korea
18	Sterile Syringe	CE	China
19	Tips	Sterellin Ltd	UK
20	Vortex mixer	Heidolph	Germany
21	VTM tube	TRUST LAB	China

3.1.2 Chemical Biological and Reagent Materials .

The chemical, biological materials and reagents used in this work are listed below in table (3-2).

Table 3-2: Chemical and Biological Materials

Materials	Manufacturer	Origin
Chloroform	SIGMA	USA
DEPC-Treated Water	Applied Biosystem	USA
Isopropanol	SIGMA	USA
RNase Zap RNase Decontamination Solution	Applied Biosystem	USA
TRIzol LS Reagent	Applied Biosystem	USA
Ultra-Pure DNase/RNase-Free Distilled Water	Applied Biosystem	USA
Ethanol (96%)	BDH	UK

3.2 Samples .

A total of 240 random samples were collected from 120 art disease patients diagnosed with COVID-19 in three hospitals (AlHussein Teaching Hospital, Nasiriyah General Hospital and Nasseria Heart Hospital). In addition, 60 positive control samples(patients with heart disease not infected with COVID19) and 60 negative control samples (patients with heart disease not infected with COVID-19) were used for troponin testing as shown in Figure 3-1. Samples for PCR and troponin were collected from patients with COVID-19 virus, respectively, the period extended from Friday, September 2023 to one month later in 2024, and also collect a questionnaire in(Table1,appendices) using VTM (viral transport medium) from the nasopharynx. VTM (viral transport medium nasopharyngeal swabs and troponinI (Tnl) tests (EDTA tubes), respectively.

3.2.1 Nasopharyngeal swab (NP):

A swab sterile was inserted into the nostril and gently advanced into the nasopharynx posterior to the nasal cavity, then rotated the swab for a few seconds to collect secretions. Swabs were stored midectly in appropriate keep samples at 2-8°C during transportation and storage.

3.2.2 Blood sampling .

First, the site of the needle's insertion was sterilized. Then, using a sterile syringe, a blood sample of about 3 ml was withdrawn from a vein in the arm and placed in the tube.

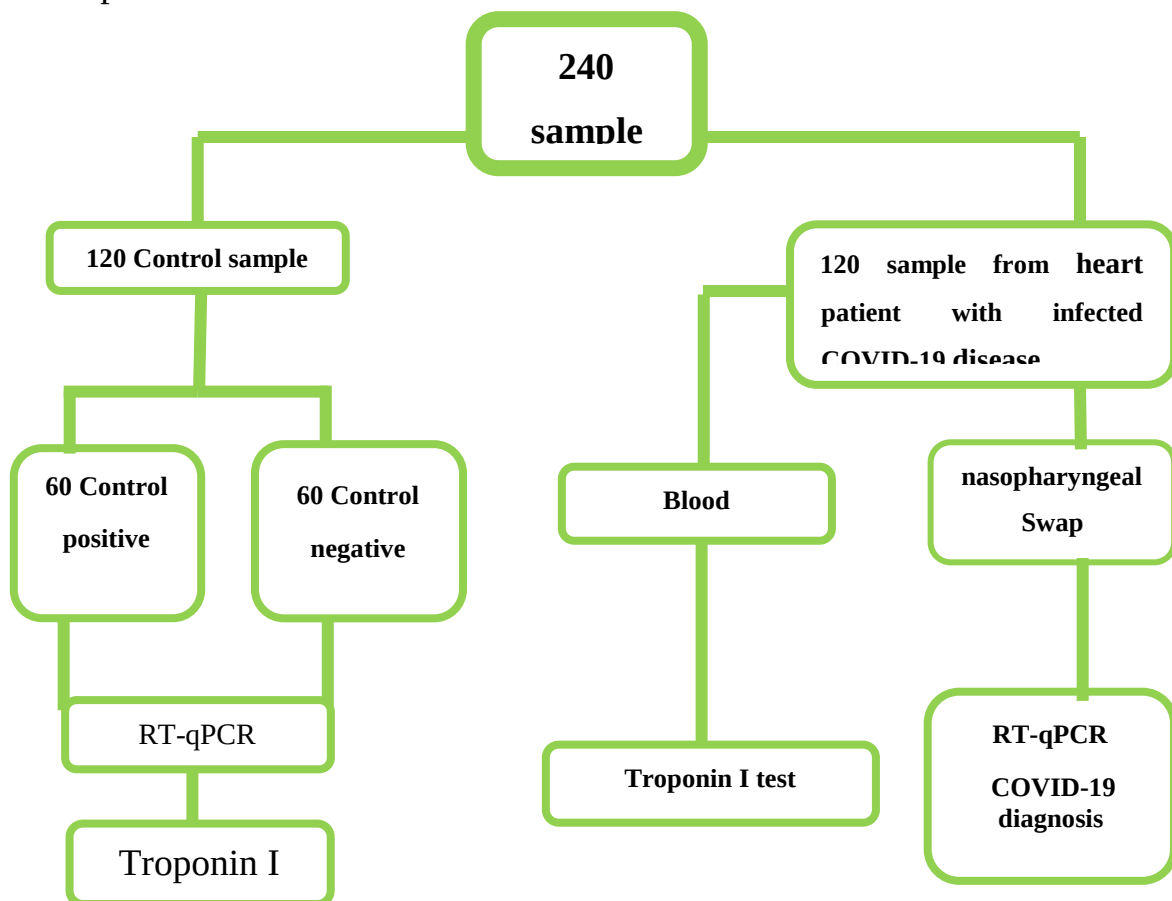


Figure 3-1: Study design

3.3 RNA extraction

EasyPure kit was used to extract Viral RNA from the studied samples. EasyPure® Viral RNA Kit utilizes a unique lysis buffer to lyse the virus and release RNA. It is suitable for isolating viral RNA from up to 200 µl of plasma, serum, whole blood, nasopharyngeal or oropharyngeal aspirate/wash, bronchoalveolar lavage fluid (BALF), tracheal aspirate, sputum, nasopharyngeal or oropharyngeal swab, and animal cell culture supernatant. Kit Contents are listed below (Table 3-3).

Component	ER201-01 (50 rxns)
Binding Buffer 5 (BB5)	15 ml
Wash Buffer 5 (WB5)	12 ml
Proteinase K (20 mg/ml)	1 ml
RNase-free Water	10 ml
RNase-free Tube (1.5 ml)	50 each
Spin Columns with Collection Tubes	50 each

Table 3-3: EasyPure kit contents

Protocol for Viral RNA Extraction:**1. Preparation of Reagents:**

- 48 ml of 96%-100% ethanol was added to 12 ml of WB5, mixed thoroughly, and labeled.

- BB5 with carrier RNA was prepared according to the kit instructions.

2. Sample Processing:

- 20 μ l of Proteinase K was added to a sterile 1.5 ml microcentrifuge tube.

- 200 μ l of the sample was added to the tube. If the sample volume was less than 200 μ l, PBS or 0.9% NaCl was added to reach a final volume of 200 μ l.

- 200 μ l of BB5 with carrier RNA was added, and the mixture was vortexed for 15 seconds.

- The mixture was incubated at 56°C for 15 minutes.

3. Ethanol Addition:

- 250 μ l of 96%-100% ethanol was added to the sample, and flocculation was noted.

- The sample was vortexed for 15 seconds and incubated at room temperature (15-25°C) for 15 minutes.

4. Centrifugation:

- The entire lysate was transferred to a spin column and centrifuged at 12,000 $\times$ g for 1 minute. The flow-through was discarded.

- 500 μ l of WB5 (with ethanol added) was added to the column, centrifuged at 12,000 $\times$ g for 1 minute, and the flow-through was discarded.

- The washing step was repeated with another 500 μ l of WB5, centrifuged at 12,000 $\times$ g for 1 minute, and the flow-through was discarded.

- An additional centrifugation step at 12,000×g for 1 minute was performed to remove residual ethanol and air-dry the membrane.

5. RNA Elution:

- The spin column was placed into a clean, RNase-free 1.5 ml microcentrifuge tube. 20-50 µl of RNase-free water was added to the center of the column, and it was incubated at room temperature for 1 minute. - The column was centrifuged at 12,000×g for 1 minute to elute the RNA. The eluted RNA was stored at -80°C. □ Addition of carrier RNA to BB5 Calculate the volume of BB5 with carrier RNA needed by selecting the number of samples to be simultaneously processed from Table 3-4. Gently mix by inverting the tube 10 times. To avoid foaming, do not vortex. For larger numbers of samples, volumes can be calculated using the following sample calculation: $N \times 0.21 \text{ ml} = A \text{ ml}$

$A \text{ ml} \times 28 \text{ µl/ml} = B \text{ µl}$ where: N = number of samples to be processed simultaneously

A = calculated volume of BB5

B = volume of carrier RNA to add to BB5

N	A	B	N	A	B
1	0.21 ml	5.9 µl	6	1.26 ml	35.4 µl
2	0.42 ml	11.8 µl	7	1.47 ml	41.3 µl
3	0.63 ml	17.7 µl	8	1.68 ml	47.2 µl
4	0.84 ml	23.6 µl	9	1.89 ml	53.1 µl
5	1.05 ml	29.5 µl	10	2.1 ml	59 µl

Table 3-4: Volumes of BB5 and carrier RNA required for different number of samples.

3.4 Measuring purity and concentration RNA with NanoDrop.

NanoDrop™ 8000 Spectrophotometer were was used to measure the amount and purity of purified RNA. NanoDrop Spectrophotometers are very convenient instruments for assessing RNA quantity and quality. This is how to use the NDS to measure RNA quantity, followed by a few points on interpreting the 260/280 and 260/230 ratios, essential indicators of RNA quality.

□ Procedure .

1. Place RNA samples and the water used for elution on ice before bringing them to the spectrophotometer.
2. Clean the sample reader with molecular-grade water and dry it with a KimWipe.
3. According to the software instructions, load 2 μL of elution water (blank) and initialize the system.
4. Set the computer to RNA mode and select the blank button.
5. Load 2 μL of the RNA sample and click the measure button.
6. Record the A260/A280 and A260/A230 ratios, as well as the RNA concentration (in ng/ μL), after the measurement is complete.
7. Clean the sample reader with a new KimWipe between samples and repeat steps 5-6. For optimal RNA purity, the A260/A280 ratio should be between 2.0 and 2.2, and the A260/A230 ratio should range from 0.1 to 0.8. These ratios reflect RNA purity, with higher values indicating lower contamination. RNA concentration can vary based on experimental conditions and extraction protocols

3.5 Primers

Oligonucleotide primer sets and probes sequence used for RT-qPCR amplification of specific COVID-19 genes used in this study are listed in table 3-5 (Corman *et al.*, 2020).

The primers were prepared according to the manufacturing instructions by dissolving the lyophilized primers with TE (Tris-EDTA) buffer to a stock solution with a concentration of 100 pmol/Ml. After spinning down and staying overnight at 4°C, the primers' working solution was prepared by diluting the stock solution with TE buffer using the equation $C_1V_1=C_2V_2$ (concentration versus volume) to get the final working solution (20 pmol/Ml) for each primer.

Gene	Oligonucleotide	Sequences 5'-3'	References
RdRP gene	RdRp_SARSr-F	GTGARATGGTCATGTGTGGCGG	Corman <i>et al.</i> , 2020
	RdRp_SARSr-P2	FAM- CAGGTGGAACCTCATCAGGAGATGC- BBQ	
	RdRp_SARSr-P1	FAM- CCAGGTGGWACRTCATCMGGTGATGC- BBQ	
	RdRp_SARSr-R	CARATGTTAAASACACTATTAGCATA	
E gene	E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT	
	E_Sarbeco_P1	FAM- ACACTAGCCATCCTTACTGCGCTTCG- BBQ	
	E_Sarbeco_R	ATATTGCAGCAGTACGCACACA	
N gene	N_Sarbeco_F	CACATTGGCACCCGCAATC	
	N_Sarbeco_P	FAM- ACTTCCTCAAGGAACAACATTGCCA- BBQ	
	N_Sarbeco_R	GAGGAACGAGAAGAGGCTTG	

W is A/T; R is G/A; M is A/C; S is G/C. FAM: 6-carboxyfluorescein; BBQ: blackberry quencher

Table 3- 5: Primers Sequences used with their references

3.6 Reverse transcriptase – quantitative PCR (RT-qPCR)

Reverse transcription-quantitative PCR (RT-qPCR) is widely acknowledged as the gold standard for virus detection and quantification, with extensive use in both diagnostic and research applications. Despite its effectiveness, direct detection of viruses is challenging due to the viral genome being encapsulated within a protein layer and surrounded by a lipid envelope. This complexity necessitates an additional RNA extraction step before RT-qPCR amplification, which can be cumbersome.

To address this challenge, we developed a direct RT-qPCR method for the detection of RNA viruses, bypassing the RNA extraction step. In a standard qPCR assay, reactions start with an equivalent amount of initial template, whether RNA (total RNA or mRNA) or cDNA. Variations in the observed outcomes post-qPCR are therefore attributed to differences in the initial concentration of the target genes. The threshold method operates on the principle that the fluorescent signal from qPCR amplification must exceed the detection limit of the instrument to be discernible. The cycle number required to achieve this threshold correlates with the initial quantity of the target in the sample: fewer cycles indicate higher initial copy numbers, while more cycles are indicative of lower copy numbers. Accurate baseline detection is critical for reliable measurements, as the baseline is determined by the system's detection limit. In this research, RT-qPCR was carried out using TranScript® II Probe One-Step qRT-PCR SuperMix (TransGen Biotech, China) with high sensitivity, synthesis, and amplification efficiency. It contains all the necessary reagents for cDNA synthesis and qPCR except probe, total RNA/mRNA template and gene specific primers.

Data analysis was performed by calculation the threshold cycle number (CT value) that presented the positive amplification for each positive sample. The components of the reaction are as in Table 3-6 (figure 3-2):

Table 3- 6: componentof for RT-qPCR

Component	volume	Final concentration
Total RNA	1pg~1ng	As required
Forward Primer	0.4μl	0.2μM
Reverse Primer	0.4μl	0.2μM
Probe (1 uM)	1μl	0.05μM
2x PerfectStarf TM M Probe One-Step qPCR SuperMix	10μl	1 X
TranScript* II Probe One-Step RT/RI Enzyme Mix	0.4μl	-
Passive Reference Dye (ROX) (Optional)	0.4μl	1 X
RNase-free Water	Variable Up To 20μl	-
Total volume	20 μl	-

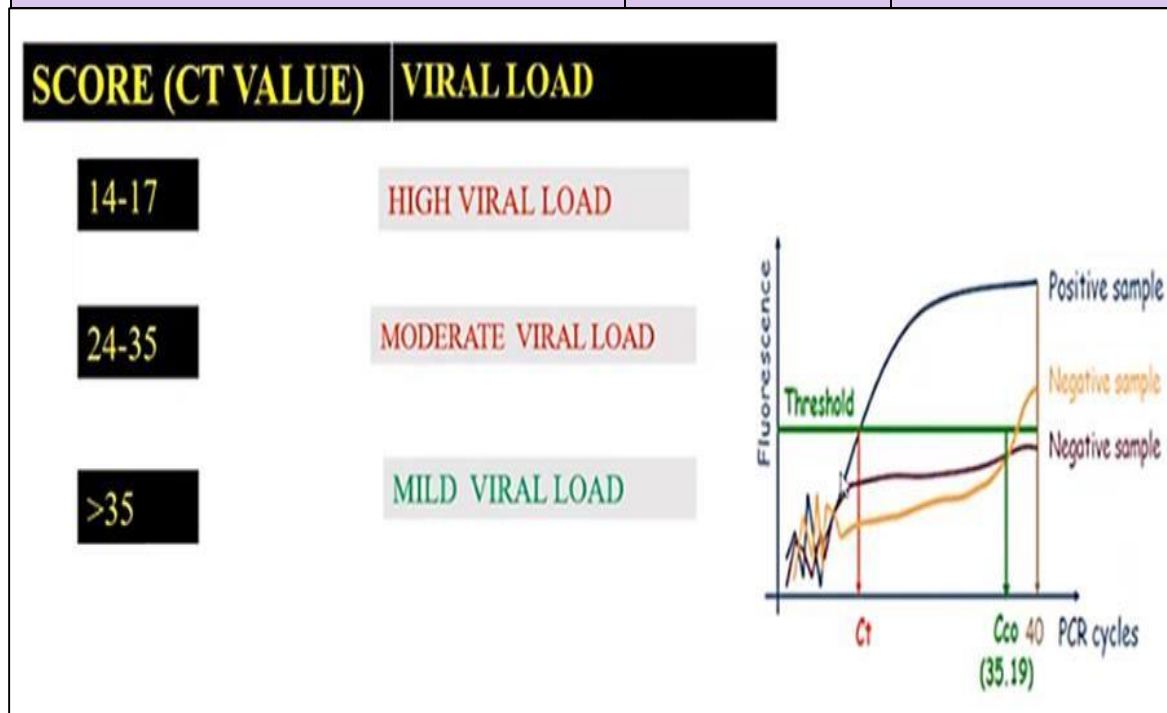


Figure 3-2: the vlues of ct score and its relation with the viral load or amplefication concentration of specific gene (TranScript® II Probe One-Step qRT-PCR (TransGen Biotech, China).

3.6.1 RT-qPCR quantitative

PCR quantitative for each gene were done by using a quantitative PCR thermocycler system, as shown in Table 3-7. After that, these PCR master mix reaction components mentioned, are placed in standard PCR tubes according to all other components needed for the PCR reaction, such as (RT-enzyme, master mix primers, and RNA template). Then, the tube was placed in a centrifuge and slightly centrifuged for 10 sec., and then transferred to the PCR thermocycler.

Table 3-7: PCR Amplification Program for RT- qPCR

Step	Temperature(C)	Time	No. of cycles
RT enzyme	50	5 min	1
Initial denaturation	94	30 sec	1
Denaturation	94	5 sec	40
Annealing	Variables according to (primers TM)	30sec	

3.6.2 Standard Curve for RT-qPCR in Viral Gene Detection

A standard curve is often needed to accurately quantify viral genes using RT-qPCR. Using a value indicator, such as the Ct (threshold cycle) or Cq (quantification cycle), can be informative for targeting a specific gene by primer and probe in qPCR. These indicators represent the cycle number at which the fluorescence signal of the amplification reaction reaches a predefined threshold level. A lower Ct or Cq value typically corresponds to a higher abundance of the target gene in the sample. Standard curve helps to establish a relationship between the cycle threshold (Ct) values obtained from the PCR reaction and the initial concentration of the target viral RNA in the samples. By plotting Ct values against known concentrations of standard samples, the standard curve enables the determination of viral RNA concentrations in unknown samples based on their Ct values. The standard curve is generated by plotting the Cq versus concentration (log concentration) for the standard curve samples (figure 3-3).

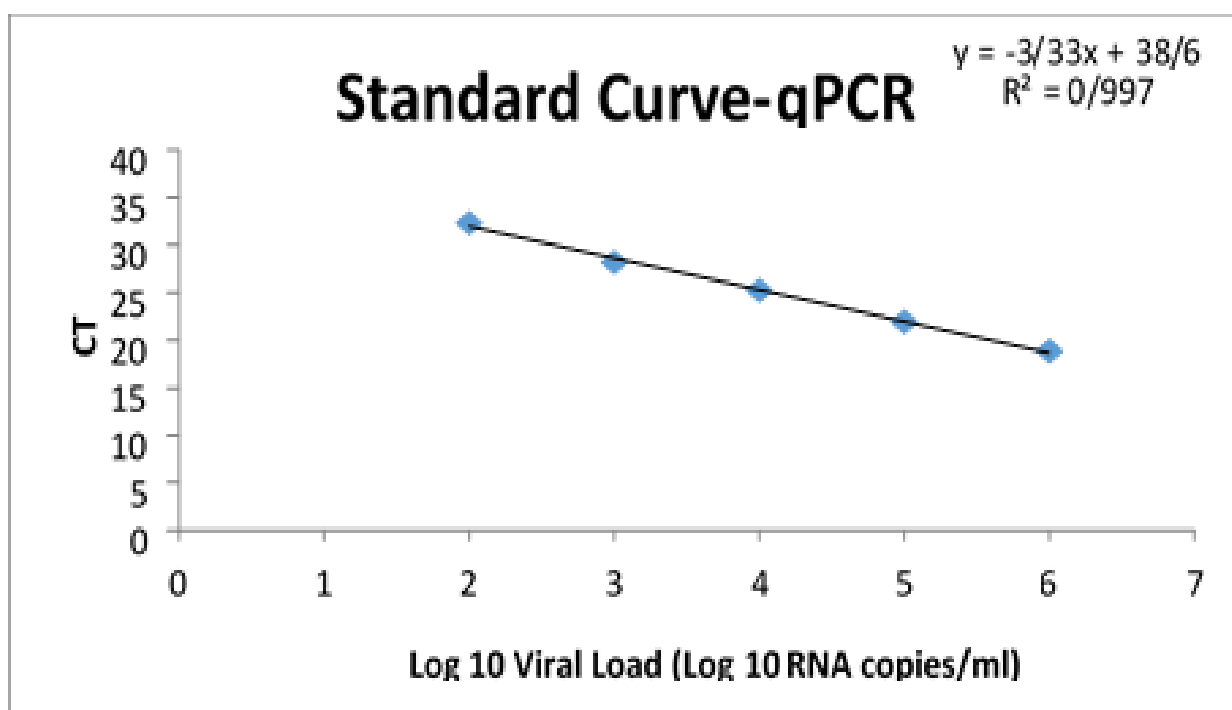


Figure 3-3: standard curve for the absolute quantification of viruses (round points). 10^6 dilution was not used for construction of calibration curve due to a non-linear response

3.7 Troponin Test

Cardiac troponin (cTn) I and T are released from myocardial cells following necrosis, i.e., cell death. An accurate measure of cTn concentrations in a patient's blood following ischemia/chest pain can enable providers to determine whether or not a myocardial infarction (MI) has occurred, (Messinger *et al.*, 2014; Amundson *et al.*, 2015).

AQT90 FLEX immunoassay analyzer evaluated troponin levels in blood samples by means of EDTA tubes. AQT90 FLEX is a comprehensive cardiac panel that tests for a variety of cardiac biomarkers, including cTnI, cTnT, CK-MB, D-dimer, and myoglobin. To begin the analysis, a minimum of 3 mL of sample in a capped blood collection tube should be placed directly into the inlet of the instrument. The two-site immunoassay utilizes an assay cup containing antibodies in a dry, stable form. Biotinylated monoclonal anti-cTn capture antibodies are immobilized to the streptavidin-coated surface of the assay cup, and tracer antibodies tagged with europium have been added above a separating layer. In the assay procedure, when the sample is added to the assay cup and incubated, capture and tracer antibodies bind to different epitopes on cTn, forming a sandwich complex. Following incubation, the assay cup is washed and dried. The europium signal emitted by the tracer antibody is then measured using time-resolved fluorometry. This signal is directly proportional to the concentration of cTnI or cTnT in the sample, depending on the specific test. The instrument calculates the analyte concentration using a built-in calibration curve (Messinger *et al.*, 2014; Amundson *et al.*, 2015).

3.8 Statistical analysis

The data were evaluated in the statistical program SPSS version 26 by using Chi-square. Statistical significance was defined as a probability P value 0.05.

CHAPTER FOUR

RESULTS

4. Result.

4.1 Purity of the extracted virus RNA

The results showed that all samples with a wavelength of 260/280 were between 2.0 and 2.2, and a wavelength of 260/230 was between 0.1 and 0.8. This indicates the presence of relatively pure RNA with acceptable purity levels.. All samples were within the acceptable range and were used in qRT.

4.2 Determination of COVID-19 virus genes in infected patients by RT-qPCR.

After extracting RNA from the examined isolates, the presence of three COVID indicator genes (RdRp, E, and N genes) in the extracted RNAs was investigated by RT- qPCR method (TranScript® II Probe One-Step qRT-PCR Super Mix, TransGen Biotech, China). The CT value was calculated for each of the 120 samples by treating them with the parameters of virus. The results showed the variation of the CT values between one sample and another, as some samples recorded a CT value between (9-25), and this indicates that the percentage of Viral Load is Very high, offset by this low value of CT. Some samples recorded values between (35-40), and this indicates a medium percentage of Viral load in those samples, then, some aggregates recorded (values higher than 35), which refers to milled Viral load in those samples.

Overall, results Ct values for RdRp, E and N genes were recorded to be around 30,32 and 15 respectively were shown that among the 120 examined samples, 95 samples contained three marker genes and were considered as COVID positive .25 samples lacked the mentioned genes and were considered as COVID negative samples. The amplification curve began at cycle 32 above the CT (cycle Threshold) line for RdRp, E and N genes as shown in Figure 4-1,4-2 and 4-3.

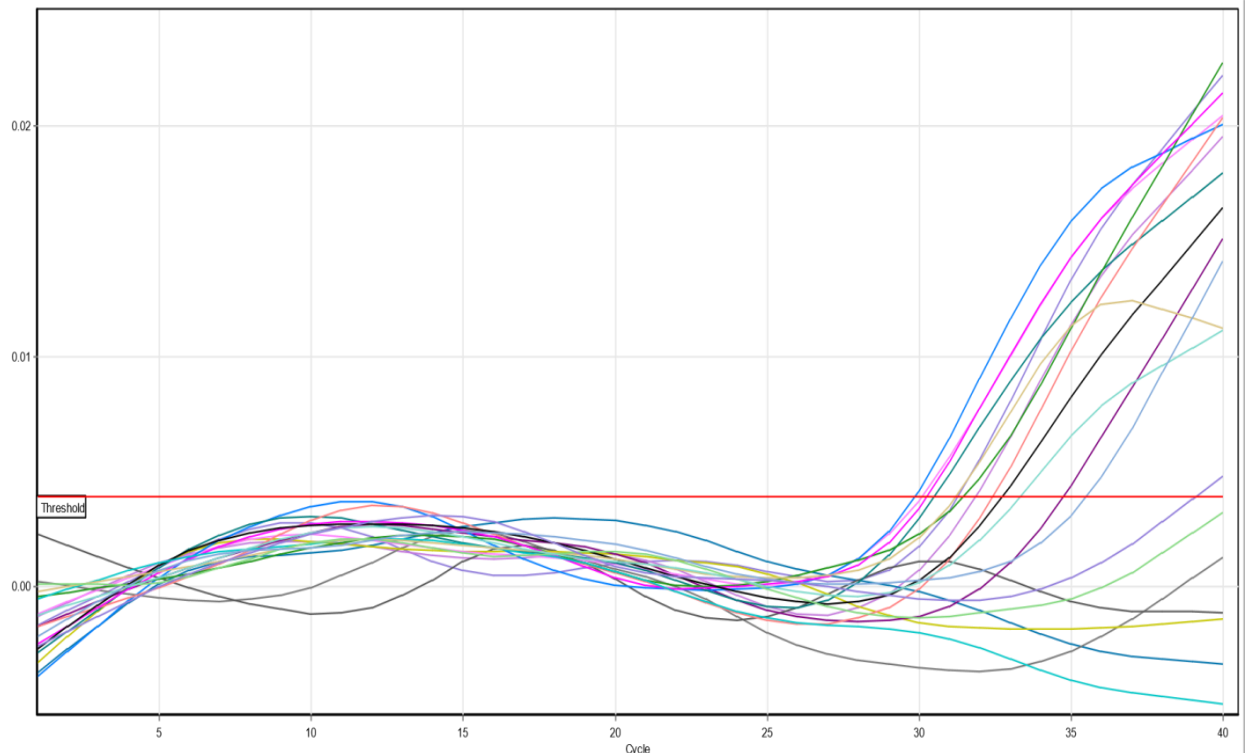


Figure 4-1: RdRp (RNA-depent RNA polymerase) + prop 1,2 gene amplification plots.

Figures 4-1, shows RdRp + P2 and RdRp + P1 genes amplification plots for 120 samples. The photograph was taken directly from qPCR machine. The amplification plot in qPCR depicts the fluorescence signal (y-axis) versus the cycle number (x-axis). Initially, there's minimal fluorescence due to background noise. As PCR cycles progress, the Viral RNA is amplified exponentially during the logarithmic phase. This results in a sharp increase in fluorescence, indicating the accumulation of amplicons. Eventually, amplification reaches a plateau phase where the reaction exhausts reagents, resulting in a minimal fluorescence increase. determining the Ct value. Ct represents the cycle number at which fluorescence surpasses the threshold, indicating detectable amplification. A lower Ct correlates with higher initial target concentration. Interpretation involves comparing Ct values among samples to quantify target abundance or perform comparative analysis. Ct for RdRp was Calculated to be around 30.

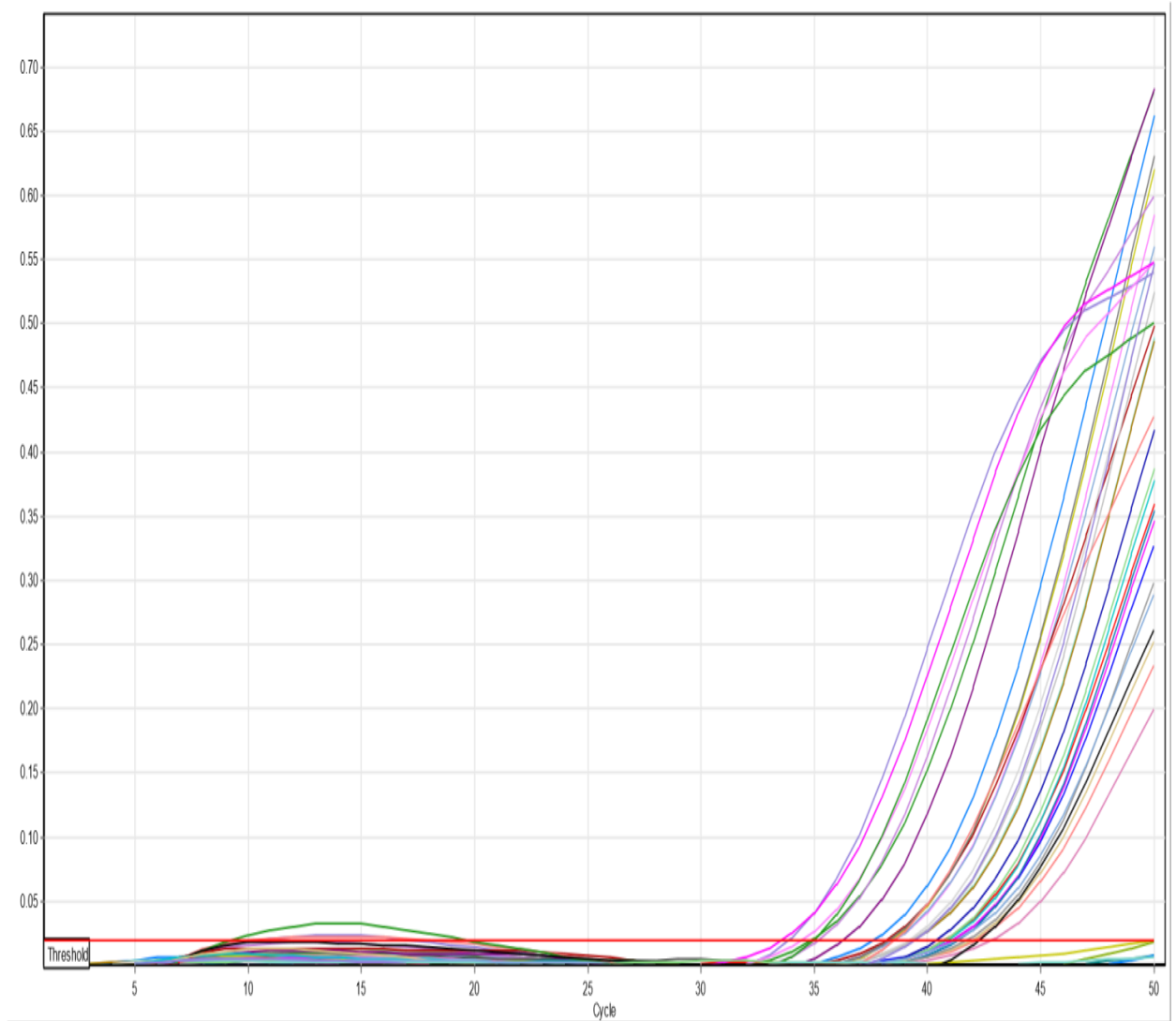


Figure 4-2: E gene (Envelope gene) amplification plots

Figure 4-2 shows E gene amplification plots for 120 samples. The threshold line, set above background noise, intersects the exponential phase's linear portion, determining the Ct value. Ct represents the cycle number at which fluorescence surpasses the threshold, indicating detectable amplification. The Ct for E was Calculated to be around 32, more than the RdRp gene.

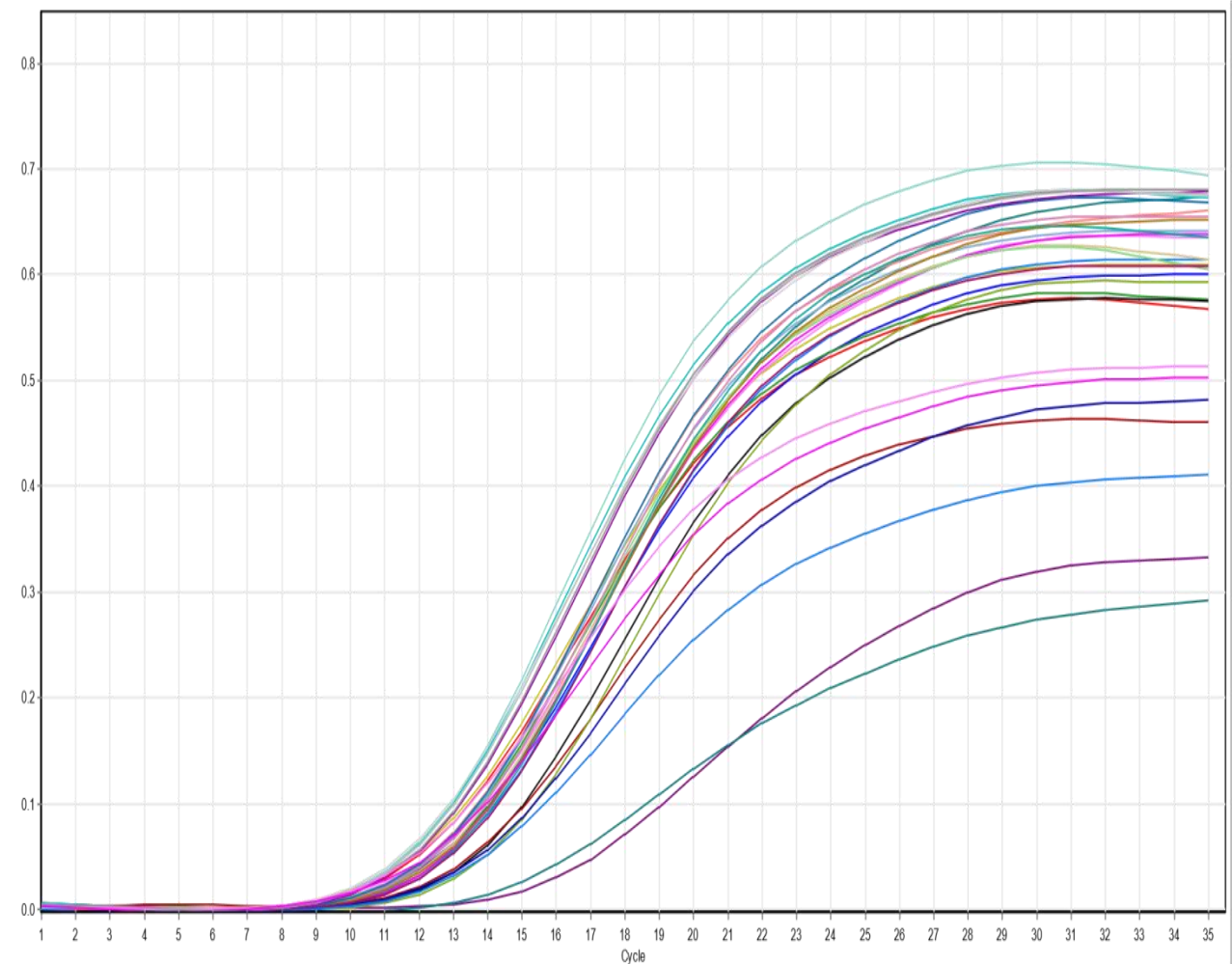


Figure 4-3: N gene (Nucleocapsid gene) amplification plots

Figure 4-3 also shows N gene amplification plots for 120 samples. The threshold line, set above background noise, intersects the exponential phase's linear portion, determining the Ct value. Ct represents the cycle number at which fluorescence surpasses the threshold, indicating detectable amplification. Ct for N was Calculated to be around 15 and less than both E and RdRp genes.

4.3 Distribution of COVID-19 virus infected patients according to sex.

The present study did not reveal statistically significant differences ($p > 0.05$) between male and female patients regarding COVID-19 infection, as indicated by a calculated chi-square value ($\text{Cal}x^2 = 0.767$) that is lower than the tabulated chi-square value ($\text{Tab}x^2 = 3.84$). However, the data showed that the majority of COVID-19 positive cases were among males more at 61.05%, while females accounted for 38.95% of the cases. In contrast, non-infected patients comprised 68.00% of the male group and 32.00% of the female group, as presented in Table 4-3.

Sex	Positive RT-qPCR		Negative RT-qPCR		Total	
	No.	%	No.	%	No.	%
Male	58/95	61.05	17/25	68.00	75	62.5
Female	37/95	38.95	8/25	32.00	45	37.5
Total	95	79.17	25	20.83	120	100
Cal.$x^2 = 0.767$ $\text{Tab}x^2 = 3.84$ DF= 1 p. value 0.381						

Table 4-3: Distribution of COVID-19 infected patients according to sex

4.4 Distribution of COVID-19 virus infected patients according to residency.

The present study demonstrated a statistically significant difference ($p < 0.05$) with a calculated chi-square value of $\text{Cal}\chi^2 = 83.2$, which is greater than the tabulated chi-square value of $\text{Tab}\chi^2 = 11.07$, regarding the residence of patients and COVID-19 infection. The highest number of COVID-19 cases was observed among residents in the city center of Nasiriyah, accounting for 67.37%, followed by those in Al-Nasr district at 10.53%. On the other hand, the lowest number of infected patients was recorded in Al-Shatrah and Al-Gharraf districts, both at 4.21%, as shown in Table 4-4.

Residency	Positive RT-qPCR		Negative RT-qPCR		Total	
	No.	%	No.	%	No.	%
Al- Nasiriyah	64/95	67.37	7/25	28.00	71	59.16
Al-Garaf	4/95	4.21	4/25	16.00	8	6.67
Al-Shatra	4/95	4.21	6/25	24.00	10	8.33
Al-Naser	10/95	10.53	2/25	8.00	12	10.0
Al-Rifai	8/95	8.42	3/25	12.00	11	9.17
Al-Suq	5/95	5.26	3/25	12.00	8	6.67
Total	95	79.17	25	20.83	120	100
$\text{Cal}\chi^2 = 83.2$ $\text{Tab}\chi^2 = 11.07$ $\text{DF} = 5$ $\text{p. value } 0.001^{**}$						

Table 4-4: Distribution of COVID-19 infected patients according to residency

4.5 Distribution of COVID-19 virus infected patients according to disease complications.

The present study revealed a highly significant difference ($p < 0.05$) with a calculated chi-square value of $Cal\chi^2 = 61.1$, which is greater than the tabulated value of $Tab\chi^2 = 11.07$, in relation to the complications observed in COVID-19 infected patients. The data indicated that a significant proportion of infected patients suffered from angina, accounting for 32.63%, followed by those with heart failure at 20.00%. In contrast, the lowest percentages were observed among patients with arteriosclerosis and myocardial infarction (14.74%) and ventricular septal defect (2.11%), as presented in Table 4-5.

Complications	Positive RT-qPCR		Negative RT-qPCR		Total	
	No.	%	No.	%	No.	%
Angina	31/95	32.63	5/25	20.00	36	30
Arrhythmia	15/95	15.79	2/25	8.00	17	14.17
Arteriosclerosis	14/95	14.74	4/25	16.00	18	15
MI	14/95	14.74	2/25	8.00	16	13.33
VSD	2/95	2.11	0/25	0.00	2	1.67
Heart failure	19/95	20.00	12/25	48.00	31	25.83
Total	95	79.17	25	20.83	120	100
Cal $\chi^2 = 61.1$ Tab $\chi^2 = 11.07$ DF= 5 p. value 0.001**						

□ MI=myocardial infarction. VSD= Ventricular Septal Defect

Table 4-5: Distribution of COVID-19 infected patients according to disease complications.

4.6 Distribution of COVID-19 virus infected patients according to collection period .

The present study recorded a highly significant difference ($p < 0.05$) with a calculated chi-square value of $\text{Cal}\chi^2 = 53.4$, exceeding the tabulated value of $\text{Tab}\chi^2 = 9.49$, concerning the collection period of samples from COVID-19 infected patients. The findings revealed that the highest percentage of infections occurred in December (27.37%), followed by November and October (19.66%), while the lowest infection rate was observed in January (10.53%), as shown in Table 4-6.

Time collection	Positive RT-qPCR		Negative RT-qPCR		Total	
	No.	%	No.	%	No.	%
September	19/95	20.00	2/25	8.00	21	17.50
October	20/95	21.05	5/25	20.00	25	20.83
November	20/95	21.05	6/25	24.00	26	21.67
December	26/95	27.37	3/25	12.00	29	24.17
January	10/95	10.53	9/25	36.00	19	15.83
Total	95	79.17	25	20.83	120	100
$\text{Cal}\chi^2 = 53.4$ $\text{Tab}\chi^2 = 9.49$ $\text{DF} = 4$ $p. \text{valu.} 0.001^{**}$						

Table 4-6: Distribution of COVID-19 infected patients according to collection period.

4.7 Distribution of COVID-19 Infected Patients According to Age Groups .

The present study demonstrated a highly significant difference ($p < 0.05$), with a calculated chi-square value ($Cal\chi^2 = 155.3$) greater than the tabulated value ($Tab\chi^2 = 15.51$) across different age groups among COVID-19-infected patients. The highest infection rate was observed in the 55-63 years age group at 24.21%, followed by the 46-54 years age group at 17.89%. In contrast, the lowest infection rates were recorded in the 91-98 years age group at 1.05% and the 82-90 years age group, as detailed in Table 4-7.

Age groups	Positive RT-qPCR		Negative RT-qPCR		Total	
	No.	%	No.	%	No.	%
19 – 27 years	10/95	10.53	1/25	4.00	11	9.17
28 – 36 years	5/95	5.26	2/25	8.00	7	5.83
37 – 45 years	11/95	11.58	4/25	16.00	15	12.50
46 – 54 years	17/95	17.89	3/25	12.00	20	16.67
55 – 63 years	23/95	24.21	7/25	28.00	30	25.0
64 – 72 years	15/95	15.79	3/25	12.00	18	15.0
73 – 81 years	11/95	11.58	1/25	4.00	12	10.0
82 – 90 years	2/95	2.11	2/25	8.00	4	3.33
91 – 98 years	1/95	1.05	2/25	8.00	3	2.50
Total	95	79.17	25	20.83	120	100
Cal χ^2 = 155.3 Tab χ^2 = 15.51 DF= 8 p. value . 0.001**						

Table 4-7: Distribution of COVID-19 infected patients according to age groups .

4.8 Distribution of COVID-19 virus infected patients according to level of troponin I.

The present study revealed a highly significant difference ($p < 0.05$) with a calculated chi-square value ($\text{Cal } \chi^2 = 350.6$) exceeding the tabulated value ($\text{Tab } \chi^2 = 12.59$) in relation to troponin levels among COVID-19-infected patients. The majority of infected patients had troponin levels ranging from 0.01 to 1.07 ng/mL (78.95%), followed by those with levels between 1.071 and 2.131 ng/mL (8.42%). Notably, no positive COVID-19 cases were recorded in patients with troponin levels of 3.193–4.253 ng/L, 4.254–5.314 ng/L, or 5.315–6.375 ng/L, as shown in Table 4-8.

Troponin I ng/L	Positive RT-q PCR		Negative RT-q PCR		Total	
	No.	%	No.	%	No.	%
0.010 – 1.070	75/95	78.95	13/25	52.00	88	73.33
1.071 – 2.131	8/95	8.42	4/25	16.00	12	10.0
2.132 – 3.192	3/95	3.16	1/25	4.00	4	3.33
3.193 – 4.253	0/95	0.00	3/25	12.0	3	2.50
4.254 – 5.314	0/95	0.00	0/25	0.00	0	0.00
5.315 – 6.375	0/95	0.00	2/25	8.00	2	1.68
6.376 – 7.436	3/95	3.16	1/25	4.00	4	3.33
7.437 – 8.500	6/95	6.32	1/25	4.00	7	5.00
Total	95	79.17	25	20.83	120	100
$\text{Cal } \chi^2 = 350.6$ $\text{Tab } \chi^2 = 12.59$ $\text{DF} = 7$ $\text{p. value } 0.001^{**}$						

* The normal range less than 0.023 ng/L

Table 4-8: Distribution of COVID-19 infected patients according to troponin level

4.9 Distribution of COVID-19 virus infected patients and control group according to heart disease and troponin I result .

The current study demonstrated a non-significant difference ($p > 0.05$), with a calculated chi-square value ($\text{Cal } \chi^2 = 0.150$) less than the tabulated value ($\text{Tab } \chi^2 = 3.84$), between the patient group, control group, and target group concerning heart disease. It was observed that 68.18% of heart disease patients infected with COVID-19 tested positive for cardiac troponin I at a higher rate, while 31.82% of the control group consisting of heart disease patients without COVID-19 also tested positive for cardiac troponin I, as shown in Table 4-9.

Heart disease	Positive Troponin I		Negative Troponin I		Total	
	No.	%	No.	%	No.	%
COVID-19 with heart diseases patients	45/120	68.18	75/120	65.79	120	66.67
Control group Heart without COVID-19	21/60	31.82	39/60	34.21	60	33.33
Total	66	36.67	114	63.33	180	100
Cal χ^2 = 0.150 Tab χ^2 = 3.84 DF = 1 p. value 0.699						

Table 4-9: Distribution of COVID-19 infected patients according to troponin result.

4.10 Association between level of troponin I and age groups in COVID-19 virus infected patients.

The present study revealed a highly significant difference ($p < 0.05$) with a calculated chi-square value ($\text{Cal}\chi^2 = 189.4$) greater than the tabulated value ($\text{Tab}\chi^2 = 15.51$) in relation to troponin levels among COVID-19-infected patients across different age groups. The highest positive troponin levels were observed in the age group of 46–54 years (24.44%) and 55–63 years (20.00%), while the lowest levels were noted in the age groups of 82–90 years (2.22%) and 28–36 years (4.44%), as shown in Table 4-10.

Age groups of patients	Positive troponin I		Negative troponin I		Total	
	No.	%	No.	%	No.	%
19 – 27 years	5/45	11.11	6/75	8.00	11	9.17
28 – 36 years	2/45	4.44	5/75	6.67	7	5.83
37 – 45 years	5/45	11.11	10/75	13.33	15	12.50
46 – 54 years	11/45	24.44	9/75	12.00	20	16.67
55 – 63 years	9/45	20.00	21/75	28.00	30	25.0
64 – 72 years	4/45	8.89	14/75	18.67	18	15.0
73 – 81 years	5/45	11.11	7/75	9.33	12	10.0
82 – 90 years	1/45	2.22	3/75	4.00	4	3.33
91 – 98 years	3/45	6.67	0/75	0.00	3	2.50
Total	45	37.50	75	62.50	120	100
Cal χ^2 = 189.4 Tab χ^2 = 15.51 DF= 8 p. value 0.001**						

Table 4-10: Association between level of troponin and age groups in COVID-19 infected patients.

4.11 Association between level of troponin I and age groups in Control group (heart disease without COVID-19 virus).

The present study demonstrated a highly significant difference ($p < 0.05$) with a calculated chi-square value ($Cal\chi^2 = 68.2$) exceeding the tabulated value ($Tab\chi^2 = 15.51$) concerning troponin levels in COVID-19-infected patients across various age groups. The highest positive troponin levels were observed in the age groups of 37–45 years and 55–63 years, both at 19.05%. Intermediate levels were found in the 28–36 years and 46–54 years age groups, each at 9.52%. The lowest positive troponin levels were noted in the 91–98 years age group, at 4.76%, as detailed in Table 4-11.

Age groups of control	Positive troponin I		Negative troponin I		Total	
	No.	%	No.	%	No.	%
19 – 27 years	3/21	14.29	4/39	10.26	7	11.67
28 – 36 years	2/21	9.52	3/39	7.69	5	8.33
37 – 45 years	4/21	19.05	5/39	12.82	9	15.00
46 – 54 years	2/21	9.52	5/39	12.82	7	11.67
55 – 63 years	4/21	19.05	9/39	23.08	13	21.66
64 – 72 years	3/21	14.29	4/39	10.26	7	11.67
73 – 81 years	2/21	9.52	3/39	7.69	5	8.33
82 – 90 years	0/21	0.00	4/39	10.26	4	6.67
91 – 98 years	1/21	4.76	2/39	5.13	3	5.00
Total	21	35.00	39	65.00	60	100
Cal $\chi^2 = 68.2$ Tab $\chi^2 = 15.51$ DF= 8 p. value 0.001**						

Table 4-11 : Association between level of troponin and age groups in control group.

4.12 Comparison between heart patients and control group (positive and negative) based on troponin I level result.

The current study revealed a statistically significant difference between the groups with a p-value < 0.05 , where the calculated chi-square ($\text{Cal}\chi^2 = 11.7$) was greater than the tabulated value ($\text{Tab}\chi^2 = 5.99$). In the control group of non-COVID-19 heart disease patients, the troponin test yielded positive results in 35.0% of cases and negative results in 65.0%. For the target group of heart disease patients with COVID-19, 37.5% tested positive for troponin, while 62.5% tested negative. In the positive control group of COVID-19 patients, 56.67% tested positive for troponin and 43.33% tested negative. The highest troponin positivity rate was observed in the positive control group.

Troponin I Test	Control group negative		Patients with COVID-19 and heart disease		Control group positive	
	No.	%	No.	%	No.	%
Positive	21/60	35.0	45/120	37.5	34/60	56.67
Negative	39/60	65.0	75/120	62.5	26/60	43.33
Total	60	25.0	120	50.0	60	25.0
$\text{Cal}\chi^2 = 11.7$ $\text{Tab}\chi^2 = 5.99$ $\text{DF} = 2$ p. value 0.003						

Table 4-12: Comparison of troponin I in COVID-19 patients and control Group.

CHAPTER FIVE

DISCUSSION

5.1 Determination of COVID-19 virus genes in Infected Patients by RT-qPCR

During the COVID-19 pandemic, the RT- qPCR is routinely used for detecting COVID19 infection (Benrahma *et al.*, 2020). The study evaluated three target genes—envelope (E), RNA-dependent RNA polymerase (RdRp), and nucleocapsid (N)—for viral detection. A result is considered positive if the viral detection threshold cycle (CT) is less than 40. (Appak *et al.*, 2021).

In a Turkish study involving 886 samples from infected patients diagnosed by RT-qPCR, the Colden method for viral detection was utilized (Pierri *et al.*, 2022).

The study of Cho *et al.* (2020) conducted, the viral gene included RbRp, N, and E genes were the most genes used in the RT-qPCR test to detect the COVID-19 virus in various samples, also showed the false positive results can be due to contamination of the testing centers or handling. The CT threshold value for the genes ranged between 14.58, 29.6, and 38.68 respectively.

In addition, the study of Gülbudak *et al.* (2021) included 355 patients with clinically diagnosed COVID-19, their age above 17 years, and they were tested using RT-qPCR technology for (N,RdRp) genes and investigated 237 (66.7%) of symptomatic patients were positive and 118 (33.2%) of asymptomatic patients, had threshold cycle CT values for the RdRp gene higher than symptomatic patient, as well as in patients who had comorbidities such as diabetes, tachycardia, and cardiovascular disease.

The study of Kostakoglu *et al.* (2021) indicates that out of 103 of COVID-19 patients who were diagnosed by RT-qPCR, 42 of patients gave negative result, while 61 patients gave positive results and their CT values ranged between (35 - 40). This study concluded that a negative RT-qPCR test result cannot be excluded in patients whose disease symptoms last more than five days.

In this study reveals RT-qPCR a great value for the detection of COVID19 infection in individuals and Ct values for RdRp, E and N genes were recorded to be around 30,32 and 15 respectively. The results showed that among the 120 patients, 95(79.17%) of samples give positive results for three involved genes and were considered as COVID positive, and the other 25 (20.83%) samples give negative results for mentioned genes and were considered as COVID-19 negative.

5.2 Distribution of COVID-19 virus Infected Patients According to the Age and Sex

Age and sex are two important independent factors related to the risk and outcome of various diseases, In the current study within positive cases approximately 61.05% of patients were males and 38.95% females with non-significant difference was observed between sex, According to age groups the study recorded that most of patients, 24.21% were in age groupe 55 to 63 years. However, it was shown that in several studies in both Iraq and out of Iraq had varying prevalence rates according to age group. This study is different from prior research that conducted by Abbas and Manhal, (2021) in Baghdad, who investigate that 41.4% of positive patients diagnosis by PCR their age between 26-40 years, also showed the age consider a risk factors predisposing to COVID-19.

The study of ABD, (2020), assessment prevalence of COVID-19 Pandemic at Al-Nasiriyah City respondents that from 460 samples, 50.9% were female and 49.1% were male with non-significant difference was observed between different sex as indicated in current study, while differ with current study with regard age was noted most samples (47.2%) were detected between the age category of 21-30 years.

don by Abd, (2021), at Al-Nasiriya city investigated prevalence and explore the risk factors of COVID-19, and recorded among 696 cases, high percentage of cases in patients with age (15-49), their study reveals that residence, chronic

disease (diabetes and heart and lung diseases), immune-compromised therapy, are play important role in increasing COVID-19 infection.

The study by Abd *et al.* (2023) assessed the prevalence of COVID-19 infections from October 2021 to June 2022 at Al Hussein Teaching Hospital in Thi-Qar. Among the 88 positive patients, 47 (53.4%) were male, while 41 (45.8%) were female, indicating a significant gender disparity. The study also found that the majority of patients were aged between 45 and 54 years, a finding consistent with the current study.

Conversely, a recent cohort study by Musa *et al.* (2024), which included 389 COVID-19 patients admitted to the same hospital during 2021, reported a different trend. This study observed that most infected patients were younger, with 255 (65.6%) aged between 19 and 40 years, while only 18 (4.6%) were older than 65 years.

A local study by Musa (2020) also investigated the epidemiological patterns of the COVID-19 epidemic during the initial phase at Al-Hussein Teaching Hospital. The results indicated that 57.2% of the patients were male, while 42.8% were female. The study found that the highest infection rate, 30.61%, was among patients aged 14 to 26 years. In contrast, only 4% of patients aged 66 to 78 years and 79 to 91 years tested positive. These findings are consistent with the results obtained in the current study.

The study of Benrahma *et al.* (2020) in Moroccan evaluated an epidemiological description and analysis for RdRp, E and N genes by RT-PCR of SARSCoV-2, and involved 376 patients, 189 (50.3 %) had negative results and 187 (49.7%) had positive results for COVID-19 RT-PCR tests. Among positives cases, the sex distribution was 41.83% women and 58.14% men which is likewise with from our results. In another study conducting by Rehman *et al.* (2022) investigates the association between SARS-CoV-2 cycle threshold to age and sex and showed that COVID-19 was more prevalent in males as compared to female, and patients that age above 50 years.

Gombos et al. (2021), in a study conducted in Hungary, analyzed RT-qPCR testing for COVID-19 and found a notable, though statistically non-significant, difference in age at diagnosis between positive and negative cases. They observed that ICU admission was significantly more frequent among PCR-positive cases compared to PCR-negative cases. Additionally, among the positive cases, 47 (54.7%) were male and 39 (45.3%) were female. This similar with results in this study.

According to sex difference favoring men has also been observed in some studies and suggests that the virus is more likely to infect men (Baggett *et al.*, 2020; Wang *et al.*, 2020; Xie *et al.*, 2020) which is consistent with the results of this study.

The results of our study agreed with previous studies that indicate that infection with Covid-19 is affected by the age factor, This is due to the immune system in the elderly being less responsive to diseases, which makes them more susceptible to infection.

5-3 Distribution of COVID-19 virus infected patients according to the residency

The current study indicated that the largest proportion of COVID-19 positive samples were obtained from the Thi-Qar Province center in Nasiriyah, accounting for 67.37% of the total cases. This was followed by the Al-Nasr district, which had an infection rate of 10.53%.

Saeed *et al.* (2021) this study investigates the epidemiological characteristics of COVID-19 in rural areas, noting that infection rates are typically lower compared to urban settings due to factors such as reduced population density and fewer opportunities for widespread transmission. However, rural communities encounter considerable challenges, including limited access to healthcare services and medical resources, which can delay diagnosis and lead to more severe disease outcomes.

Hirsch *et al.* (2022) this research explores the effects of rural settings on the spread of COVID-19, emphasizing that preventive measures such as social distancing and mask-wearing may be less rigorously enforced due to lower levels of awareness or perceptions of reduced risk. Consequently, despite lower infection rates, rural areas may face more severe consequences due to inadequate healthcare infrastructure.

Barouki *et al.* (2021) this article examines the intersection of the COVID-19 pandemic with global environmental changes, including urbanization and climate factors. It highlights the necessity for further research into how these environmental factors influence the spread and outcomes of COVID-19, particularly in regions with limited healthcare resources, such as rural areas.

5-4 Distribution of COVID-19 virus infected patients according to collection period

Climatic characteristics play a major role in influencing several diseases that are caused by different pathogen including viruses, SARS-CoV-2 is a seasonal virus, and its transfer can be predicted (Al-Jabri, 2022).

Another factor that has been investigated ,In this study is the months of sample collection. The results showed significant difference (p. value < 0.05) between collection period and in December showed the highest frequency of COVID-19 patients with 27.37%.

Smith *et al.*(2021)this article analyzes the trends in COVID-19 cases observed specifically in December 2020. It highlights a notable surge in infections during this month, attributed to factors such as increased indoor gatherings and the effects of colder weather on viral transmission. The study discusses the implications of this surge for public health policies and emphasizes the need for targeted interventions to manage the increased case loads and mitigate further spread of the virus during the winter season.

The study of Al-Obaidi *et al.* (2023) similar with current study investigated that the COVID-19 distributed in different manners with weather in Iraq, they recorded highest COVID-19 infection in September and the lowest in January during 2020.

Jebril (2020) reported that the highest air temperatures in Iraq occurred during the indoor environment summer season , from June 22 to September 22. Given that the summer weather in Iraq is characterized by hot, dry conditions, the relative humidity during this period is notably lower than in other seasons. These environmental conditions are considered highly conducive to the transmission and spread of the COVID-19 virus.

5-5 Distribution of COVID-19 virus Infected Patients According to the Disease Complications.

Several studies showed that the COVID-19 had a greater prevalence of in the patients with chronic disease, and in patients with cardiovascular disease register >7% of the patients with the infection experienced myocardial injury (Naz and Billah ,2021).

In a particular study conducted by Zhou *et al.*, (2020), involved 191 patients in Wuhan, China, 48% of patients had co-morbidities, and 8% had cardiovascular disease. Moreover, Eight studies conducted had meta-analysis results that showed that amongst comorbidities, hypertension ($17 \pm 7\%$), Diabetes mellitus ($8 \pm 6\%$), and cardiovascular disease ($5 \pm 4\%$) were the most prevalent in COVID-19 (Yang *et al.*, 2020).

COVID-19 has been associated with various cardiovascular complications such as heart attacks, arrhythmias, and cardiomyopathy, which are considered risk factors for cardiovascular disease. The virus is believed to potentially harm the heart directly, likely through a cytokine storm that leads to non-ischemic myocardial injury (Mishra *et al.*, 2020; Adu-Amankwaah *et al.*, 2021).

The progression of heart deterioration involves multiple factors and stages, often resulting in severe outcomes. In addition to direct viral damage, early infection stages trigger inflammatory responses and immune cell reactions that contribute to cardiac injury. Later stages of infection may involve a combination of viral and immune-mediated damage. (Zhu *et al.*, 2020).

The complex cytokine storm induced by SARS-CoV-2 infection involves various host immune response factors and can lead to significant inflammation and lung issues, as well as potential cardiovascular complications (Adu-Amankwaah *et al.*, 2021; Alqahtani *et al.*, 2022). Although COVID-19 virus primarily affects the respiratory system, it can also lead to conditions like acute myopericarditis, acute coronary syndrome, congestive heart failure, cardiogenic shock, and cardiac arrhythmias (Liu *et al.*, 2021).

The virus enters cells through ACE2 receptors found in certain organs, contributing to its impact on cardiovascular health. Epidemiological data suggests that patients with COVID-19 who have pre-existing cardiovascular risk factors are at higher risk of severe illness and death. Those with more severe symptoms are more likely to experience heart damage or irregular heart rhythms, (Zhu *et al.*, 2020, Liu *et al.*, 2021; Alqahtani *et al.*, 2022).

In this study, the relationship between patients with COVID-19 and the presence of other underlying diseases in the tested samples were investigated, it was determined that Ventricular septal defect Angina and Heart failure with 32.63% and 20.00% are predominant and VSD and MI with 2.11% and 14.74% have the lowest frequency.

Coromilas *et al.* (2021) conducted a retrospective analysis of patients hospitalized globally with COVID-19, examining the occurrence of cardiac arrhythmias. Their study found that cardiac arrhythmias were prevalent and associated with high morbidity and mortality in these patients. Among the 4,526 patients analyzed, 827 developed cardiac arrhythmias during hospitalization. The

mean age of this cohort was 71.1 ± 14.1 years, with 65% being male. These findings contrast with those of the current study.

5.6 Evaluation of level troponin I in COVID-19 virus infected patients

The American College of Cardiology suggested that the high troponin level can be predicate for myocardial infarction in suspected COVID-19 patients (Cordeanu *et al.*, 2020). Since then, myocardial infection has been described in approximately one-third of COVID-19 patients in several studies (Guo *et al.*, 2020; Lala *et al.*, 2020; Shi *et al.*, 2020; Wang *et al.*, 2020; Zou *et al.*, 2020; Majure *et al.*, 2021).

The National Health Commission of China reported that 12% of hospitalized patients without known cardiovascular disease exhibited high levels of troponin (Zheng *et al.*, 2020). Troponin, a component of cardiomyocytes' contractile apparatus, is a specific biomarker for acute myocardial infection (Thygesen *et al.*, 2012).

Immunocompromised patients and those with comorbidities generally experience more severe symptoms of COVID-19. Particularly, individuals with pre-existing atherosclerosis may be more vulnerable to ischemic events due to SARS-CoV-2's activation of inflammatory pathways and coagulation abnormalities. This susceptibility may elucidate the pathological effects of SARS-CoV-2. Moreover, the increased expression of ACE2 receptors in myocardial cells could further explain the link between COVID-19 and cardiac health issues. Elevated levels of cardiac biomarkers, indicating myocardial injury, (Naz and Billah, 2021). were observed in early cases in China, with 7.2% of hospitalized patients in Wuhan exhibiting increased high-sensitivity cardiac troponin I levels (Wang *et al.*, 2020).

The current study was noted high significant difference (p. value < 0.05) according to level of troponin in COVID-19 infected patients was showed the

high COVID-19 infected patients with (0.01-1.07) 78.95% troponin level, while the lowest infected patients in group of troponin level was (2.132-3.192) and (6.376-7.436) 3.16%. of patients with heart disease were positive for troponin and COVID-19.

The study of Khalid *et al.* (2022), evaluated serum levels of homocysteine, Troponin-I, and high sensitive CRP in Iraqi COVID-19 Patients that hospitalized in Al-Ataa, and Dhare Alphaeadh hospitals, during the period from October/2021 to February/2022, their results indicated the troponin level evaluation significantly in patients with cardiac problem specially Troponin-I in compare to control group.

5.7 Association between Troponin I Level and Age Groups in COVID-19 Infected Patients.

The relationship between troponin levels and age groups was also evaluated. The results show that there is a significant relationship at (p. value < 0.05) between the age groups and troponin level. The highest positive troponin level was showed in the age group (46-54years) 24.44%, and in the age group (55-63 years) 20.00%, while in control group (patient with heart disease and without COVID-19), highest positive troponin level was showed in the age group (37-45 years) and (55-63) 19.05%.

An American study (Lami *et al.* ,2021) indicated that high troponin levels may be common in the elderly and may not always be an indicator of a severe heart attack, but rather may be the result of age-related heart changes.

The study conducted in London of Papageorgiou *et al.* (2022), 434 patients diagnosed with Covid-19 and suffering from comorbidities such as chronic kidney disease and high blood pressure, in addition to their advanced age, they were more susceptible to elevated troponin levels.

Evaluating the association between troponin levels and disease severity in patients infected with Covid-19, it emerged that age is a critical factor, as older

patients showed lower survival rates at ($p < 0.001$) than those over 50 years of age. (John *et al.*, 2024)

The study of Shi *et al.* (2020) A large COVID-19 patients, it was observed that have myocardial infection detected upon admission was associated with an increased likelihood of in-hospital mortality, Among 416 patients hospitalized with COVID-19 in China, 82 patients had initial levels of cardiac troponin I (cTn-I) exceeding the normal value.

5.8 Comparison between cardiac patients with COVID-19 and the control group (positive and negative) based on Troponin I level result.

The current study identified a significant disparity in troponin I levels between COVID-19 patients and the control group. Specifically, 56.67% of COVID-19 patients exhibited positive troponin I results. Among those, 37.5% also had concurrent heart disease. In comparison, only 35.0% of individuals in the control group with heart disease but without COVID-19 tested positive for troponin I.

A recent study by Moll-Bernardes *et al.* (2022) supports the findings of the current study. This research, which examined the utility of routine troponin measurement for predicting adverse outcomes in a large multicenter cohort. The prevalence of myocardial infarction did not significantly differ by sex. Troponin levels were slightly elevated in 127 patients (8.6%), moderately elevated in 87 patients (5.9%), and severely elevated in 139 patients (9.4%). Myocardial infarction, identified by elevated troponin levels during the initial week of hospitalization, was found in 23.9% of patients who had troponin assessments. Patients with cardiac infection had a higher in-hospital mortality rate, which increased with the severity of troponin elevation. Additionally, in-hospital mortality was associated with age.

Also, the study of Shi *et al.* (2020) agreed with this study, was reported that cardiac infection was a common complication in COVID-19 patients was (19.7%)

associated with a greater risk of in-hospital mortality in 416 patients hospitalized with in Wuhan, China.

Furthermore, the study of Guo *et al.* (2020) demonstrated that patients with COVID-19 virus and myocardial infection were in high prevalent in elderly and had a greater prevalence of chronic cardiovascular conditions.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions and Recommendations

Conclusions

The study concluded the following statements:

1. The highest incidence of heart diseases associated with Angina and Arrhythmia was recorded in patients infected with COVID-19.
2. There is a notable increase in COVID-19 incidence among patients aged 55 to 63 years.
3. COVID-19 infection rates rise in southern Iraq during December.
4. The sex of patients is not considered a contributing factor.
5. Troponin I levels are elevated specifically in COVID-19 patients compared to those with both heart disease and COVID-19, as well as patients with heart disease alone.

Conclusions and Recommendations

Recommendations

- 1- An analysis of alternative approaches for detecting mutations in the SARS-CoV-2 virus in heart disease patients.
- 2- Study of other troponin types related to COVID-19 infected patient by other serological technique.
- 3- Study the relationship between COVID-19 infections with other types of heart disease.
4. Evaluate the efficacy of vaccination in reducing severe outcomes in heart disease patients infected with new SARS-CoV-2 variants.
5. Investigate the impact of long COVID symptoms on the progression of heart disease in patients with a history of SARS-CoV-2 infection.

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# APPENDICES

## Appendices

| <i>COVID-19 Patients Questionnaire</i>  |      |        |
|-----------------------------------------|------|--------|
| Sex                                     | Male | Female |
| Age                                     |      |        |
| Place of residence                      |      |        |
| Accompanying heart diseases             |      |        |
| according to duration of months in year |      |        |

Table (1) : Questionnaire for COVID-19 Patients .

MINISTRY OF HEALTH  
THI-QAR HEALTH DIRECTORATE  
Department of Training and Human Development  
No. :  
Date : / /

وزارة الصحة  
دائرة صحة ذي قار  
قسم التدريب والتنمية البشرية  
شعبة إدارة البحوث والمعرفة  
العدد :  
التاريخ : ٢٠٢٣/٩/١٠

الى / مستشفى الناصرية التعليمي

م / تسهيل مهمة

تحية طيبة..  
إشارة الى كتاب جامعة الشطرة / مكتب رئيس الجامعة ذي العدد ٤٨١ بتاريخ ٢٠٢٣/٩/١٠ وبناء على موافقتكم المبدئية على استمارة مشروع البحث المقدمة من قبل الباحث، تم عرض الاستمارة على لجنة البحوث في دائرتنا خلال جلسته الأسبوعية وكان قرار اللجنة:

((الموافقة على إجراء مشروع البحث بصيغته المقدمة ولا مانع من تنفيذه في مؤسسات الدائرة))

نرفق ربطا قرار لجنة البحوث ذي العدد (٢٠٢٢/١٩٢) لا مانع لدينا من تسهيل مهمته على ان لا تتحمل دائرتنا هي تبعات مالية أو قانونية وان يلتزم الباحث بالاعتبارات الأخلاقية أثناء إجراء البحث.

- علما تم استيفاء أجور تسهيل المهمة بموجب الوصل المرقم (٠١٧١٣٣٣) بتاريخ ٢٠٢٣/٩/٢٤

... للتعاضد بالاطلاع مع الاحترام...

الطبيب الاختصاص  
مشعل زوري جبار  
مدير قسم التدريب والتنمية البشرية  
٢٠٢٣/٩/٢٤

م.م. الطبية  
٢٠٢٣/٩/٢٤

نسخة منه الى:  
قسم التدريب والتنمية البشرية / شعبة إدارة البحوث والمعرفة ... للحفظ مع الاوليات

العنوان : شارع الجنوبي - البصرة / ذي قار  
البريد الإلكتروني : thiqr.health@yahoo.com  
الموقع الإلكتروني : www.thiqrhealth.gov.iq  
الهاتف : ٠٠٩٦٤٤٢٢٣٠١١٣

Post. Address : Habbobist. , Nasiriya , ThiQar  
Email : thiqr\_health@yahoo.com  
Web site : www.thiqrhealth.gov.iq  
Telephone : 0096442230113

Figure(1) : Facilitating document issued by the Dhi Qar Health Directorate to the relevant health institutions (Al-Nasiriyah Teaching Hospital)



وزارة الصحة  
دائرة صحة ذي قار  
مركز التدريب والتنمية البشرية  
لجنة البحوث



وزارة الصحة العراقية  
Iraqi Ministry of Health  
Founded 1920

استمارة رقم ٢٠٢١/٠٣  
رقم القرار: ٢٠٢٣/١٩٢  
تاريخ القرار: ٢٠٢٣/٩/٢٤

**قرار لجنة البحوث**

درست لجنة البحوث في دائرة صحة ذي قار مشروع البحث ذي الرقم ( ٢٠٢٣/١٩٢ ) المعنون:  
((Serological and immunological covid19 virus in the heart diseases patients in thi-qar province))

والمقدم من الباحث (طالبة دراسات عليا/ ماجستير) تبارك ربيع لفته) إلى وحدة إدارة البحوث و التنمية في مركز التدريب والتنمية البشرية في دائرة صحة ذي قار بتاريخ ٢٠٢٣/٩/٢٤ وقررت

"الموافقة على تنفيذ مشروع البحث بصيغته المقدمة ولما نعت من تنفيذه في مؤسسات الدائرة."

الطبيب الاختصاص

راشد نجم جاسم

رئيس لجنة البحوث

٢٠٢٣/٩/ ٢٤

الدكتور الصيدلاني  
**علي غالب سائي**  
اختصاص تخليط استراتيجي  
مدير قسم التخطيط



٢٠٢٣/٩/ ٢٤

٢٠٢٣/٩/ ٢٤

٢٠٢٣/٩/ ٢٤

**المرفقات:**

تعديلات و ملاحظات لجنة البحوث

**ملاحظات:**

- تم تحويل رئيس لجنة البحوث او مقرر اللجنة للتوقيع على هذا القرار استنادا الى النظام الداخلي للجنة البحوث .
- الموافقة تعني ان مشروع البحث قد استوفى المعايير الاخلاقية والعلمية لإجراء بحث والمعتمدة في وزارة الصحة، اما التنفيذ فيعتمد على التزام الباحث بتعليمات المؤسسة الصحية التي سينفذ فيها البحث.

: Supplement

Thi\_Qar Health Directorate  
Imam Hussein Teaching Hospital  
Human Resources

NO :  
Date :

دائرة صحة ذي قار  
مستشفى الإمام الحسين (ع) التعليمي  
التدريب والتنمية البشرية  
العدد : ١٤١٤١  
التاريخ : ٢٠٢٣ / ٩ / ٢٠

الى /دائرة صحة ذي قار - قسم التدريب والتنمية البشرية

م/ تسهيل مهمة

تحية طيبة

أشارة الى كتاب دائرة صحة ذي قار قسم التدريب والتنمية البشرية ذي العدد ٦٦٣ في ٢٠٢٣/٩/١٩ باشر طالب الدراسات العليا - الماجستير ( تبارك ربيع لفته ) لا مانع لدينا من تسهيل مهمته لغرض الحصول على بيانات لاستكمال متطلبات بحثه الموسوم .

Serological and immunological study covid19 virus in the heart disease patients in thi-qar province

على ان لا تتحمل دائرتنا اي تبعات قانونية ومالية ويلتزم الباحث بالاعتبارات الاخلاقية اثناء اجراء البحث .

مع التقدير

الصادرة  
دائرة صحة ذي قار  
مستشفى الإمام الحسين (ع) التعليمي

نسخة منه الى:  
م. الحسين التعليمي/مركز تعليم الطبي المستمر

مدير المستشفى  
٢٠٢٣ / ٩ /

Figure (2) : Facilitating document issued by the Thi-Qar Health Directorate to the relevant health institutions (Imam Hussein Teaching Hospital)

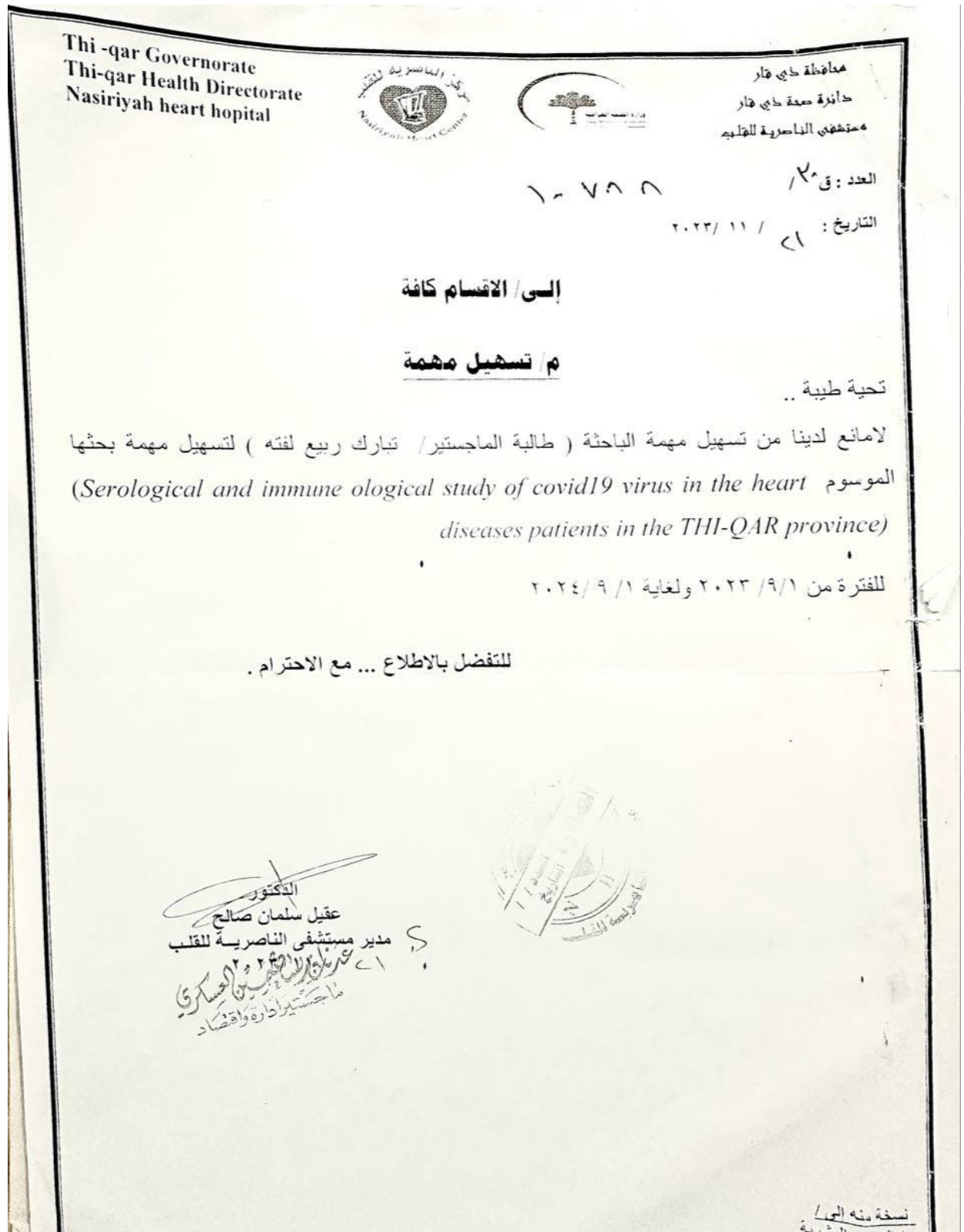


Figure (3): Facilitating document issued by the Thi-Qar Health Directorate to the relevant health Institutions (Nasiriyah Heart Center).

## Appendices

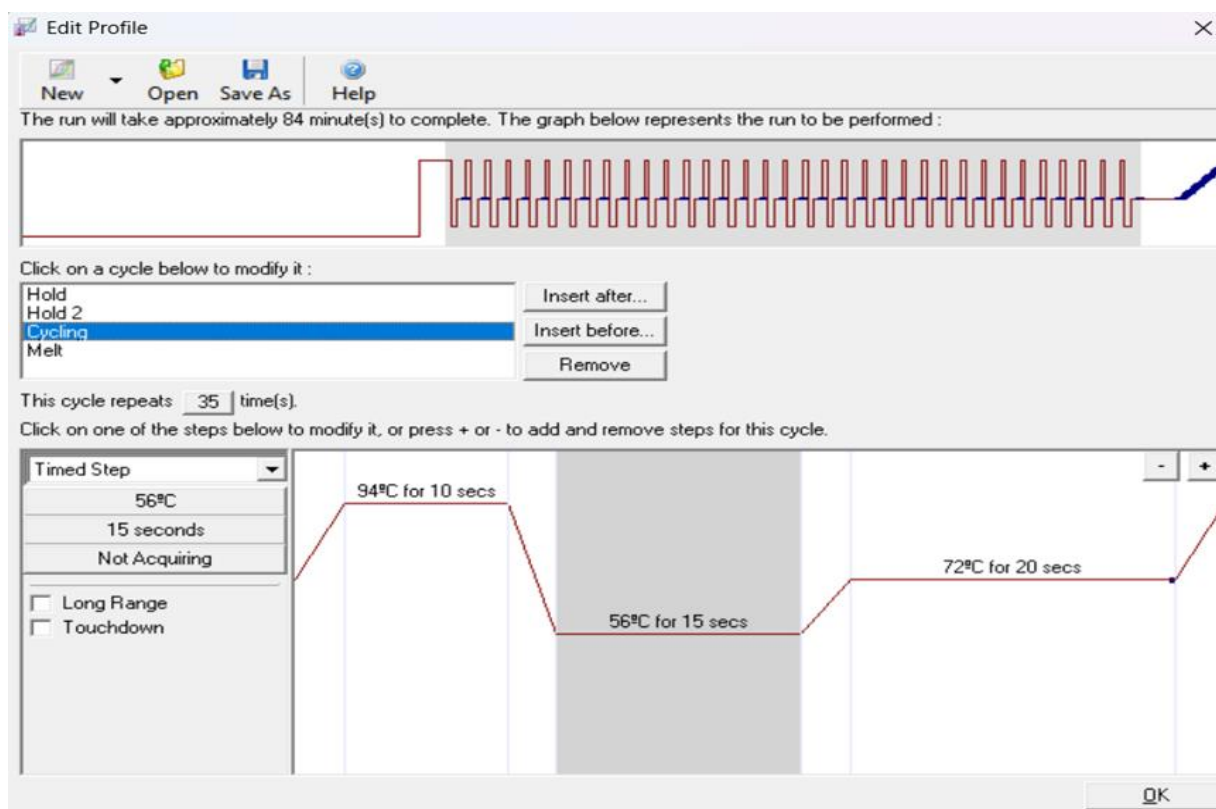


Figure (4): Thermal profile used in expression of RdRp + P2 gene. The profile was taken directly from qPCR machine . T<sub>m</sub> for RdRp + P2 and RdRp + P1 gene (56°C).

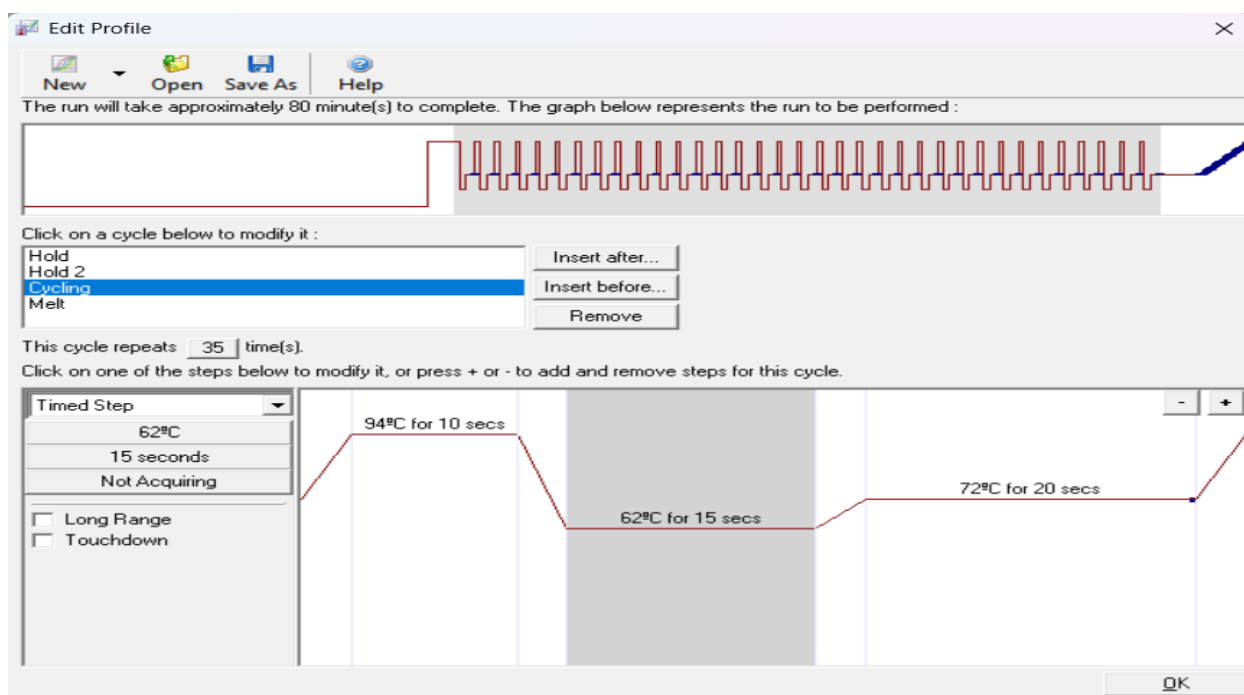


Figure (5): Thermal profile used in expression of N gene. The profile was taken directly from qPCR machine . T<sub>m</sub> (62°C).

## Appendices

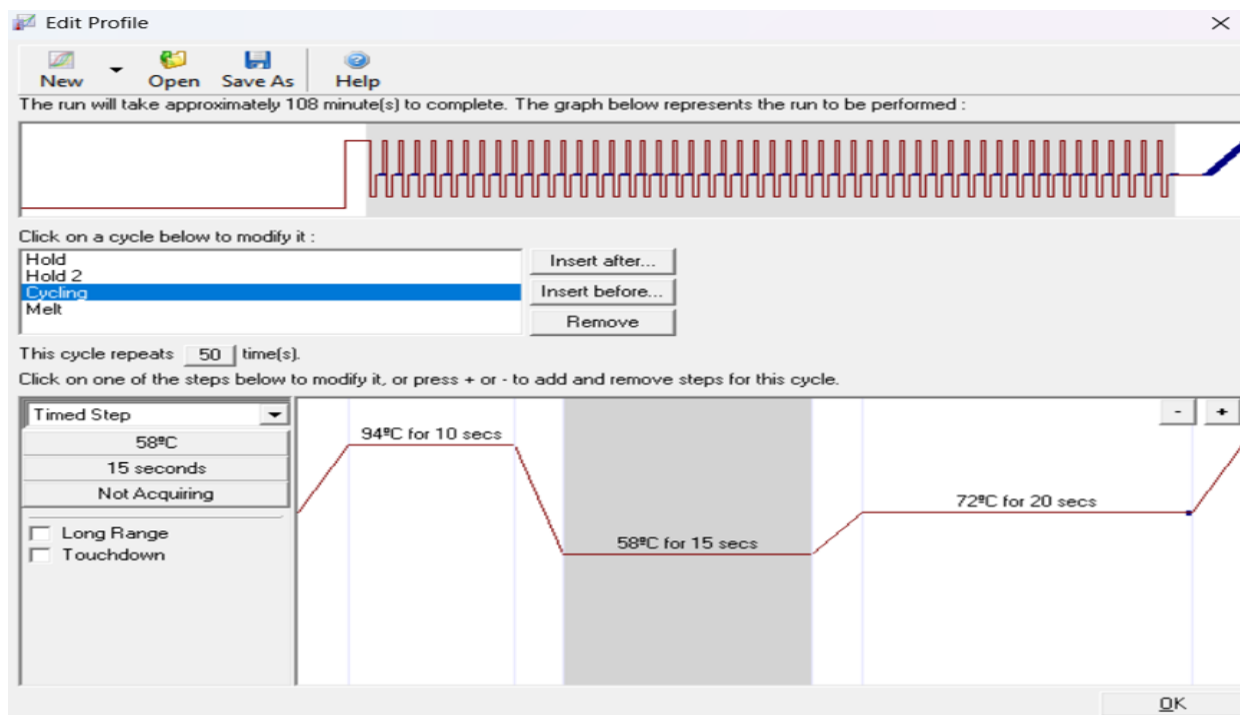


Figure (6): Thermal profile used in expression of E gene. The profile was taken directly from qPCR machine . T<sub>m</sub> (58°C)



Figure (7) :The device RADIOMETER (AQT90) FLEX, examination accuracy 96.8% used to test troponin I with some of its results.

## Appendices



Figure (8): Nanodrop device used to measure the concentration of RNA samples.



Figure (9) :The real-time PCR device used in the study.

وفقاً لتحليل توزيع العلامات المرضية المرتبطة بالإصابة بفيروس كوفيد-١٩، فقد تبين أن أعلى معدل إصابة بالفيروس كان بين المرضى المصابين Angina (٣٢,٦٣%)، تليها حالة Arrhythmia بنسبة (١٥,٧٩%). أما أدنى معدلات الإصابة فقد سجلت بين مرضى Myocardial infarction بنسبة (١٤,٧٤%) و مرضى Ventricular Septal Defect (٢,١١%). أظهرت النتائج وجود فروق إحصائية مهمة في معدل الإصابة بالفيروس بناءً على المضاعفات القلبية المصاحبة. ( $P<0.05$ )

عند مقارنة عينات الدراسة مع عينات السيطرة (المجموعة الإيجابية التي تضم مرضى القلب والمجموعة السلبية التي تضم مرضى القلب المصابين بكوفيد-١٩)، أظهرت الدراسة أن (٣٧,٥%) من عينات مرضى القلب المصابين بكوفيد-١٩ كانت إيجابية لاختبار التروبونين I. أما في المجموعة الإيجابية من مرضى القلب، فكانت نسبة التروبونين I الإيجابية (٥٦,٦٧%)، بينما في المجموعة السلبية كانت النسبة (٣٥,٥%). وقد أظهرت النتائج فروقاً إحصائية كبيرة لاختبار التروبونين I بين مجموعات الدراسة ( $P<0.05$ )، مما يشير إلى أن مرضى كوفيد-١٩ أكثر عرضة للإصابة بأمراض القلب.

وفيما يتعلق بتحليل نتائج اختبار التروبونين I حسب العمر في عينات الدراسة، أظهرت الدراسة أن أعلى نسبة إيجابية كانت في الفئة العمرية (٤٦-٥٤) سنة بنسبة (٢٤,٤٤%)، وأدنى نسبة في الفئة العمرية (٨٢-٩٠) سنة بنسبة (٢,٢٢%). كما كانت هناك فروق إحصائية مهمة بين الفئات العمرية المختلفة في اختبار التروبونين I ( $P<0.05$ ).

أما في مجموعة السيطرة السلبية (مرضى القلب)، فكانت أعلى نسبة إيجابية لاختبار التروبونين I في الفئة العمرية (٣٧-٤٥) و (٥٥-٦٣) سنة بنسبة (١٩,٠٥%)، وأدنى نسبة في الفئة العمرية (٩١-٩٨) سنة بنسبة (٤,٧٦%)، مع وجود فروق إحصائية مهمة لاختبار التروبونين I ( $P<0.05$ ).

بالنسبة لتحليل نسب التروبونين، سجلت أعلى نسبة إيجابية لاختبار التروبونين في النطاق (٠,٠١٠-٠,٠٧٠ نانوغرام/مل) بنسبة (٧٨,٩٥%)، مع وجود فروق إحصائية كبيرة بين النسب المختلفة لاختبار التروبونين I.

## الخلاصة:

يعد فيروس كورونا المستجد (SARS-CoV-2) المسؤول عن الجائحة الحالية (المتلازمة التنفسية الحادة والشديدة)، ولا يزال يسجل عددًا كبيرًا من حالات المرض والوفيات. هذا الفيروس يسبب مرضًا تنفسيًا خطيرًا يهدد الحياة. هدفت هذه الدراسة إلى استقصاء تأثير فيروس SARS-CoV-2 على مرضى القلب المصابين بالفيروس، وذلك باستخدام الاختبارات الجزيئية (PCR) للتحقق من وجود ثلاثة جينات (N، E، RdRp) لتأكيد الإصابة، إلى جانب تحليل التروبونين I كأختبار مصلي.

شملت الدراسة ٢٤٠ عينة عشوائية من الدم ومسحات من البلعوم الأنفي ١٢٠ عينة كانت مجموعة الدراسة اما مجموعة السيطرة شملت (٦٠ عينة سيطرة إيجابية و ٦٠ عينة سيطرة سلبية) من مرضى القلب المصابين بفيروس SARS-CoV-2 في مستشفى الناصرية العام، مستشفى الحسين التعليمي، ومستشفى القلب في محافظة ذي قار جنوب العراق، خلال الفترة من سبتمبر ٢٠٢٣ إلى يناير ٢٠٢٤. أظهرت نتائج تشخيص RT-qPCR، للعينات نسبة (٧٩,١٧%) نتائج إيجابية، بينما أعطت النتائج السلبية نسبة (٢٠,٨٣%). أظهرت النتائج فروقًا كبيرة في معدل الإصابة بين الأشهر، حيث سُجلت أعلى نسبة إصابة في شهر ديسمبر بنسبة (٢٧,٣٧%).

فيما يتعلق بالخصائص الديموغرافية، أظهرت النتائج أن نسبة الإصابة بفيروس كورونا في محافظة ذي قار كانت (٦١,٠٥%) بين الذكور مقارنة بـ (٣٨,٩٥%) بين الإناث، ولم تكشف الدراسة عن فروق إحصائية مهمة بين الجنسين في معدل انتشار الفيروس ( $P > 0.05$ ). أما من حيث مكان الإقامة، فقد كانت أعلى نسبة إصابة في مدينة الناصرية بنسبة (٦٧,٣٧%)، تليها قضاء النصر بنسبة (١٠,٥٣%)، بينما كانت أقل نسبة في قضاء الشطرة والغراف بنسبة (٤,٢١%) كما لوحظت فروق إحصائية حسب مكان الإقامة ( $P < 0.05$ ).

بالنسبة للفئات العمرية، كانت أعلى نسبة إصابة في الفئة العمرية (٥٥-٦٣) سنة بنسبة ٢٤,٢١%، تليها الفئة العمرية (٤٦-٥٤) سنة بنسبة (١٧,٨٩%). بينما كانت أقل نسبة إصابة في الفئة العمرية (٩٨-٩١) سنة بنسبة (١,٠٥%) والفئة العمرية (٨٢-٩٠) سنة بنسبة ٢,١١%. أظهرت النتائج فروقًا إحصائية في معدل الإصابة بالفيروس حسب الفئات العمرية ( $P < 0.05$ ).



جمهورية العراق – وزارة التعليم

العلمي والبحث العلمي

جامعة ذي قار

كلية الطب البيطري

فرع الاحياء المجهرية



## الكشف عن فيروس المتلازمة التنفسية الحادة و الشديدة (SARS-CoV-2) وقياس نسبة التروبونين I المصلي بين مرضى القلب في محافظة ذي قار.

رسالة ماجستير مقدمة الى مجلس كلية الطب البيطري ، جامعة الشطرة كجزء من متطلبات نيل  
درجة الماجستير في علوم الطب البيطري / الاحياء المجهرية .

مقدمة من قبل الطالبة.

تبارك ربيع لفتة الحجامي .

بكالوريوس كلية تربية للعلوم الصرفة \ علوم حياة (٢٠٢١)

بأشراف

أ.د. باسم عبد الحسين جار الله.

