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Study of Human Gene Polymorphism Associated with Covid-19 Infection in Type 2 Diabetes Mellitus Patients

A Thesis

Submitted to the College of Science/University of Babylon as Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology

By

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بسم اللّه الرحمن الرحيم

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ
وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ

صدق اللّه العلي العظيم

سورة المجادلة :الآية 11

Dedication

***many thanks to Allah*

*** To my family.*

***To everyone who has supported me to bear the difficulties.*

*** To everyone who sees that science is the light of life. Respect*

I dedicate my humble efforts.

Maryam

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Maryam

2022

CERTIFICATION

Certify that this Thesis **(Study of Human Gene Polymorphism Associated with Covid-19 Infection in Type 2 Diabetes Mellitus Patients)** has been prepared under my supervision at the Department of Biology, College of Science, University of Babylon, a partial fulfillment of the requirements for the degree of Master of Science in Biology by the student**" Maryam Ali Hussain"**

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Summary

Compared with previous epidemic infections, Coronavirus has the highest transmission rate and risk of death. On the other hand, diabetes is one of the most prevalent diseases. Thus, diabetics infected with the COVID-19 virus are the most affected. The aim of this research was to find out if there was a correlation between infection with the COVID-19 virus and patients with type 2 diabetes. The study concluded that there has been a relationship between diabetes and patients with COVID-19.

The current study was conducted in the molecular laboratory in the Department of Biology, College of Science, University of Babylon. The samples were collected and the practical part of this study was completed during October (2021-2022). The samples were obtained from patients with Covid-19 and Covid-19, diabetes mellitus and healthy people. These samples were collected from Marjan Medical Hospital and Al-Saadiq Teaching Hospital in Babylon Governorate, where the total number of samples was 92 including 31 blood samples from healthy people (3 females, 28 males), 30 blood samples from the patients infected with COVID-19 (16 females, 14 males), and 31 blood samples from the patients infected with COVID-19 + diabetes (17 females, 14 males).

The current study has been divided into three parts: the first study was demographic, the second included measuring blood parameters, and the third included a molecular study. The first part included the social and demographic distribution of the study coefficients in terms of gender, level of injury, age, duration of injury, and body mass. The second part included an assessment encompassing the estimation of physiological parameters (HBA1C, FBG, insulin, insulin resistance, insulin sensitivity, CRP, D-dimer, Ferritin) and the third part involved the study of the effects of some genes (ACE2, AT1R, rs657152) determined by PCR.

The results of this study showed that the gender revealed significant differences at the probability value ($p \leq 0.01$) (0.0002) between the two groups of males and females, where the males in the CDM2 group were (46.67 %) while in the C group it was (43.3%). The value of female was (56.7%) in group C and it was (48.38%) in group CDM2. The level of infection was classified into three sub- levels including: mild, moderate, and severe. The percentage of the level of cut infection in CDM2 (48.30%) was higher than that of group C (26.66%), while the average level was higher in CDM2 group (38.70%) than it was before. Therefore, the minor level was lower in CDM2 (12.9%) than in group C (43.33%), and the severity of infection increased in CDM2 than in group C.

As for the age groups, there were statistically significant differences for the three categories adopted in the current study (> 30, 30-50, <50 years). The group of less than 30 years old was more in group C (36.7%) than in the CDM2 category (6.5%), while the 30-50 category was rounded off in the C and CDM2 groups (26.7%, 22.6%). A higher percentage of greater than 50 categories was observed in CDM2 (71%) and the of the total C was (36.7%). There were three BMI categories (BMI) according to the current study including (normal, overweight, and obesity), and there were no statistically significant differences among these groups.

As for the injury period, the duration periods were classified into 3 subgroups including (<7, 7-14 >,14). days). It was less than 7 days in group C (73.3%) while it was (51.61%) in CDM2, the 7-14 days in group C was (13.33%) and it was (32.25%) in CDM2 group, and more than 14 days was (13.33%) in the CDM2 group and it was (16.12%) in the C group. The differences among groups were significant (p. 0.0311) with a value of ($p \leq 0.05$).

The results of the physiological study showed significant differences among healthy people and Covid patients along with patients with Covid + diabetes mellitus compared to healthy people in ferritin (108.59±18.4, 322.28±30.1 and 583.10±44.6) respectively, while significant differences at ($p \leq 0.01$), showed significant differences In FBG, cumulative glucose and insulin resistance only in the COVID-19 + diabetes group (253.09±16.2, 9.15±2.0,1 and 17.51±4.1 respectively) at ($p \leq 0.05$) ($p \leq 0.01$) and at the same time there was no significant difference among the healthy groups. C-reactive protein or D-dimer showed high values in the Covid-19 group and the covid-19 + diabetes groups compared with the healthy groups (43.72±5.3, 61.18±5.3, 1731.41±114.5 and 1282.97±221.3). at ($p \leq 0.01$) respectively but it did not show significant differences in insulin and insulin sensitivity.

In the molecular study, single nucleotide polymorphism (SNPs) of ACE2 gene was detected by using PCR technique in patients compared with the healthy groups. The group (DD; ID) showed statistically significant differences in relation to the genetic makeup of patients compared with healthy controls at (0.04), whereas I, II did not show any significant relationship. The ACE D genotype was dominant in three groups: (72.6%, 85%,54%) in the healthy control group, the COVID-19 group, and the COVID-19 + diabetes group respectively, followed by the alleles DD; ID; I. It was observed in AT1R that there was a very significant difference between the healthy group and the group of patients by using polymerase chain reaction techniques PCR-SSCP, where the distribution of DNA polymorphisms was found to be (96.77%, 3.23%) in the healthy group and (6.67%,93.33%), respectively. In covid-19 + diabetes group and (12.9%, 87.1) and in covid-19+ diabetes patients, the results showed that there was no association between DNA polymorphisms according to the

number of bands with patients compared to control groups. Meanwhile, there were significant differences in Frequencies of the AT1R polymorphism among patients with COVID-19 and Covid-19 with diabetes mellitus and healthy subjects at (P-value ≤ 0.01).

As for the rs567152 SNP, the study showed that there were no significant statistically significant differences among the genetic makeup of patients compared with the genetic makeup of healthy people.

Finally, it was observed that patients with diabetes who developed contracted Covid-19 appeared to have significantly higher levels of (CRP, D-dimer, and Ferritin) and parameters related to diabetes than patients who suffered from Covid-19 alone, also high levels of ACE2 gene, AT1R receptor as such this would make them more likely to develop illness and death.

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List of Abbreviation

Abbreviation	Meaning
6HB	Six-helical Bundle
ACEi	Angiotensin-converting enzyme inhibitors
AEC2	Angiotensin-Converting Enzyme2
ARB	Angiotensin-Receptor Blockers
ARDS	Acute Respiratory Distress syndrome
AT1R	Angiotensin II Type I Receptor
BGM	Blood Glucose Monitor
TEMED	Tetramethylethylenediamine
CRP	C-Reactive Protein
CT	Computer Tomography
CVD	Cardiovascular Disease
DKA	Diabetic Ketoacidosis
DNA	Deoxyribonucleic Acid
FBS	Fasting Blood sugar
HbA1C	Glycated Hemoglobin A1C
HBGAs	Histo-blood Group Antigens
ICU	Intensive Care Unit
IN	Insulin
IR	Insulin Resistance
IS	Insulin Sensitivity
MCP-1	Monocyte Chemoattractant Protein-1
MERS-CoV	The Middle East Respiratory Syndrome Coronavirus
RAAS	Renin-Angiotensin-Aldosterone System
RBD	Receptor-Binding Domain
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SGLT	Sodium-Glucose Transporter
SSCP	Single-Strand Conformation Polymorphism
T1DM	Type One Diabetes Mellitus
T2DM	Type Two Diabetes Mellitus
TMPRSS2	Transmembrane Protease Serine 2
TNF	Tumor Necrosis Factor
NETs	Neutrophophil Extracellular traps

CONTENTS

IDF	International Diabetes Federation
BGM	blood glucose monitor
HBGAs	histo blood group antigens
AGEs	advanced glycation end
ROS	reactive oxygen species
TNFR	tumor necrosis factor receptor
AGEs	glycation end products
PCR	Polymerase chain reaction
SPSS	Body mass index

Chapter one

Introduction

Introduction

The SARS-CoV-2, or syndrome coronavirus 2, was appeared Coronavirus illness in late 2019 in Wuhan, China. The illness (Covid-19) produced by SARS-CoV-2, quickly became a global pandemic (Zhu *et al*., 2020). In June 15, 2020, there have been over 8.03 million confirmed cases globally, amounting to over than 436,900 fatalities. Early on in the epidemic, Italy and Spain were particularly hard to hit. The outbreak peaking at 61,507 fatalities were recorded by June 15, 2020, and by the second half of February 2020, covid-19 has a wide range of symptoms with the vast majority of infected people experiencing relatively little or no symptoms at all, the significant majority of fatality rates have been caused by a subset of individuals suffering from severe respiratory failure due to interstitial pneumonia in both lungs and acute respiratory distress syndrome (Berlin *et al*., 2020).

In comparison to the infected non-diabetic patients, diabetic patients with Covid-19 are at significant risk of severe pneumonia and have a pronounced pro-inflammatory and pro-thrombotic condition. CRP, interleukin.6, ferritin, and D-dimers are all higher in diabetes individuals than in non-diabetic people. Some patients with SARS-CoV.2 pneumonia experienced syndrome of acute respiratory distress (ARDS) in the past, and some of them quickly deteriorated and died of multiple organ failure. Individuals with diabetes will inevitably suffer from Covid-19 in comparison to those who do not have diabetes. It has been seen that Covid-19 illness 2019 (Covid-19) has a higher rate of morbidity and death (Chen *et al*., 2020).

Diabetic patients with COVID-19 have greater levels of diabetes biomarkers than patients with DM s alone. Increased levels of FBS and

Hb1Ac have been linked to the advancement of COVID-19 in diabetic patients (Ling *et al*., 2021). Hyperglycemia inhibits neutrophil chemotaxis by reducing macrophage, neutrophil, and monocyte phagocytosis, and compromising innate cell-mediated immunity. FBS is linked to inflammatory reactions in the body the immune system as well. Blood glucose levels can be measured and tracked in clinical settings by a handy and easy-to-detect diagnosis. For COVID-19 patients with increased admission FBS, continuous glucose monitoring is very important. Infections and the associated consequences can be prevented and controlled with the help of glucose management. Diabetic patients can have more inflammation leading to IR and the other way round Covid-19 Infections which result in an elevated inflammatory response. Moreover, their coexistence with diabetes can also result in hyper inflammation and severe/fatal outcomes due to the underlying inflammation. (Santos *et al*., 2021). In the higher IR causes, more ACE2 receptors are to be produced in the pancreas, which increase the INR patients to have a higher propensity for the spike protein to bind, rendering them more vulnerable to COVID-19 infections (Roca-Ho *et al*., 2017).

The CRP is a plasma protein that is formed in response to inflammatory mediators produced by the liver, including IL-6. This acute-phase protein is useful despite its non-specificity as a diagnostic technique for a number of inflammatory diseases in clinical settings. According to previous studies, it has been seen that CRP levels were substantially greater in COVID-19 patients who died compared to those who lived (Chen *et al*., 2020).

The DM with COVID-19 patients had a greater level of D-dimer than the other two research groups. D-dimer was also shown to be the most accurate predictor of severe COVID-19 in diabetic individuals. One of the

most prevalent laboratory results in COVID-19 patients requiring hospitalization has been identified as a D-dimer increase (Zhang *et al*., 2020). At that time, the non-survivors had much higher D-dimer levels (Tang *et al*., 2020). Other coagulation indicators, including PT and APTT, were shown to be higher in the patients studied by (Connors and Levy, 2020).

Patients with DM and COVID-19 had considerably greater ferritin levels than the other two research groups. Ferritin levels were also considerably higher in diabetic patients than in the control group. The findings of this study were similar to those of (Cheng *et al*.,2020)

The ACE2 receptor has been found in human heart failure and lymphoma cDNA libraries before being identified as the SARS-CoV receptor. SARS-CoV-2 binds to ACE2 more strongly than does SARS-CoV-1, and it has been recently shown that ACE2 can also be the virus's cellular entry site (Xu *et al*., 2020). The expression and distribution of ACE2 in the human body may indicate infection pathways and tissues that are particularly susceptible to SARS-CoV-2 infection. ACE2/Ang-(1–7) system protects the lungs against Acute Respiratory Distress Syndrome by acting as an anti-inflammatory and antioxidant (ARDS). As an adaptive reaction to counterbalance the raised levels of Ang-II and Ang-I, ACE2 gene expression is dramatically enhanced in patients with DM who are treated with ACEi or ARBs. As a result, using ACE2-stimulating medicines might make it easier for SARS-CoV-2 to enter pneumocytes and other ACE2-rich tissues, potentially leading to a more severe and deadly illness. Angiotensin-converting enzyme (ACE or ACE1) converts Ang-I into Ang-II, whereas ACE2 hydrolyzes Ang-II into Ang-1–7. Vasoconstriction, fibrosis, inflammation, and thrombosis, among other things, happen when Ang-II binds to the AT1-receptor. Ang-1–7 binds to the AT2-receptor,

resulting in an increased vasodilation and a reduced fibrosis, inflammation, along with thrombosis. As a result, ACE and ACE2 are seen as competing factors in the equation that influences hypertension, diabetes, and cardiovascular disease risk (pal and Bhansali, 2020).

It has been seen that the ABO gene Chr 9q34.2, which is responsible for ABO determination, is one of the risk factors that has been linked to COVID-19 severity in research conducted in the United Kingdom and Denmark (Kaiser 2020; Barnkob *et al*., 2020). The purpose of this study aimed to look at the frequency of haplotypes of the ABO Transferase A Gene (rs657152) in COVID -19 infected and non-infected people. This SNP has been linked to respiratory failure in COVID-19 according to genome-wide association studies. The rs657152 gene is present in the ABO intron region, and it has been discovered that this location has an influence on the severity of the disease (Group SCG *et al*., 2020).

Aim of the Study: -

Covid-19 and Diabetes are one of the risk diseases currently prevalent among people, so this study aimed to determine the relationship between Covid and diabetes mellitus by a set of physiological and molecular markers.

Chapter two

Review of Literatures

2- Review of Literatures

2-1 Definition of Covid-19

The SARS-CoV-2 is an RNA virus with positive strands having a single-stranded genome encased in a lipid bilayer adorned with proteins. SARS-CoV is a human virus that causes severe acute respiratory syndrome and shares 82 percent of homology with SARS-CoV-2 (SARS) (Chen *et al*., 2019). In cells from humans, the major entrance of the Angiotensin-converting enzyme 2 (ACE-2) is SARS-receptor. CoV-2's (ACE2) (Walls *et al*., 2020) is found in high amounts. Other cell types include Alveolar cells in the lungs, cardiac myocytes in the heart, vascular endothelium and other cells. (Zhang *et al*.,2020). However, SARS-CoV-2 is likely to be transmitted through virus-bearing respiratory droplets with a viral load at its maximum occurring within 5–6 days before the beginning of symptoms, and with an average of 4–5 days before the onset of symptoms (Guan *et al*., 2020; Pung *et al*., 2020).

2-2 SARS-genesis the Structure of CoV2

Spike (S) glycoprotein, small envelope (E) glycoprotein, membrane (M) glycoprotein, nucleocapsid (N) glycoprotein, and a few additional auxiliary proteins are all found in the nucleocapsid (N) glycoprotein. All of these viral proteins make up SARS-CoV.2 (Jiang *et al*., 2020). The spike, also called S glycoprotein, is a 150-kilodalton trans membrane protein present in the virus's outer region. The S protein forms homotrimers that protrude from the surface of the virus and attract angiotensin by making it easier to envelope viruses in order to bind to the host cells. Lower respiratory tract cells express converting enzyme 2 (ACE2). This glycoprotein is cleaved into two subunits, S1 and S2, by a furin-like protease in the host cell. Part S1 regulates the host viral range and the

cellular tropism, while part S2 promotes the virus fusion in transmitting host cells, due to the nature of the receptor-binding domain make-up (Walls *et al*., 2020). Protein (N) is also phosphorylated to a higher degree. It is assumed that this causes structural changes which improve viral RNA binding. The Covid's nucleocapsid, also known as N protein, is a structural component found in the endoplasmic reticulum-Golgi region that is structurally linked to the virus's nucleic acid content because such a protein is attached to RNA. What involved in the processes of a virus's genome, are the replication cycle, and the cellular response of the host cells of the viral infections (Schoeman and Fielding, 2019) (Tai *et al*., 2020) As shown in Figure(2-1).

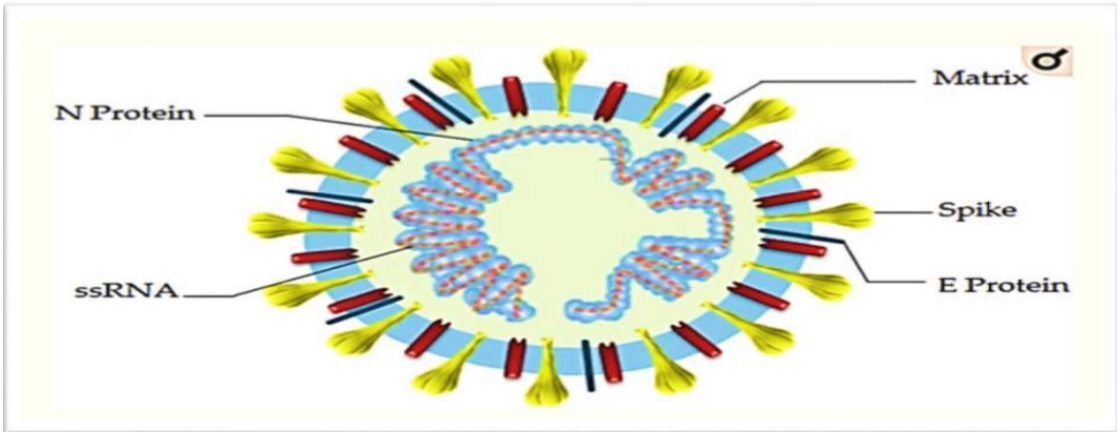


Figure 2-1 Structure of Severe Acute Respiratory Syndrome Covid-19 (Tai *et al*., 2020)

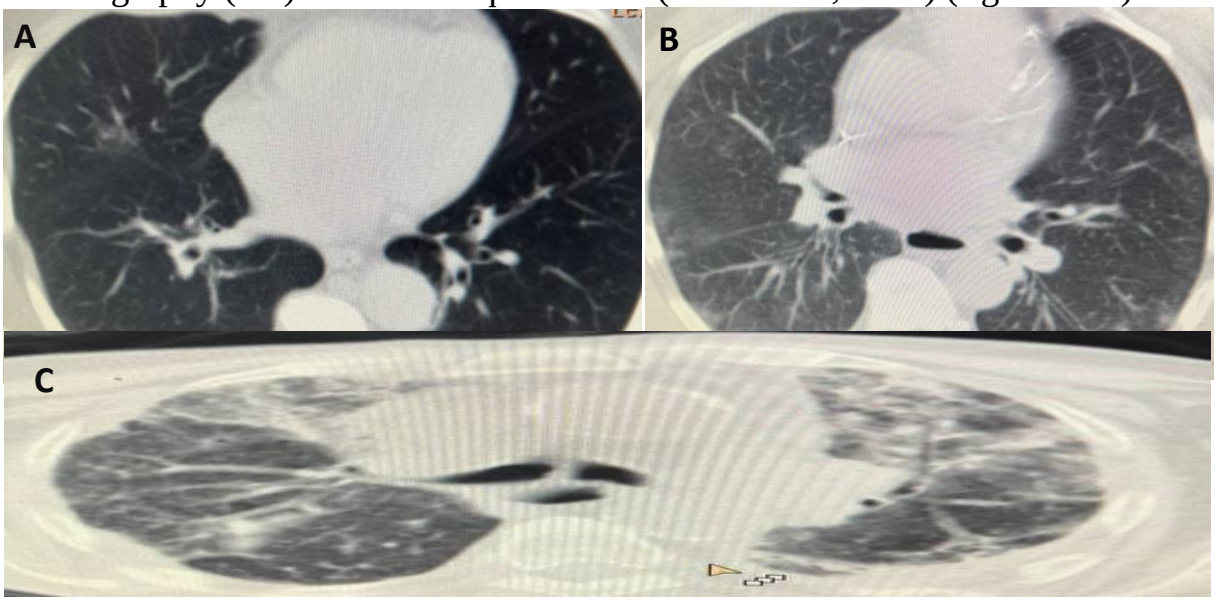
Another important membrane, or M protein, is a component of this virus that maintains the form of the viral envelope. It is the most physically organized protein in the virus. All structural proteins can bind to this protein. Affiliation with the M protein can happen by stabilizing the N protein-RNA complex within the internal virion. It aids in the stabilization of the nucleocapsids or N proteins and boosts the encouragement of viral assembly. The E protein, also known as the envelope protein, is the

smallest protein in the SARS-CoV structure which is crucial for viral replication and maturation (Schoeman and Fielding, 2019).

2-3 Symptoms and Harms of Covid-19

Fever, cough, anosmia, ageusia, weariness, myalgia, headache, sore throat, cold diarrhea and dyspnoea are some of the symptoms that people experience along with the shortness of breath all of which are signs of COVID-19 (Chen *et al*.,2020; Song *et al*.,2020).

Acute respiratory distress syndrome (ARDS) would be developed in severe COVID-19 infected patients in around 8–9 days after the starting of the symptom (Wang *et al*., 2020). Pneumonia, pulmonary edema, oxygen saturation (SpO2) of 93 percent, respiratory failure requiring invasive ventilation, and ICU admission, coagulopathy, lymphopenia, cytokine storm, viremia, and multi-organ destruction are all signs of this illness (Guan *et al*.,2020; Keeley *et al*., 2020). To confirm severe COVID-19 patients, physical and laboratory testing including SpO2, D-dimer, Ferritin, CRP, inflammatory indicators, leucocyte counts, and computed tomography (CT) scans are required here (Zhou *et al*., 2020) (figures 2-2).



Figures 2-2: Comparing Three Cases (A) Mid, (B) Moderate, (C) Severe, from the domestic center.

Pulmonary edema occurs when the lungs become swollen. Excess fluid leaks from the lungs' blood vessels results in gas obstructing exchange, lower SpO2, respiratory failure, and ICU admission due to the lack of oxygen and carbon dioxide. Microvascular thrombosis and the diffuse of intravascular coagulopathy are two different types of coagulopathy(DIC) (Connors and Levy,2020),(Fei *et al*., 2020). Lymphopenia might be a sign of impending death in severe circumstances. In severe Covid-19illness survivors suffer from Scarring and fibrosis of the lungs which are frequent hyper-inflammatory signs that indicate tissue damage caused by hyperactive, dysregulated inflammation (Tavakolpour *et al*., 2020).

Further, in the Covid-19 pandemic, the autopsies of deceased people's lungs Neutrophophil Extracellular traps (NETs) have been linked to lung damage caused by inflammation thrombosis, and fibrosis (Radermecker *et al*.,2020).In every case analyzed, autopsies have revealed type-2 pneumocyte hyperplasia (Carsana *et al*., 2020). The time-varying reproduction number (R) of SARS-CoV-2 and the introduction and the removal of non-pharmaceutical therapies potentially suggest a compensation for the lung's loss of cells that express angiotensin-converting enzyme 2 (ACE2) (Li *et al*., 2020). Severe infections have been linked to a persistently elevated viral load in the upper respiratory tract (Liu *et al*., 2020; Sungnak *et al*., 2020). SARS-CoV-2 virions have been found in practically all organs, including the brain of individuals who have died from severe COVID-19 (Vasquez-Bonilla *et al*., 2019; Al-Sarraj *et al*., 2020;).

Asymptomatic infections may be caused by the host immune system's efficacy. Previous infection of the immunity system of other human coronaviruses, low viral load, and cross-reactivity of existing

immune effectors are also possible causes of the longer duration of the viral RNA detection in symptomatic patients' upper respiratory tracts and cross-immunity against SARS-CoV-2 (Lee *et al*., 2020; Sette *et al*., 2020).

2-4 Diabetes Mellitus

Diabetes mellitus (DM) is a glucose metabolic disorder marked by insulin production and action both of which produce chronic hyperglycemia. (Yates *et al*., 2016). There are two kinds of this disease: Type 1 diabetes (T1) is characterized by an absolute insulin shortage produced by the pancreas' failure to secrete insulin, whereas type 2 diabetes (T2) is characterized by insulin resistance and relative insulin insufficiency, both of which may be present at the time when diabetes is diagnosed. According to the eldest data from the International Diabetes Federation (IDF) diabetes poses a significant hazard to human health. The IDF attempts to understand of how the pathogenesis of DM is ongoing. T1DM has traditionally been considered as an autoimmune disease and the early research has demonstrated that T cells could have been involved in various pathogenic steps in T1DM, including the initiation of insulitis and the injury to β cells (Gao *et al*., 2017) T cells have also been identified to play a function in the development of insulin resistance in the past study (Jagannathan-Bogdan *et al*., 2011), and the various complications in T2DM, including atherosclerosis (Zetterqvist *et al*., 2013) nephropathy and neuropathy T cells may thus be a crucial player in the development of diabetes mellitus, as well as a potential diagnostic and therapeutic target (Zhang *et al*., 2016).

Diabetes can be classified into the following general categories:

1. Type 1 Diabetes is an autoimmune-cell destruction resulting in absolute insulin insufficiency, including the adult-onset latent autoimmune diabetes

2. Type 2 Diabetes is caused by a gradual loss of sufficient -cell insulin production, often in the context of insulin resistance ADA (2021).

3. **Gestational Pregnancy Diabetes Mellitus** is a condition that occurs when a woman is pregnant (diabetes diagnosed in the second or third trimester of pregnancy which is not a clearly overt diabetes before gestation.

4. Other Types of Diabetes are monogenic diabetes syndromes (such as newborn diabetes and young-onset diabetes), exocrine pancreas illnesses (such as cystic fibrosis and pancreatitis), and drug- or chemical-induced diabetes (such as following glucocorticoid usage, HIV/AIDS treatment, or organ donation) (ADA, 2014).

Both types of diabetes (type1 and 2) are heterogeneous diseases resulting from interaction among different factors including genetic and environmental factors that cause loss of beta-cell function and hyperglycemia, where the chronic hyperglycemia contributes to the complications development (Skyler *et al*., 2017).

2-4-1 Type 1 Diabetes Mellitus (T1DM)

T1DM is an autoimmune disease in which the insulin-producing beta cells in the pancreas are destroyed. Insulin is an anabolic hormone that regulates growth as well as glucose, fat, protein, and mineral metabolism. Insulin, too, has a big role to play. It allows glucose to enter the muscle and adipose cells to promote the liver to store glucose as glycogen and produce fatty acids. It increase amino acid absorption, slows fat breakdown in adipose tissue, and enhances potassium uptake into cells. T1DM sufferers will need insulin replacement medication for the rest of their lives. A shortage of insulin causes diabetic ketoacidosis (DKA), which is a life-threatening illness (Saxby *et al*.,2020).

2-4-2 Type 2 Diabetes Mellitus

Non-insulin-dependent diabetes mellitus or adult-onset diabetes is a metabolic disorder characterized by high levels of blood glucose caused by two different things which are insulin resistance and relative insulin insufficiency.T2DM symptoms include frequent urination, increased thirst, tiredness, and weight loss. T2DM is caused by a combination of lifestyle and genetic factors, while recent increases in T2DM rates may be due to environmental contaminants. Having relatives with T2DM significantly raises the family's risk of having such illness. In addition to any genetic component, environmental factors, particularly nutrition and obesity, have a significant role in the development of T2DM. Insulin resistance refers to the inability of human tissues to respond effectively to insulin when it is present (Ahima and Flier, 2000). Insulin resistance, unlike Type I diabetes, is usually caused by cells' insulin receptors failing to respond correctly to insulin rather than it is an issue with insulin synthesis. Increased glucose levels on two occasions are defined by the World Health Organization (WHO) as T2DM: first, a glucose tolerance test two hours after an oral dose with plasma glucose of 11.1 mmol/Lans, and second, a fasting plasma glucose of 7.0 mmol/L. T2DM can be avoided by following a healthy diet and exercising regularly. Past studies have pinpointed that obesity and T2DM have been related to insulin resistance in the brain and neurodegeneration. (Ginter *et al*., 2013).

2-5 The Relationship Between COVID-19 and Diabetic Mellitus

The covid-19 and diabetes mellitus interact in a two-way manner The unholy state of DM and COVID-19 coexistence is one in which one disease entity tends to complement the other. The potential interactions between the two pandemics have been investigated in old studies (Pal and Bhadada, 2020) (Figure 2-3).

2-5-1 The Impact of COVID-19 on Diabetes Mellitus

Diabetes is an independent predictor of ICU admission in COVID-19, invasive ventilation, and death. (Hazard Ratio: 1.59, with a 95% chance). Although there is no apparent distinction in the COVID-19, type 2 diabetes mellitus (T2DM) are potential predictor of poor outcomes (Pal *et al*., 2020).

Multiple pathophysiological explanations for the correlation between the severity of DM and COVID-19 have been proposed. Patients with uncontrolled diabetes have an impaired innate immune system since it is the primary line of defense against SARS-CoV-2 (Jafar *et al*., 2016). As demonstrated in Covid-19 patients, DM is a pro-inflammatory state defined by an inappropriate and excessive cytokine response where blood levels of interleukin-6 (IL-6), C-reactive protein, and ferritin have been considerably greater in DM patients than in non-DM patients. This indicates that diabetics are more susceptible to an inflammatory cytokine storm which can result in ARDS, shock, and rapid COVID-19 breakdown. According to the a forementioned study, COVID-19 participants with diabetes posses higher D-dimer levels than those without diabetes (Guo *et al*., 2020). Overactivation of the hemostatic system could be the probable cause. In the context of a Hypercoagulable pro-thrombotic disease could be made worse by the presence of DM (Guo *et al*., 2020). In COVID-19, the excessive activation of the coagulation cascade can result in catastrophic thromboembolic consequences and eventual death (Hussain *et al*., 2020).

Diabetes has been linked to lower levels of angiotensin-converting enzyme 2 (ACE2), which is found in the lungs (particularly type II pneumocytes), kidney, stomach, and vascular endothelium. Angiotensin II and, to a lesser extent, angiotensin can be degraded by ACE2 into smaller peptides.

It's also important to consider the interaction between angiotensin-converting enzyme inhibitors (ACEi) and angiotensin-receptor blockers (ARB). As regards those with diabetes, ACEi/ARBs are commonly used as antihypertensive and renoprotective medications. As an adaptive response to increasing levels of angiotensin-II, the increased expression of ACE2 is connected to the use of ACEi/ARBs (Cure *et al*.,2020). Unfortunately, SARS-CoV-2 requires ACE2 as a receptor for entry into the host pneumocytes, therefore ACE2 overexpression would allow the coronavirus to enter and replicate more easily. ACE2 is downregulated after the virus uses the enzyme to gain access to the host tissue, and it is no longer able to guard against lung harm (Pal and Bhansali; 2020).

According to a new research, the non-structural proteins from SARS-CoV-2 attack hemoglobin's 1-chain causing iron to dissolve from porphyrin and reducing hemoglobin's oxygen transport capability. SARS-CoV-2 may have a stronger affinity for glycated hemoglobin than non-glycated hemoglobin, albeit this is only a theory (Wenzhong *et al*., 2021).

2.5.2 Effect Does COVID-19 Have on Diabetes Mellitus?

The covid-19 can affect glycemic control in persons with pre-existing diabetes according to the preclinical evidence and data collected from research based on the previous SARS outbreak (2003). This is in addition to the stress caused by a critical disease (i.e., stress hyperglycemia). According to researchers, individuals with SARS (caused by SARS-CoV, a relative of SARS-CoV-2) who never took glucocorticoids had considerably higher fasting plasma glucose levels than patients with non-SARS pneumonia. This is because ACE2 is expressed on the islets of the pancreas (Yang *et al*., 2010). This could be interpreted as SARS-CoV-mediated injury, in which individuals have died of SARS due to immunohistochemistry and in-situ hybridization. This may explain why

persons with T2DM who have some functional b-cells in reserve have a deteriorating glucose control (Ding *et al*., 2004).

Furthermore, COVID-19 may exacerbate T2DM patients with insulin resistance (especially those who are fat and have some form of insulin resistance in addition to a complete lack of insulin). Increased levels of IL-6, IL-1, tumor necrosis factor (TNF), monocyte chemoattractant protein-1 (MCP-1), and inducible protein-10, leading to a loss of insulin sensitivity, can be found even when COVID-19 levels are low. Obesity, which is usually associated with T2DM, is also known to exacerbate the cytokine response, leading to a greater insulin resistance (Kassir,2020). That is, SARS-CoV raises serum levels of fetuin-A, a 2-Heremans glycoprotein associated with decreased insulin sensitivity (Wan et al,2006). One needs to investigate if SARS Cov-2 can cause an increase in fetuin-A levels. Finally, COVID-19 has been linked to hypokalemia, which it in turn has been linked to decreased pulmonary ACE2, decreased angiotensin-II degradation, and increased aldosterone production (Liamis *et al*.,2014).

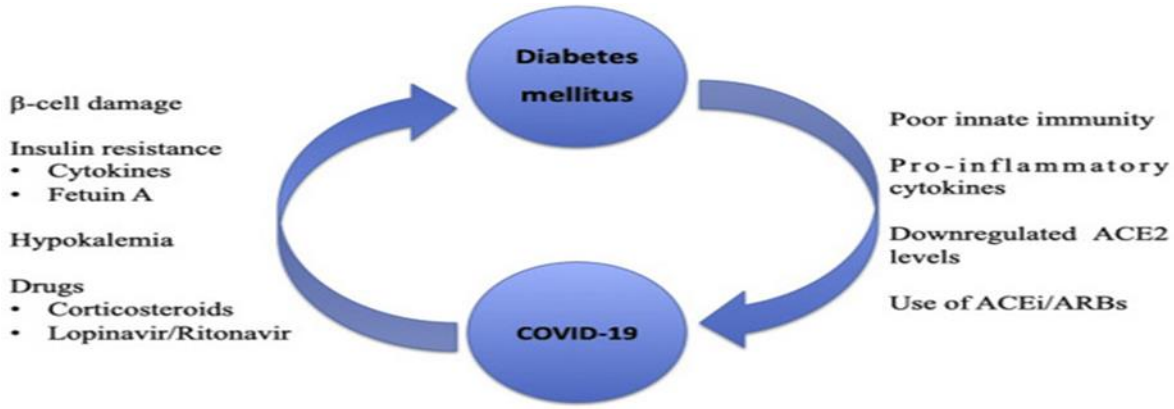


Figure: 2-3 This Diagram Displays a Unique Coronavirus Illness that Has a Two-way Interaction: (Pal and Bhadada, 2020)

Covid-19 and diabetes mellitus. By impairing innate immunity, creating an increased pro-inflammatory cytokine response, and reducing angiotensin-converting enzyme 2 expression, diabetes mellitus worsens

COVID-19 illness (ACE2). Furthermore, in COVID-1In of diabetic patients, the usage of angiotensin-converting enzyme inhibitors (ACEi)/angiotensin-receptor blockers (ARBs)have been linked to disease severity. COVID-19, on the other hand, exacerbates diabetes mellitus by increasing insulin resistance via cytokines and fetuin-A, and via hypokalemia. Additionally, Corticosteroids and lopinavir/ritonavir, which are used to treat COVID-19, may induce dysglycemia (Pal and Bhadada, 2020).

2-6 Stress Hyperglycemia is Caused by the Viral Virus COVID-19

Stress hyperglycemia, a disease characterized by hyperglycemia, insulin resistance, and glucose intolerance, can be caused by the body's protection mechanisms during acute sickness (Saqib et al., 2020). As a result of intubation and other therapies such as glucocorticoids, hospitalized patients may incur additional stress on their bodies. (Pourfridoni *et al*., 2020). Patients in intensive care units are more likely to develop stress hyperglycemia (Ceriello *et al*., 2020). As patients heal and are discharged from the hospital, many of these cases of stress hyperglycemia disappear (Bronson *et al*., 2020). Patients with admission hyperglycemia and no known diabetes have performed worse in recent trials, both in terms of severity of illness and mortality rates than patients with previously diagnosed diabetes along with patients with normoglycemia (Carrasco-Sánchez *et al*.,2021).

2-7 Insulin Resistance Can Occur as a Component of Stress Hyperglycemia after Severe Sickness

In fact, the virus causes a severe form of insulin resistance in type 2 diabetic individuals (Marik *et al*., 2013). COVID-19 and diabetes have a bidirectional relationship. It is possible that inflammation, prevalent in

both illnesses, is to blame: "Chronic illnesses [like diabetes] have several common characteristics with infectious diseases, such as a proinflammatory state and a weakened innate immune response. (Yang *et al*., 2020). Insulin resistance is caused by inflammation in the liver, which can progress to type 2 diabetes (Asrih *et al*., 2013). Diabetic people infected with COVID-19 are said to be "at higher risk of excessive uncontrolled inflammatory responses and hypercoagulable condition, which may lead to the development of cardiovascular disease" (Guo *et al*., 2020). SARS-CoV-2 is most likely to affect pancreatic beta-cells, causing insulin output to decrease. If combined with the cytokine storm, this would result in insulin resistance, with the virus acting as a trigger for hyperglycemia and DKA stress (Ceriello *et al*., 2020; Fadini *et al*., 2020). Interleukin-6 has been found in both DKA and COVID-19 quite frequently (Gentile *et al*., 2020).

2-8 Diabetes and COVID-19 Clinical Course

It has been recently demonstrated that diabetics can have a greater prevalence of cardiovascular disease (32.4 percent vs. 14.6 percent) and less fever than non-diabetics (59.5 percent vs. 83.2 percent). Really, diabetes patients have been showing higher levels of inflammatory blood markers such as LDH, CRP, ferritin, D dimer, lower lymphocyte counts, and more evident of computer tomography (CT) imaging abnormalities all of which indicate a more severe overall and especially lung involvement. In COVID-19, greater D dimer levels are closely associated with increased mortality (Tang *et al*.,2020), which are substantially higher in diabetic patients showing a proclivity for hypercoagulation (Dong *et al*.,2020; Guo *et al*.,2020).

One of the first observations of COVID-19 diabetic patients manifests that they are more likely to require intensive care, which generally entails

invasive ventilation. As regards this study, diabetes has been detected in 22.2 percent of ICU patients, compared to 10.1 percent of COVID-19 hospitalized patients. As a result, diabetes can have a comparable impact similar to other risk factors including hypertension and cardiovascular disease (Bai *et al*.,2019).

Diabetes has been found to be present in 7.4% of the COVID-19 population in a comprehensive assessment of 1099 patients in China; however, 16.2% of those with severe disease have been discovered to have the disease (Guan *et al*.,2020). Furthermore, diabetes has been found in 26.2 percent of patients who have experienced the primary composite outcome, subsuming admission to an intensive care unit, mechanical ventilation, and/ or death, in addition to a 3.6-fold enrichment for those seriously ill. Diabetes patients are more likely to be admitted to an intensive care unit, according to a recent meta-analysis (Li *et al*.,2020). With a hazard ratio of 2.3, diabetes has been seen to be strongly linked to the development of acute respiratory distress syndrome (ARDS) (Meiri *et al*.,2021). The pooled diabetes ratio among COVID-19 patients having a more severe course compared to those having a more favorable course has been 2.26. Consequently, this points out to a considerably higher risk of diabetes (Fadini *et al*.,2020).

Looking at 2003 COVID-19 fatalities in china and Italy, diabetes was nearly twice as common in the non-surviving COVID-19 population as it was in the surviving COVID-19 population (Wu *et al*.,2020). These data referred to the higher mortality rates of diabetes patients in SARS and MERS (Gupta *et al*.,2020). As a matter of fact, diabetic complications increase the risk of diabetes-related death. In individuals with SARS, plasma glucose levels and diabetes are the independent predictors of mortality and morbidity. As a result, it is acceptable to conclude that

diabetes not only increases the probability of SARS-CoV 2 infection but also greatly increases the severity and mortality of COVID-19 (Dong *et al*.,2020; Guo *et al*.,2020).

2-9 Treatment for Diabetes During COVID-19

The covid-19 initial influence on glycemic control appears to be similar to that of other viral diseases. Insulin resistance and consequent hyperglycemia results from an initial inflammatory reaction necessitate the intensification of pre-existing diabetic treatment (Dungan *et al*., 2009). This has been supported by Wuhan, China, research, in which 56% of the blood glucose readings of the 881 of Covid-19 hospitalized patients have been seen as normal. This emphasizes the importance of a proactive approach to diabetes management for these patients, even though blood sugar is not the primary concern. Furthermore, roughly 70% of T2DM patients have been recommended to initiate or enhance insulin therapy while in the hospital (Vickers *et al*.,2017).

Inadequate glycemic management has been proven to have a deleterious impact on outcomes in a variety of conditions, including length of stay, complication rate, and mortality. Patients who have preprandial blood glucose levels of >180 mg/dl (>10 mmol/l) should begin or increase their antidiabetic therapy. Target glucose varies depending on various patient characteristics, including comorbidities, yet for individuals with severe COVID-19 symptoms, the target usually falls between 140 and 180 mg/dl preprandial. Due to its well-established effectiveness and safety profile, insulin is the treatment of choice for these patients to be preferably repeated daily in the form of injections or to be admitted in intensive care units along with continuous intravenous infusion via a syringe pump. Dosing recommendations are included in the appropriate sections of the DG guidelines (Mader *et al*.,2019).

Metformin and sodium-glucose transporter (SGLT2) inhibitors, which are both commonly used in type 2 diabetes, can have serious, even fatal side effects in acute illness, and that is why they should be temporarily stopped during COVID-19. This is because certain diabetics have an increased risk of acute renal injury (James *et al*., 2015; Mansfield *et al*., 2016). ACE inhibitors and angiotensin receptor blockers (ARB) should be avoided during acute sickness; however, in COVID-19 a complete withdrawal of renin-angiotensin-aldosterone inhibitors has not been referenced due to concerns about their ability to up-regulate the heart (Vaduganathan *et al*.,2020).

2-10 Increased COVID-19 Severity

Diabetes mellitus has been identified in 58 percent and 33 percent of severely sick COVID-19 patients admitted to ICUs in the United States. (Arentz *et al*.,2020; Bhatraju *et al*., 2020). There has been a relationship between severe COVID-19 and diabetes mellitus, according to the findings. The increased clinical severity of COVID-19 in patients with diabetes can be caused by several processes. In individuals with diabetes mellitus, glucotoxicity, Inflammation-induced endothelial damage, oxidative stress, and cytokine production all lead to an increased risk of thromboembolic complications and organ damage as shown in (figure 2-4) (Walls *et al*.,2020). Furthermore, Systemic corticosteroids and antiviral

medications, which are commonly used in the treatment of COVID-19 patients, might exacerbate hyperglycemia (Cui *et al*., 2020).

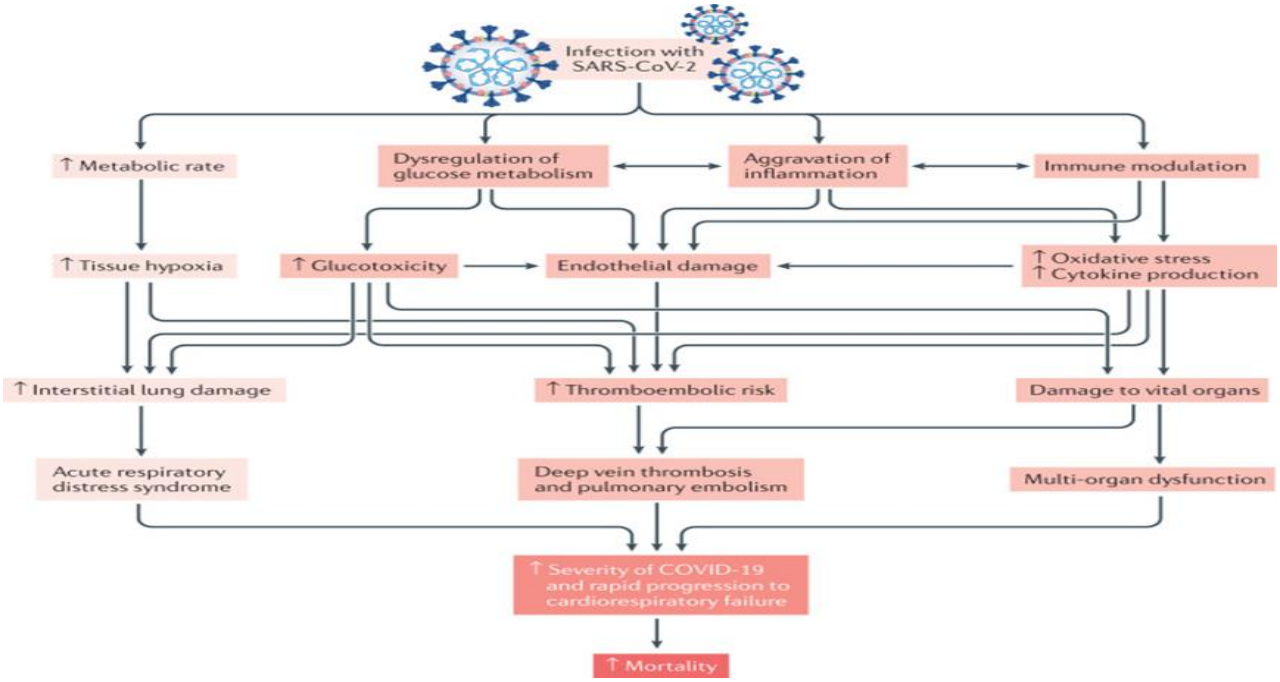


Figure:2-4 Potential Pathogenic Pathways in T2DM and Covid-19 Patients (Walls *et al*.,2020).

2-11 Infections in People with Diabetic Mellitus.

The SARS-CoV-2 infection raises metabolic rate, causing tissue hypoxia, which leads to interstitial lung damage and acute respiratory distress syndrome (Tang *et al*.,2020). Diabetes mellitus patients infected with coronavirus 2019 (COVID-19) disease have disrupted glucose homeostasis, increased inflammation, and immune system dysfunction. (Chen *et al*.,2020; Teuwen *et al*.,2020). These conditions increase oxidative stress cytokine production, endothelial dysfunction thromboembolism, and at the same damage to important organs are more likely occur. As for diabetic individuals, all of these variables contribute to COVID-19's enhanced severity and to the quick development of cardiorespiratory failure. (Kuba *et al*.,2005).

2-12 Indications Related to COVID-19 and Diabetes Type 2

2-12-1 Fast Blood Glucose

The use of a personal blood glucose monitor (BGM) to self-test blood glucose (BG) is an important part of diabetes management. BGMs are used for a variety of purposes including:

1) determining blood glucose levels to make therapeutic decisions

2) calibrating continuous glucose monitoring devices

3) detecting or confirming hypoglycemia.

BGM systems must accurately assess BG levels to be both safe and clinically useful (Klonoff *et al*.,2018; Singh *et al*., 2020). During previous pandemics of Middle East Respiratory Syndrome Coronavirus (MERS-CoV), H1N1 influenza, and Severe Acute Respiratory Syndrome Coronavirus-1 are all related to such pandemics, where poor glycemic management has been linked to a greatly a higher risk of complications and mortality. Although several research and meta-analyses have recently indicated that individuals with diabetes have a considerably greater risk of severe COVID-19 and increased mortality, no studies examining the relationship between glycemic control and COVID-19 severity and death have been available until recently (Badawi *et al*., 2016).

2-12-2 Hemoglobin A1c Test-glycated

Glycosylated hemoglobin (HbA1c) is a reliable indicator of long-term glucose management that gives an average of the previous three months' values. (Wang *et al*.,2020). It can be used to determine diabetes status and to identify COVID-19 high-risk patients who are unaware of their diabetes (Liu *et al*., 2021). Unlike some other risk factors for COVID-19-related mortality of the advanced age, HbA1c may be altered by healthcare

measures and is readily available in everyday practice (Holman *et al*., 2020). According to a new meta-analysis, COVID-19 patients with pre-existing DM have a threefold greater risk of in-hospital mortality (Guo *et al*., 2020).

2-12-3 Insulin

It is a peptide hormone that controls blood glucose levels by assisting cellular glucose absorption, regulating carbohydrate, lipid, and protein metabolism, as well as stimulating cell division and proliferation through its mitogenic actions (Lebovitz 2001).

2-12-4 Insulin Resistance

Insulin resistance is the inability of target organs to respond appropriately to insulin's action. Insulin resistance leads to higher insulin needs due to inadequate reduction of hepatic glucose production and decreased insulin-mediated glucose absorption in the peripheral (skeletal muscle and adipose tissue). Hyperglycemia occurs when higher insulin demands are not met by increasing insulin levels. Insulin resistance has also been linked to other disorders such as central obesity, hypertension, and dyslipidemia, all of which are risk factors for heart disease (Ahima and Flier, 2000).

Insulin resistance can be the source of inflammation. Insulin resistance leads to adipose tissue macrophage infiltration which triggers an inflammatory response in insulin signaling (IRS-1/2:P I3K:AKT:mTORC2) where pathwaymTORC2 suppresses the MCP1 gene in adipose tissue. The downregulation of insulin signaling in conjunction with lower mTORC2 activity will depress MCP1 and draw monocytes to adipose tissue, where they will be transformed into M1 macrophages as it is shown in figure (2-5) (Santos *et al*.,2021). The mystery behind the link

between insulin resistance and inflammation has yet to be unraveled. As a result, we could speculate that inflammation generated by insulin resistance may be a contributing factor to the cytokine storm seen in obese Covid-19 patients (Shimobayashi *et al*.,2018).

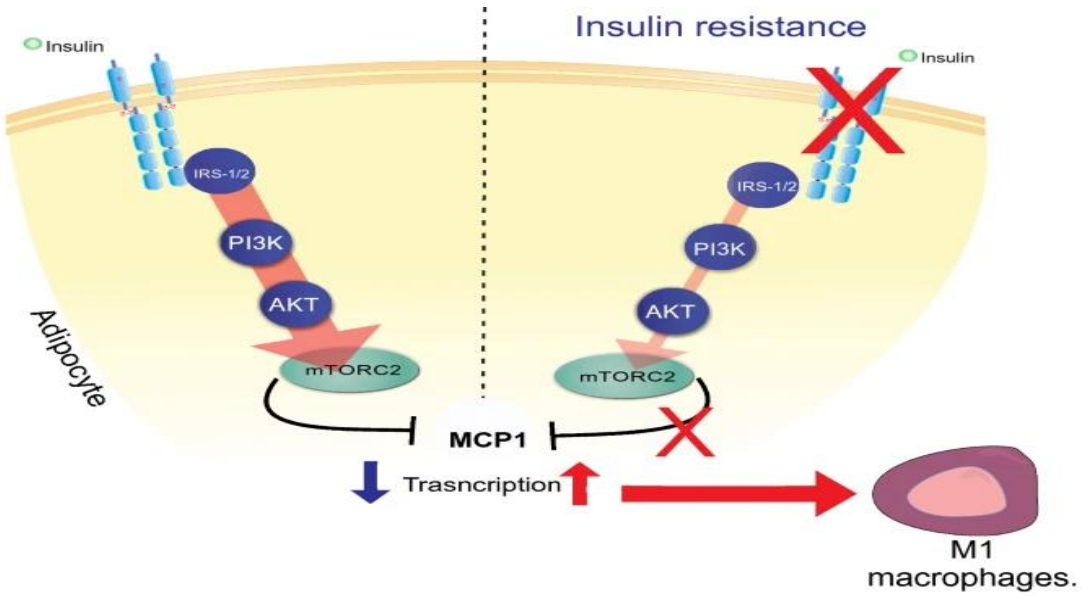


figure 2-5 Insulin Resistance Relationship with Inflammation (Santos *et al*.,2021)

2-12-5 Insulin Sensitivity

Dysregulation of carbohydrate and lipid metabolism causes glucose intolerance. Genetic or lifestyle/environmental variables that influence the physiological activity of beta-cells and insulin sensitivity are frequently linked to this disorder (Iqbal *et al*., 2015). Adipose tissue releases leptin, resistin, adiponectin, and tumor necrosis factor (TNF-), where all of them disturb glucose metabolism by lowering insulin sensitivity. It is clearly seen that T2DM is more frequent in obese people (Duvnjak and Duvnja, 2009). Tumor necrosis factor can also suppress GLUT4 expression required for glucose transfer (Olson, 2012). Insulin resistance, as a symptom of T2DM, is caused by a loss of insulin sensitivity, whereas a

lack of insulin secretion and function results in an elevated blood glucose level (Iqbal *et al*., 2015).

2-12-6 C-Reactive Protein

The c-reactive protein (CRP) is a highly conserved ancient molecule that belongs to the pentraxin protein family. The liver produces CRP in response to several inflammatory cytokines. CRP levels rise swiftly in reaction to trauma, inflammation, and infection, and fall just as quickly when the disease is terminated. As a result, CRP testing is used to monitor a variety of inflammatory diseases. CRP binds to damaged tissue, nuclear antigens, and some pathogenic organisms in a calcium-dependent way. CRP's role in the innate immune system is assumed to be linked to its engagement (Du Clos *et al*., 2000).

Some patients infected with SARS-CoV-2 have not developed hypoxemia or respiratory stress during COVID-19, demonstrating that SARS-CoV-2 infection is a diverse disease. To predict the severity of COVID-19 pneumonia, one accurate and convenient biomarker is required. Several studies have recently found that C-reactive protein (CRP) is linked to severe dengue infection and that patients with greater plasma CRP during the early stages of the disease are more likely to develop plasma leakage (Eppy *et al*.,2016; Chen *et al*., 2020).

2-12-7 D-dimer level

D-dimer is a soluble fibrin degradation product that results from the fibrinolytic system's orderly breakdown of thrombi. D-dimer has been established in numerous studies to be a useful measure of coagulation and fibrinolysis activation (Weitz *et al*., 2017).

A three- to four-fold increase in D-dimer levels is associated with a bad prognosis. Furthermore, underlying disorders like diabetes, cancer,

stroke, and pregnancy may cause D-dimer levels to rise in COVID-19 patients. Controlling and managing COVID-19 disease can also be done by measuring D-dimer levels and coagulation parameters early on in the disease (Rostami *et al* ., 2020).

2-12-8 Ferritin level

The ferritin, through direct immuno-suppressive and pro-inflammatory actions, is a significant modulator of immune dysregulation, especially in extreme hyperferritinemia, leading to the cytokine storm (Abbaspour *et al*., 2014)

The COVID-19-infected individuals have had lower hemoglobin levels and higher ferritin levels according to the past research. Regardless of the underlying diseases, anemia associated with hyperferritinemia is a powerful predictor of mortality. Elevated ferritin levels in COVID-19 could signal an impending inflammatory response linked to viral propagation in the human body, affecting iron metabolism (Wessling, 2018). In this aspect, iron is an important micronutrient for both human cells and pathogens. During infections, the natural immune response may reduce iron turnover to restrict the pathogen's ability to use it. This process, on the other hand, can cause anemia, which lowers oxygen delivery to tissues and leads to multi-organ failure. As a result, one can assume that it is critical for the investigation of the link between iron metabolism and the evolution of covid-19(Weiss *et al*., 2019).

2-13 SARS-CoV and ACE2 as SARS-CoV Receptors

In vitro and in vivo, the functional SARS-CoV receptor has been identified as angiotensin-converting enzyme 2 (Imai *et al*., 2005; Monteil *et al*., 2020). On the human X-chromosome, the ACE2 gene has 22 exons covering around 40 kb of genomic DNA. It has a 40 percent amino acid

sequence similar to ACE1's N- and C-terminal domains which makes it a homolog (Tipnis *et al*., 2000; Harmer *et al*., 2002).

Angiotensin-Converting Enzyme II (ACE2) is a membrane-bound carboxydipeptidase found throughout the human body, including the heart, kidney, small intestine, and to a lesser degree, the lungs. Type II alveolar cells and macrophages express the most ACE2, with bronchial and tracheal epithelial cells expressing the least (Hamming *et al*., 2004). Angiotensin II is degraded by ACE2 to produce angiotensin 1-7 which activates the mas oncogene receptor and inhibits several angiotensin II activities mediated by the type 1 receptor (AT1R). Thus, the ACE2/angiotensin 1-7/mas receptor axis is hypothesized to counterbalance the highly active ACE/angiotensin II/AT1R axis found in hypertension, cardiac hypertrophy, heart failure, and other CVDs (Santos *et al*., 2017).

Human ACE2, SARS-CoV, on the other hand, enters the host target cells through a well-established functional receptor (Turner *et al*., 2004). The SARS-transmembrane SARS-CoV binds to the target cells, then to SARS-CoV-S protein primed by cellular surface proteases including transmembrane protease serine 2 (TMPRSS2) which allows viral and cellular membranes to merge, resulting in SARS-CoV infection which can enter and reproduce in the target cells. Furthermore, in SARS-CoV-infected mice, the elimination of ACE2 significantly lowers viral infection and replication. As a result, it is believed that the SARS-CoV-S protein's binding to ACE2 is critical for SARS-CoV infection (Kuba *et al*., 2005).

2-13-1 ACE2'S Normal Physiological Role

The major physiological function of ACE2 is to serve as a metalloprotease, digesting a variety of proteins in the renin-angiotensin-aldosterone pathway. ACE2 transforms the vasoconstrictors angiotensin-I

and angiotensin-II into angiotensin-(1–9) and angiotensin-(1–7), both of which are vasodilators (Carey *et al*., 2003; McKinney *et al*., 2014). As a such, ACE2's activity tilts the vascular control balance in favor of vasodilation (Schulz *et al*., 2011). AT1R, an angiotensin-II receptor, is found on macrophages, dendritic cells, T-cells, mesangial cells, and vascular smooth muscle cells, suggesting that the ACE2 processing of angiotensin-II affects their activity (Benigni *et al*., 2010). ACE2 possesses normal physiological activities that are closely linked to COVID-19 characteristics in the lung, causing inflammation, oxidative stress, and fibrosis, in addition to their role to sustain SARS-CoV-2 cellular entrance (Schulz *et al*.,2011).

The ACE2 inactivates the vasodilator bradykinin, in addition to its involvement in the RAAS. It is vital to remember that the RAAS and the bradykinin system have a complicated relationship. Because of its impartiality in silico approach to identify the underlying reason for variable severity in COVID-19 symptoms, the role of bradykinin in the COVID-19 tale has lately acquired attention. Endothelial nitric oxide (NO) and prostacyclin (PGI2) induce vascular permeability and local oedema, while Bradykinin binds to particular G-protein-coupled receptors to mediate vasodilation. Leaky capillaries in the lungs of COVID-19 patients would not only make breathing difficult due to oedema, but they would also enhance the trafficking of inflammatory cells and the inflammatory mediators they carry between the blood and lung tissue (Garvin *et al*., 2020).

2-13-2 ACE2 and the S Protein

SARS-S CoV-2's protein is a homotrimer, and it is a class I fusion protein, much like SARS- CoV-1's which recognizes the ACE2 receptor on the host cell as a crucial step in the viral internalization process (Chen

et al., 2020). ACE2 is a zinc metalloprotease having ACE and collectrin-like properties (CLTRN) (Kuba *et al*., 2010). The SARS-CoV-2 binding interface is comparable to that of the SARS-CoV-1 S protein, according to X-ray crystallography (Lan *et al*., 2020), and in *vitro* investigations have demonstrated that SARS-CoV-2 internalization needs ACE2. (Hoffmann *et al*., 2020)

When the S protein binds to ACE2, so its conformation changes allow virus particles and host cells to create a membrane fusion permitting the viral DNA to be internalized. The receptor-binding domain (RBD) of the protein is found in the S1 subunit, where it interacts with the N-terminus of ACE2 on target host cells. The S2 subunit has two different heptad repeated domains as well as a fusion domain which are all required for viral entrance into host cells (Walls *et al*., 2020). S2's heptad repeats interaction with each other after the binding of the viral to ACE2, generating a six-helical bundle (6HB) complex (Xu *et al*., 2020). The development of 6HB in SARS-CoV-1 exposes the hydrophobic fusion region of the S2 subunit embedded into the host cell membrane to initiate fusion (Liu *et al*., 2004).

2-14 A1166C at1r Polymorphism Receptor

The AT1R receptor is one of the important receptors in modulating blood pressure. High blood pressure is directly linked to the SARS-CoV-2 virus, caused by the inhibition of this receptor (Gurwitz,2020).

Overactivation of the AT1R is exceedingly damaging to the human body, resulting in the formation of COVID-19 in particular. The activated receptor causes vasoconstriction, hypertension, organ hypertrophy (heart, blood vessels), tissue fibrosis (heart, lungs, kidneys, and liver), ageusia (loss of taste), anosmia (loss of smell), neurological disorders, intestinal

and vascular inflammation, obesity and glucose metabolism (diabetes), as well as skin and testicular lesions (Coto *et al*., 2021; Rysz *et al*.,2021).

2-14-1 Angiotensin-Converting Enzyme 2 and Renin–Angiotensin–Aldosterone System

As reviewed elsewhere, the renin-angiotensin-aldosterone system (RAAS) is a signaling pathway that acts as a homeostatic regulator of vascular function (Iwai *et al*., 2009). It regulates systemic and local blood flow, blood pressure, natriuresis, and trophic responses to a variety of stimuli in a dynamic manner. Low intratubular sodium concentration and sympathetic nerve stimulation cause the macula densa to produce renin in the liver where angiotensinogen is converted to angiotensin I. Angiotensin-converting enzyme (ACE) converts angiotensin I (Ang I) to angiotensin II (Ang II) which is produced mostly in the lungs. The adrenal cortex is then stimulated by Ang II to release aldosterone (Murray *et al*.,2020; Silhol *et al*., 2020).

Figure (2-6) is a diagram of the RAAS signaling pathway. ACE/angiotensin II (Ang II)/Ang II receptors AT1 and AT2 make up the RAAS' primary axis. The kind of receptor that Ang II binds to can cause distinct effects. For example, AT1 receptor binding increases the conventional Ang II effects of AT2 receptor binding to promote the opposing effects of increased oxidative stress, inflammation, fibrosis, and vasoconstriction. Furthermore, persistent RAAS activation is linked to Ang II/AT1 toxicity worsens inflammation, fibrosis, apoptosis, antidiuretic hormone (ADH) and aldosterone secretion, and eventually water and salt retention (Murray *et al*., 2020; Silhol *et al*.,2020).

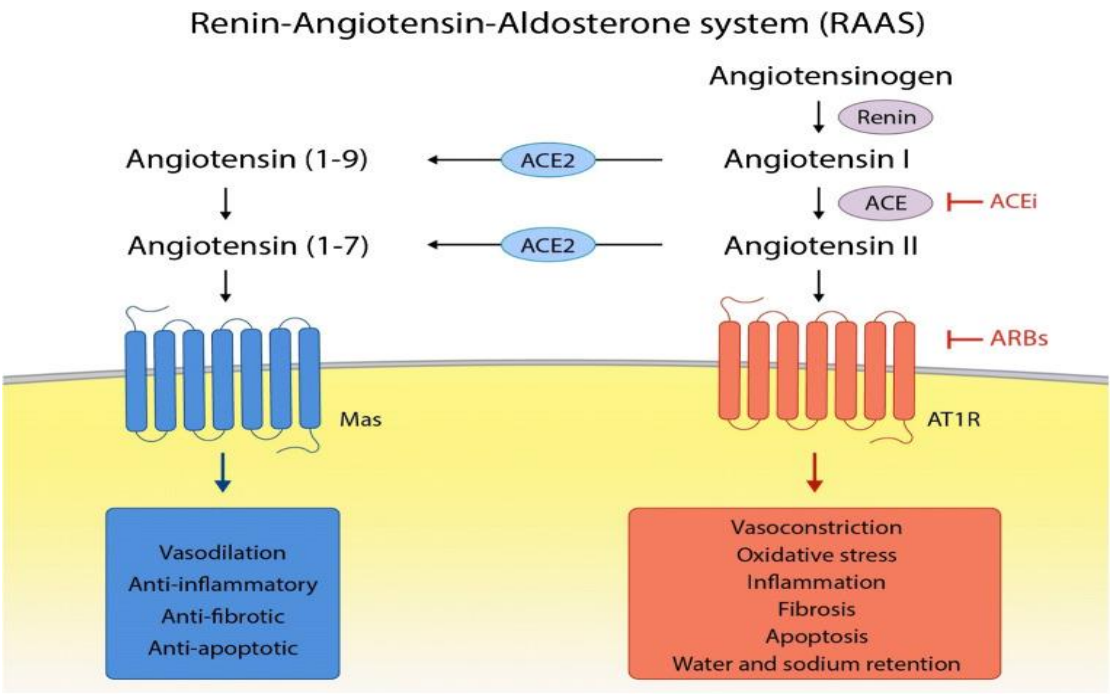


Figure: 2-6 Renin-Angiotensin-Aldosterone System(RAAS) (Silhol *et al*.,2020).

2-15 ACE2 Distribution and COVID-19 Manifestations

The SARS-CoV-2 infection is linked to ACE2 expression in multiple organs. Lower respiratory tract (2%), lung (> 1%), heart (> 7.5%), ileum (30%), esophagus (> 1%), kidney (4%), and bladder (4%). (4%) are all classified as high-risk tissues (2.4 percent). It is worth noting that the number of ACE2-positive cells in stomach and liver samples is less than 1%, indicating that SARS-CoV-2 infection has not been a concern for these organs (Zou *et al*., 2020). Importantly, ACE2 nasal gene expression is age-dependent, which may explain, at least in part, why younger persons have a lower COVID-19 incidence because they have the lowest ACE2 nasal gene expression (Bunyavanich *et al*., 2020). As compared to adults, children can have a reduced ACE2 expression not just in the bronchial epithelial cells but also in nasal epithelial cells, and this may also explain the non-respiratory COVID-19 symptoms/clinical presentations in the youngest COVID-19 patients (Sharif-Askari *et al*., 2020).

The SARS-CoV-2 infection in the oral cavity is also a possibility (Xu *et al*., 2020) since ACE2 is expressed throughout the oral mucosa, particularly in tongue epithelial cells. This conclusion has been in line with previous research that shows in COVID-19 patients, SARS-CoV-2 induces gustatory and olfactory impairment. Anosmia is not simply attributable to ACE2 expression in the olfactory cavity of these patients. (Brann *et al*., 2020), but it also raises concerns regarding SARS-CoV-2 (Vaira *et al*., 2020).

To note, neurological manifestations can potentially be explained by a fever or nasal obstruction and conductive olfactory loss due to mucosal congestion (Lechien *et al*., 2020). ACE2 expression in sensory and olfactory bulb neurons has not been found. However, magnetic resonance imaging (MRI) has revealed no aberrant findings in the olfactory bulb or tract, where there has been no evidence of nasal blockage (Galougahi *et al*., 2020). As a result, anosmia and ageusia in COVID-19 might indicate a problem with the olfactory and gustatory receptors in various brain locations where ACE2 is expressed (Kabbani *et al*., 2020). and encephalopathy characteristics have been observed by MRI along with neurologic symptoms including headache and dizziness which boost the ability of viruses to reach the brain and spinal cord (CNS) (Helms *et al*,.2020). The SARS-CoV epidemic has presented a putative pathophysiological mechanism for CNS involvement, in which the virus might pass past the blood-brain barrier and attach to the epithelial ACE2 receptor via hematological dispersion or via the cribriform plate. Furthermore, SARS-CoV-2 has been discovered in post-mortem samples of the frontal lobe (PanizMondolfi *et al*., 2020) and in the cerebrospinal fluid proving the virus's neurotropic capability (Moriguchi *et al*., 2020).

In addition, evidence of ACE2 expression in the oral cavity suggests the SARS-CoV-2 pathways infection. The major route of viral infection has been thought to be the respiratory transmission; however, recent studies may help us better comprehend alternate pathways transmission. ACE2 expression has been found in various digestive system regions, while SARS-CoV-2 RNA has been found in fecal samples from COVID-19 patients (Amirian *et al*., 2020). Furthermore, the presence of Days after nasopharyngeal swabs have been confirmed to be negative. The viral RNA in rectal samples have shown the viral shedding for days following IgG seroconversion. As a result, ACE2 expression and distribution imply that SARS-CoV-2 might be transmitted from feces to the mouth; nevertheless, viral viability in stool samples must be investigated further (Smyk *et al*., 2020; Wölfel *et al*., 2020).

It's worth noting that male patients can have a little higher infection rate and a substantially greater death rate than female patients (> 60% vs. 40%). ACE2 expression might explain these results and, as such, the impact of COVID-19 susceptibility in different ethnic groups, genres, and ages (Chen *et al*., 2020; Li *et al*., 2020) reveals, with the help of single-cell RNA-seq, that ACE2 expression is 3-fold greater in male lung samples than in female lung samples (Beyerstedt *et al*., 2020). In virto experiments, estrogen has also been shown to influence ACE2 expression in different airway epithelial cells (Stelzig *et al*., 2020). In salivary glands, however, there has beens no gender difference in ACE2 and TMPRSS2 expression (Song *et al*., 2020) and bronchial epithelium (Bradding *et al*., 2020).

Therefore, other variables and habits, such as smoking, which is more widespread in males, might influence enzyme expression. Smoking has been linked to 1.4 time of greater risk of developing severe manifestations of the disease and 2.4 times of higher requirement for

intensive care in studies employing epidemiological data from COVID-19 participants (Vardavas *et al*., 2020). ACE2 expression is high in the lungs and bronchial tubes in patients with chronic obstructive pulmonary disease (COPD) (Higham *et al*., 2020; Pinto *et al*., 2020) Smoking is the major cause of COPD, as it damages the respiratory system and its function. Because ACE2 expression in the lungs and bronchial epithelium is increased by smoking, (Brake *et al*., 2020; Leung *et al*., 2020), the nicotinic acetylcholine receptor may also upregulate ACE2 in neurons, glia, and endothelial cells (Kabbani *et al*, 2020). As a result, smoking increases the susceptibility of these tissues to SARS-CoV-2 infection and is linked to COVID-19 pneumonia. Importantly, ACE-2 gene expression in bronchial epithelial cells can not be changed in patients with asthma compared to healthy people. This explains why asthma and COVID-19 severity are not linked, but can be aggravated in COVID-19 (Bradding *et al*., 2020).

The researchers discovered that the major male hormone imbalances have been connected to possible harm to Leydig cells. Longer follow-up is still required to determine the impact of SARS-CoV-2 on male fertility. a mystery because ACE2 is expressed in the female reproductive system. This is suggesting that it might be a target for SARS-CoV-2, aside from genre distinctions. ACE2 is prominently expressed in the testicles, raising awareness of the virus's impact on reproductive and sexual transmission. SARS-CoV-2 has not been found in the semen according to a single-center analysis, or male tissues of verified COVID-19 patients during the acute and post-recovery stages (Song *et al*., 2020). SARS-CoV-2 transmission by sexual intercourse, for example, is improbable. This leads to investigate whether or not the illness may result in infertility (Yu *et al*., 2020). Pregnant women are thus a high-risk category for COVID-19. ACE2 expression is increased in the kidneys, uterus, and placenta during

pregnancy enhancing RAAS activation (Brosnihan *et al*., 2004). This has clearly seen in murine models (Levy et al., 2008). ACE2 has a time-dependent expression pattern in the placenta, and it may be found in numerous fetal organs in the late stages of pregnancy (Bunyavanich *et al*., 2020). SARS-CoV-2 has not been detected in the placenta, although intramural fibrin deposition and the foci of villous stromal–vascular karyorrhexis, as well as infarction, can be seen (Baergen *et al*., 2020). Furthermore, 15.4% of pregnant women hospitalized for delivery have been tested positive for SARS-CoV-2 though they are asymptomatic. Such a finding can have serious clinical implications (Sutton *et a*l.,2020).

The virus has not been found in amniotic fluid, cord blood, breastfeeding, or newborn throat swabs. The symptoms of COVID-19 levels in a small group of pregnant women have been similar to those in non-pregnant situations. Furthermore, a case-control study of 225 pregnant women indicated that COVID-19-related symptoms have been comparable to controls and that the cumulative rate of early pregnancy loss has been different but similar to controls (Cosma *et al*., 2021). However, moms hospitalized owing to COVID-19 pathology signal a higher chance of having their pregnancy terminated by cesarean section, and the neonates can have a higher risk of being born prematurely (Marín Gabriel *et al*., 2020).

In late pregnancy, there are no signs of vertical transmission. Many issues remain, including what implications the SARS-CoV-2 could have on fetal development due to the reduced ACE2 activity during the early stages of pregnancy. Of importance, the COVID-19 pandemic is still ongoing and further investigation is required (Bharadwaj *et al*., 2011).

2-16 Infants with SARS-CoV-2

Numerous studies have emphasized the low prevalence of SARS-CoV-2 infection in newborns compared to adults (Castagnoli *et al*., 2020). Approximately 2% of Covid-19 cases are in children. (Wu *et al*., 2020). SARS-CoV-2 infection and death are less likely in children (Patel *et al*., 2020). Furthermore, reduced ACE2 expression in the upper airway nasal epithelium has just been found in newborns (Bunyavanich *et al*., 2020).

The ACE2 gene expression is an age-dependent in the nasal epithelium, where it is the first site of contact between SARS-CoV-2 and the human body. The ACE2 gene has the lowest expression in infants (aged 2.5 years). The positive and quasi-linear relationship between ACE2 gene expression and age appears to be unaffected by sex or asthma. The fact that children having lower nasal ACE2 gene expression than adults might explain why SARS-CoV-2 is less frequent in neonates and why they have a lower mortality rate (Dong *et al*., 2020).

2-17 The ABO Transferase A Gene Polymorphism (rs657152)

The ABO blood group system contains four blood types: A, AB, B, and O, which is the most significant blood group system in humans. The ABO blood group is found on chromosome 9 in humans (9q34.2) (Guillon *et al* 2008; Wiggins *et al*., 2009). According to several researches, the ABO blood type has been linked to a variety of human illnesses, including cardiovascular, oncological, infectious, and non-infectious disorders (Chen *et al*., 2016; Wolpin *et al*., 2010). Meanwhile, the system can play a direct role in infection by acting as receptors or co-receptors for bacteria, parasites, and viruses. One of the most prevalent antigens identified on the surface of human red blood cells are histo blood group antigens (HBGAs). They have a variety of forms and qualities that are passed down across

generations in individuals and groups. Antigen expression differences across blood groups can enhance or reduce a host's susceptibility to a variety of illnesses. Several blood-types antigens influence the innate immune response to infection by facilitating intracellular absorption, while the architecture of membrane microdomains can be used for signal transduction or adhesion (Behal *et al*., 2010; Singh *et al*., 2016; Chakrani *et al*., 2018; Liu *et al*., 2018). Since March 2020, several papers have reported a link between the ABO blood group and COVID-19. Among these are works by (Wu *et al*., 2020; Zeng *et al*., 2020) Everyone agrees that group A people would be at a higher risk of infection, whereas group O people would be at a reduced risk. In conformity with epidemiological data, Group O people can have a decreased risk of infection during the last SARS-CoV-1 outbreak in Hong Kong (Cheng *et al*., 2005), However, due to the swift management of the pandemic and its limited impact on the world population, this discovery has not been thoroughly discussed or validated by subsequent research. Findings from epidemiological experimental investigations and the present human understanding of how coronavirus enters our cells imply that the link between ABO blood group and infection risk is extremely feasible. In a research setting, the anti-A antibodies have been shown to prevent SARS-Cov-S protein-expressing cells from adhering to ACE2-expressing cell lines. As a result, Researches have come to the conclusion that the lower susceptibility of blood type O to COVID-19 and the higher susceptibility of blood group A to COVID-19 might be due to the existence of natural ABO antibodies in the blood, specifically anti-A antibodies (Guillon *et al*.,2008).

Although the majority of individuals infected with SARS-CoV-2 do not become ill, some do die within days. Some of the differences might be attributed to age and past illnesses andobesity as well. Genetics, on the

other hand, plays a role in COVID-19 where several genes and loci have been linked to an increased risk of respiratory failure in genome-wide association studies. By studying the SNP rs657152 situated in the intron region of the ABO gene which defines blood type, it has been discovered that the ABO gene, Chr 9q34.2, the determines of blood type, may enhance sickness severity (Lehrer and Rheinstein, 2021), This outcome, however, has not been repeated. In a study of 2200 Covid-19 patients in United Kingdom,it has been seen that there has been no connection between ABO blood type and sickness severity (Kaiser, 2020). ABO blood group has been found to be a risk factor for SARS-CoV-2 infection in Danish research, but it has not been the result of hospitalization or mortality caused by COVID-19 (Barnkob *et al*., 2020) In the current investigation, we will look at the association between ABO blood types and rs657152, a cancer-linked gene, and cardiovascular disease risk as well as COVID-19 testing positive which leads to death (Li *et al*., 2020).

Chapter three

Materials and methods

3- Materials and Methods

3-1 Chemical and Instrument

A chemical that was used during this study is shown in table (3-1)

Table (3-1): Chemical Materials Utilized in the Study

N0. Chemical	Company
1-Acetic acid	Scharlau
2- Acrylamide	Bio-Basic / Canada
3- Agarose	Bio-Basic / Canada
4- Ammonium persulfate	Bio-Basic / Canada
5- Bis-Acrylamide	Bio-Basic / Canada
6- D200 DNA ladder	Biosharp/Korea
7-DNA Extraction Kit	Fibrogene
8- EDTA	Himedia(India)
9- Ethanol	Biosolve company/USA
10- Ethidium Bromide	Promega/USA
11-Formaldehyde	BDH chemical/ England
12- Glycerol	Bio Basic/Canada
13- Loading Dye (bromophenol blue)	Promega/USA
14-Master Mix	Promega/USA
15- Primer	Macrogen
16- proteinaes K	Biolabs/England
17- silver nitrate	Scharlau
18- Sodium hydroxide	Scharlau
19- TBE Buffer	Bio-Basic / England
20- TEMED	Bio-Basic/ Canada

3-2 An Instruments Used During this Study is Shown in The Table

Table (3-2): Instruments and Equipment its Supplying Company

NO. Tools	supplying company
1-Autocluve	Haramaya/Japan
2-Balance	pricisa/UK
3-centrifuge; cooling centrifuge	Amal/Turkey
4- Conical flask	China
5- Cylinder	China
6- Deep freeze	GFL/Germa
7-Digital Camera	Sony/Japan
8-Distillater	Ogawa Seki (Japan)
9-EDTA tube	AFCO/ Jordan
9-Electronic sensitive Balance	Denevr / Instrument / Germany
10-Gel tube	AFCO/Jordan
11- Horizontal Gel electrophoresis unit	Cleaver scientific/Germany
12- Hot plate with a magnetic stirrer	Heidolph/ Germany
13- Microcentrifuge tubes	Hlalab
14-Micropipette	Dragon med. (Germany)
15- PCR tube	Hlalab
16- photo documentation, UV source	Cleaver Scientific/ UK
17- Polyacrylamide gel electrophoresis	Cleaver scientific/UK
18-Sodium Citrate tube	AFCO/Jordan
19- UV transilluminator	Cleaver scientific/ Japan
20- Vertical Gel electrophoresis unit	Cleaver scientific/ Germany
21- vortex mixer	Bioneer/Korea
22-water bath	GFL/Germany

3-3 Experiment Design

Figure (3-1): The Scheme of Experiment Design is Explained as Following:

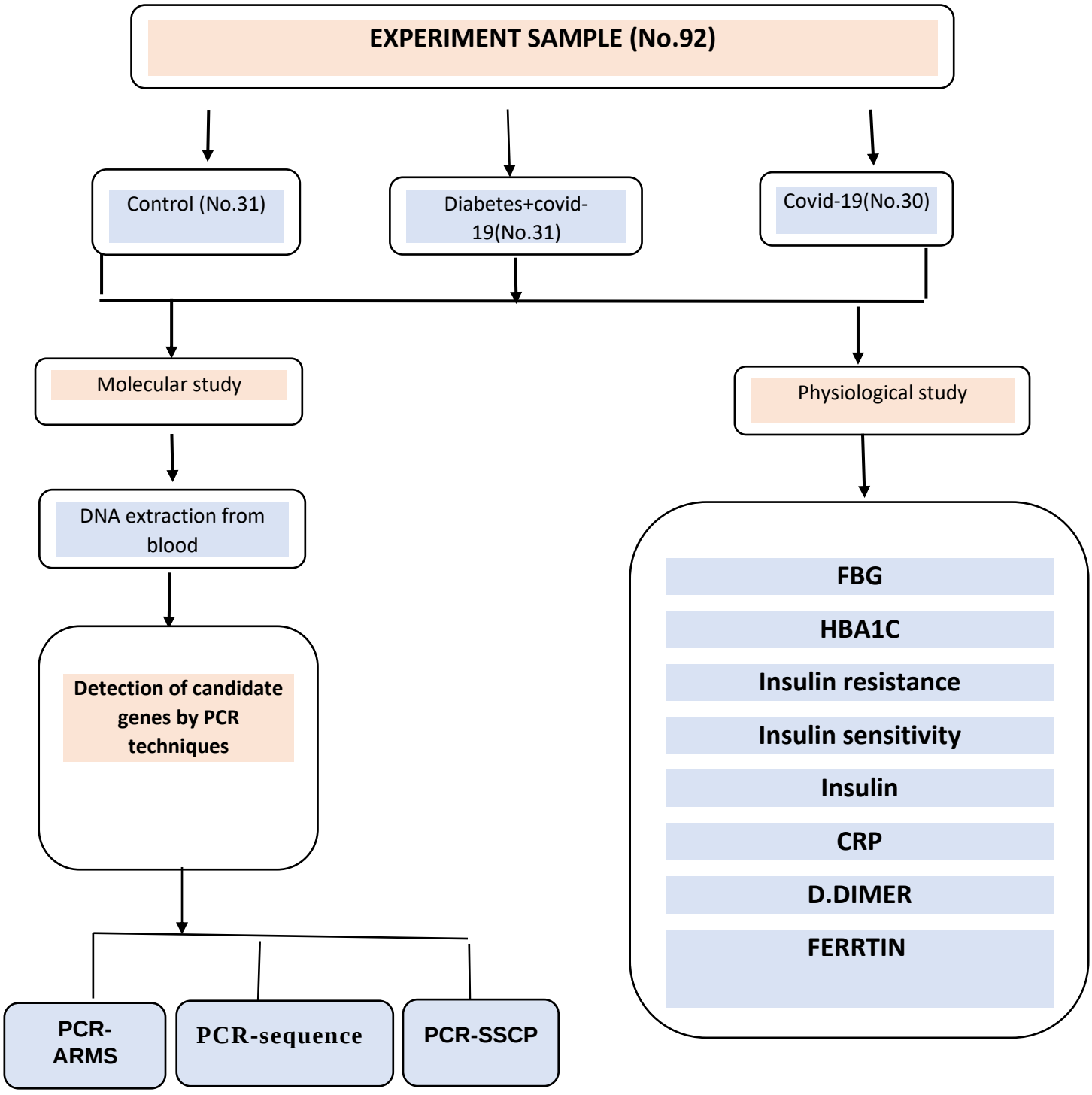


Figure (3-1): scheme of experiment design

3-4 Study Subject

3-4-1 Study Design, Sample, and Data Collection Time

The current study was conducted in the molecular laboratory in the Department of Biology, College of Science, University of Babylon. Samples were collected in the month of October 2021. They included males and females patients from Marjan Teaching Hospital and Al-Saadiq Hospital. The percentage of patients ranged from mild, moderate, and severe in Intensive Care Units (ICU). 31 control (3 females, 28 males), 30 covid only (16 females, 14 males), and 31 were covid and diabetes (17 females, 14 males), while the physiological analyses for them were conducted in the Al-Sudair Specialized Laboratory where all the patients and control were from the same ethnic group (Arabs). The samples and data collection were carried out according to the ethical approval of environment and in conformity with the criteria issued by The Ministry of Health Iraq.

2-4-2 Questionnaire

The questionnaire was given to the patient and the case sheets included control individuals and covid -19 patients in addition to diabetes patient. The Questionnaire sheets included: name, age, sex, duration of disease, weight, work, place of injury, covid-19 treatment type, diabetes treatment type + dose quantity, CT scan, and some other questions like: Do you have other diseases? Do you have diabetes? Do you have injured or deceased relatives with covid-19? Have they been injured or have deceased due to diabetes?

3-4-3 Blood Collection

About five milliliters of venous blood were collected from each patient and control subject in the study. The blood was collected into (EDTA, gel, and sodium citrate) tubes. 2 ml of blood was put in the EDTA tube used to measure Hba1c levels and extract DNA, stored at - 20˚C (deep freeze). 2 ml of Blood was pushed slowly into disposable serum tubes containing separating gel. The blood in gel tubes was allowed to clot at room temperature for 10-15 min then it was centrifuged at 2000 r.p.m for about 10-15 min. After the serum was obtained by using the Gel tube to measure FBS, Ferritin, CRP, IN. 1 ml in was placed sodium citrate being used to measure D-dimer levels.

3-5 Methods

3-5-1 Fasting Blood Glucose Measurement (FBG)

The RanDox kit was used to determine serum Sugar levels based on the PAP enzymatic determination of glucose (Barham and Trindoer method,1972). Glucose was determined after its enzymatic oxidation in the presence of glucose oxidase to gluconic acid and hydrogen peroxide reacted under the catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinonimine dye as an indicator according to the following reaction**:**

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$$

$$2H_2O_2 + 4\text{-aminophenazone} + \text{phenol} \xrightarrow{\text{POD}} \text{quinoneimine} + 4H_2O$$

Procedure

Test tubes were prepared for sample, blank, and standard as following the instructions in the table (3-3)

Table (3-3): The Procedure for Measuring the levels of Fasting Blood Sugar in Serum (mg/dl).

Reagents	Blank	Standard	Sample
Standard	-	10 µl	-
Serum	-	-	10 µl
Working reagent	1000 µl	1000 µl	1000 µl

The solution was mixed and incubated for 10min at 370C, while the absorbance of color was read at 505 nm by spectrophotometer within 60min using 1cm lightpath cuvette.

3-5-2 Hemoglobin A1c (HbA1c)

The test used a sandwich immunodetection method; the detector antibody in buffer could bind to antigen in sample, forming antigen-antibody complexes, and migrating onto nitrocellulose matrix to be captured by the other immobilized antibody on test strip. The more antigen in the sample forms the more antigen-antibody complex which led to stronger intensity of fluorescence signal on detector antibody. Instrument for ichromaTM tests would display the content of glycated hemoglobin in terms of percent of the total hemoglobin in blood.

Normal range: 4.5-5.6%

Test procedure

1- Drawing 100 µL of hemolysis buffer and transferring it into detection buffer tube.

2-Drawing 5 µL of fingertip blood or tube blood using 5 µL capillary tube while puting the capillary tube into the detection buffer tube

3-Closing the lid of the detection buffer tube and mixing the sample thoroughly by shaking it about 15 times.

5-Taking out the cartridge half form i-Chamber slot.

6-Pipetting out 75 µL of the sample mixture and loading it into a sample well in the test cartridge.

6-Waiting till the sample mixture flow would appear in the windows.(about 10 seconds)

7-Inserting the cartridge into i-Chamber slot.

8-Leaving the cartridge in i-Chamber for 12 minutes before removing it.

9- Scanning the sample-loaded cartridge and inserting it into the cartridge holder of the instrument for ichromaTM tests. Proper orientation of the cartridge was ensured before pushing it all the way inside the cartridge holder. An arrow would be marked on the cartridge, especially for this purpose.

10-Pressing the‘ Select’ button on the instrument for ichromaTM tests to start the scanning process.

11- Scanning immediately the sample–loaded cartridge by the instrument for ichroma TM tests.

12- Reading the test result on the display screen of the instrument for ichromaTM tests.

3-5-3 Insulin Assay

Insulin level was detected by name of the company with a catalog number as follows:

1- A 50µl of the appropriate serum was added to the assigned well.

2- A 100µl of the Insulin Enzyme Reagent was added to all the wells.

3- The microplate was swirled gently for 20-30 seconds to mix and was covered with plastic wrap.

4- Incubated for 60 minutes at room temperatures.

5-The contents of the microplate were discarded by decantation or aspiration and if decanting, the plate was tapped and plotted dry with absorbent paper.

6- A 350 µl of wash buffer, decanted or aspirated was added. Two additional times were repeated for a total of three washes, then the wash was decanted and repeated for two additional times.

7- A100 µl of substrate solution was added to all wells provided that the plate should not be shaken after the substrating addition then the microplate was incubated at room temperature for 15 minutes.

8- A 50 µl of stop solution was added to each well and gently was mixed for 15-20 seconds.

9- Reading the absorbance in each well at 450 nm by using a microplate reader and the results should be read within 15 minutes after adding the stop solution.

10- Reading the concentration of insulin hormone from the standard curve, as in the figure (3-2).

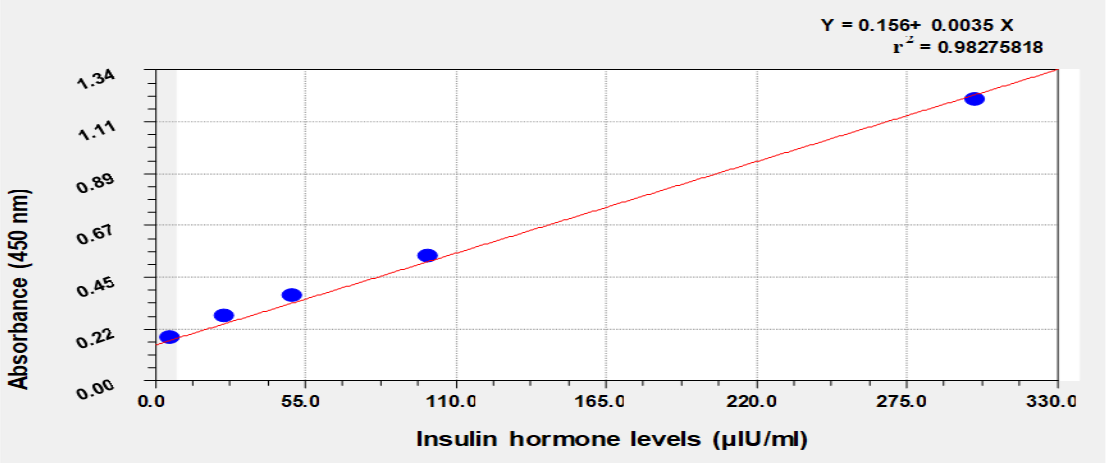


Figure (3-2): Insulin standard curve

3-5-4 Insulin Resistance Test

Insulin resistance (IR) can be evaluated by the determination of homeostasis model assessment of insulin resistance (HOMA-IR) (Mathews *et al*., 1985; Stumvoll and Gerich, 2001) and calculated by using the following equation

IRHOMA = (I0 × G0) / 22.5

Where: IRHOMA: insulin resistance according to homeostasis model assessment

I0: Fasting insulin level.

G0: Fasting glucose level.

3-5-5 Insulin Sensitivity Test

The quantitative (IS) insulin sensitivity check index (QUICKI) is derived by using the inverse of the sum of the logarithms of the fasting insulin and fasting glucose (Katz *et al.*, 2000).

1 / (log (fasting insulin μU/mL) + log (fasting glucose mg/dL)

3-5-6 C-Reactive Protein Test

The AFIAS CRP is a fluorescence immunoassay (FIA) for the quantitative determination of C-reactive protein (CRP) in human whole blood/serum/plasma. It is useful as an aid in the management and control of autoimmune diseases and infectious processes, such as rheumatoid arthritis. For *in vitro* diagnostic use only.

*The serum CRP level may rise from a normal level of <5 mg/L to 500 mg/L.

The test used a sandwich method to detect immunity. The reagent antibodies in the buffer would bind to the antigens in the sample to form antigen-antibody complexes, and migrate to the nitrocellulose matrix which could be captured by other antibodies attached to the test strip. More antigens in the sample would form more antigen-antibody complexes resulting in a stronger fluorescence signal by the reagent antibody, which was processed by the AFIAS assay instrument to show the CRP concentration in the sample. (Pepys *et al*., 2003)

1-A 100 μL of sample was taken with a pipette and dispensed into the sample well in the cartridge.

2-The cartridge was inserted into the cartridge holder.

3-A tip was inserted into the tip hole of the cartridge.

4- The 'START' icon was tapped on the screen.

5-The test result would be displayed on the screen after 3 minutes

3-5-7 D-dimer Test

The test used a sandwich immunodetection method; the detector of antibody in buffer would bind to the antigen in the sample, forming antigen-antibody complexes, and migrating onto nitrocellulose matrix to be captured by the other immobilized antibody on the test strip. The more antigen in the sample could form a more antigen antibody complex leading to stronger intensity of fluorescence signal on detector antibody. This was processed by Instrument for ichroma™ tests to show D-dimer concentration in the sample: normal range: 1.0 ug/ml or lower

Test procedure

1- A 10 μL of sample was transferred (Human whole blood/plasma/control) by using a pipette annexed to a tube containing the detection buffer.

2- The lid of the detection buffer tube was closed and the sample was mixed thoroughly by shaking it about 10~15 times.

3- out 75μL of a sample mixture was pipetted and loaded into the sample well on the cartridge.

4- The sample-loaded cartridge was left at room temperature for 12 minutes.

5- The sample–loaded cartridge was inserted into the cartridge holder of the instrument for ichromaTM tests after it was being scanned The proper orientation of the cartridge should be ensured before pushing it all the way inside the cartridge holder. For this purpose, an arrow was marked on the cartridge.

6-The ‘Select’ was pressed and the ‘START’ button was tapped on the Instrument for ichromaTM tests to start the scanning process.

7-The Instrument for ichromaTM tests started canning the sample-loaded cartridge immediately.

8- The test result was read on the display screen of the Instrument for ichromaTM tests.

3-5-8 Ferritin Test

The test used a sandwich immunodetection method; the detector antibodies in buffer would bind to antigen in a sample, forming antigen-antibody complexes, and migrating onto nitrocellulose matrix to be captured by the other immobilized antigen on a test strip. The more antibody in the sample forms the more antigen-antibody complex and leading to stronger intensity of fluorescence signal by director antibodies, which was processed by Instrument for ichroma™ tests to show ferritin concentration in the sample.

Procedure

1. Thirty of the blood samples (human serum/plasma/control) were transferred. The pipette was transferred to a tube containing detection buffer.

2. The cap of the detection buffer tube was closed and the sample was mixed well by shaking it about 10 times.

3. seventy-five µl of the sample mixture was withdrawn and the sample was loaded into a test cartridge

4. The test cartridge loaded with the sample was left at room temperature for 10 minutes.

5. The ferritin identification code was put into place.

6. The "Select" button was pressed on the device for ichroma™ tests to start the scanning process.

7. The instrument for ichroma™ tests started scanning the sample and the cartridge was loaded immediately.

8. The test result was read on the display of the ichroma™ test instrument. Interpretation of test results. An instrument for ichroma™ tests calculatesd the test result automatically and displayed the ferritin concentration of the test sample in terms of ng/mL. The cut off (reference range) was:

Women: 20~250 µg/mL Men: 30~350 µg/mL

Genetic study

3-6 DNA Extraction

The DNA isolation from whole blood of study groups by Total DNA Extraction Kit (Favorgen)was as in the following:

Genomic DNA from white blood cells (WBCs) for both diabetes, covid-19, and the control group were extracted by using a DNA extraction kit as described below in **Table (3-4)**

Table (3-4) Kit Contents Used in DNA Extraction

Items	Quantity
RBC Lysis Buffer	405 ml
FATG Buffer	75 ml
FABG Buffer	100 ml
W1 Buffer	130 ml
Wash Buffer (concentrate)	50 ml
Elution buffer	75 ml
FABG Columns	300 pcs
Collection Tubes	600 pcs
Use manual	1

Procedure

Protocol: Isolation of DNA from Frozen Blood.

Sample Preparation

1-A 200 µl of blood was transferred to a 1.5ml microcentrifuge tube (not provided).

2-A 30 µl of Proteinase K (10 mg/ml, not provided) was added to the sample and was briefly mixed. Then incubate for 15 min at 60 °C.

Cell Lysis

3- A 200 µl of FABG Buffer was added to the sample and was mixed by the vortex.

4- Was incubated in a 70 °C water bath for 15 min to lyse the sample. During incubation, the sample was inverted every 3 min.

5- The required Elution Buffer was preheated in a 70 °C water bath for DNA Elution.

DNA Binding

6- A 200 µl of ethanol (96-100%) was added to the sample and was vortexed for 10 sec. The sample was pipetted to mix well if any precipitate could be formed.

7- The FABG Column was placed in a Collection Tube. The sample mixture was transferred carefully to FABG Column. It was centrifuged at speed 14,000 rpm m for 1 min. Then the collection tube was discarded and placed the FABG Column in a new Collection Tube.

Column Washing

8- A 400 µl of W1 Buffer was added to the FABG column and was centrifuged for 30 sec at a speed 14,000 rpm. The flow was discarded through and placed the FABG Column back in the Collection Tube.

9- A 600 µl of wash buffer was added to the FABG column and was centrifuged for 30 sec at a speed 14,000 rpm; the flow was discarded through and placed the FABG Column back to the Collection Tube. It was assured that ethanol was added to Wash Buffer when first opened.

10- It was centrifuged for an additional 3 min at speed 14,000 rpm to dry the column.

Elution

11- The dry FABG Column was placed to a new 1.5 ml microcentrifuge tube.

12- A 100 µl of Preheated Elution Buffer or TE was added to the membrane center of the FABG Column. -- Important Step! For effective elution, it was assured that the elution solution would be dispensed onto the membrane center and absorbed completely.

13- The FAGB Column was incubated at 37 °C for 10 min in an incubator.

14- Centrifuged for 1 minute at full speed 14,000 rpm to eluted the DNA.

15- The DNA fragment was stored at 4°C or -20°C.

3-7 Reconstituting and Diluting Primers

Primer was constructed by Bioneer in a clean room under strict ISO 9001:2000 to confirm DNase/RNase and DNA-free environment. Bioneer® primers were commonly shipped in a lyophilized state. The units of a lyophilized primer were given as a mass, in picomoles. To create a stock of primers, one would reconstitute the primer in sterile 1X TE (1 mM Tris, 0.1 mM EDTA, pH 8.0) or sterile, nuclease-free H_2O. The company supplied the amount of TE or sterile, nuclease-free H_2O to be added to each primer to obtain a master stock that would be used again to obtain a working stock.

The following steps were followed for reconstituting and diluting the primers:

1- The tube was spun down before opening the cap

2- The desired amount of water was added according to the oligos manufacturer to obtain 100 pmoles/µl (Master Stock).

3 It was vortexed properly to re-suspend the primers evenly.

4- A10 µl was transferred from the master stock to a 0.2 ml Eppendorf tube that containing 90 µl of sterile, nuclease-free H_2O (Working Stock).

5- The master stock was stored at -20 C°.

6-The working stock was stored at -20 C°.

7-The working stock was thawed on ice and vortex before it would be used in PCR and then was stored at -20 C°

Table (3-5): Sequences of Primers Used for PCR Amplification of ACE2, AT1, rs657152:

PRIMER NAME	SEQUENCE	Amplicon size (kb)	References
ACE-F *ACE-R*	5-CTGGAGACCACTCCCATCCTTTCT-3 5-GATGTGGCCATCACATTCGTCAGAT-3	I 490 bp D 190 bp	(Takayanagi *et al* ., 1992)
AT1-RF *AT1-RR*	5-GCAGCACTTCACTACCAAATGAT-3 5′-TGTTCTTCGAGCAGCCGT-3′	200 bp	(Knoblauch *et al* ., 1999)
rs657152-F *rs67152-R*	5-GGGACCTTACTGGGGTGATT-3 5-ACAAGGGCAAGCCTGAAAGG-3	300bp	(Zhou *et al* ., 2020)

3-8 Amplification by Polymerase Chain Reaction

Conventional PCR was used to amplify target DNA using specific primer pairs. PCR typically consists of three consecutive steps (denaturation, annealing, and elongation) of repeated cycles to get PCR product (amplicon). The PCR conditions were mentioned in tables (3-7)(3-8)(3-9). The size of PCR products (5 µL) was analyzed in 1.5% (w/v) agarose gel by electrophoresis using 1X TBE buffer and visualized by UV and Product size had been determined by comparison with size 100 bp DNA ladder (Intronbio/Korea).

Table (3-6) Recommended Volume and Concentration for Applying PCR into PCR Tuber

Component	**Volume**	**Concentration**
PCR master mix	**12.5**	**2X**
Forward primer	**1**	**DNA 10 PMOL/ul**
Reverse primer	**1**	**DNA 10 PMOL/ul**
Templet DNA	**2**	**50 ng**
The final volume (dH2O)	**8.5**	

Table (3-7): The Condition Used for ACE2 Polymorphism Amplification

Stage	Tamp(C°)	function	Time(min)	Cycle
1	94	Initial denaturation	5:00	30
	94	denaturation	0:30	
2	57	Primer annealing	0:30	
	72	Template elongation	0:30	
3	72	Final elongation	10:00	
	4	incubation	HOLD	

Table(3-8): The Condition Used for ATIR Polymorphism Amplification

Stage	Tamp(C°)	Function	Time(min)	Cycle
1	94	Initial denaturation	5:00	30
	94	Denaturation	0:30	
2	56.6	Primer annealing	0:30	
	72	Template elongation	0:30	
3	72	Final elongation	10:00	
	4	Incubation	HOLD	

Table(3-9): The Condition Used for rs657152 SNP Amplification.

Stage	Tamp(C°)	Function	Time(min)	Cycle
1	94	Initial denaturation	5:00	30
	94	denaturation	0:30	
2	58	Primer annealing	0:30	
	72	Template elongation	0:30	
3	72	Final elongation	10:00	
	4	incubation	HOLD	

3-9 Agarose Gel Electrophoresis

Electrophoresis through agarose is the standard method used to separate, identify, and purify DNA fragments. The technique is simply rapid to perform, and capable of resolving fragments of DNA that cannot be separated adequately by other procedures. Furthermore, the location of DNA within the gel can be determined directly by staining with low concentrations of the fluorescent intercalating dye ethidium bromide; bands containing as little as 1-10 mg of DNA can have been detected direct examination of the gel in ultraviolet light. If necessary, these DNA bands can be recovered from the gel and used for various cloning purposes (Sam Brook and Russell, 2001).

This method, charging molecules in solution including chiefly proteins, and nucleic acids migrating in response to an electric field, is allied to electrophoresis. Their rate of migration, or mobility, through an electric field, depends on the strength of the field, the net charge, size, and shape of the molecules, and the ionic strength, viscosity, and temperature of the medium the molecules are moving. The DNA movement in the gel depends on its molecular weight, conformation, and concentration of the agarose,

the voltage applied, and strength of the electrophoresis buffer. In this study, the same procedure was used (Harisha, 2007).

3-9-1 Preparation of Solution

a- Ethidium Bromide Staining Solution

Ethidium bromide was put in four drops of concentration 1.25 mg/ml in TBE buffer 100 ml 0.5X. The solution was stored in a dark bottle at room temperature (Robinson and Lafleche, 2000).

b-Loading Buffer for Agarose Gel Electrophoresis

Bromophenol blue 0.25% and Sucrose 40%, then were stored at 4ºC (Sambrook and Rushell, 2001).

c- TBE (10X) Stock Solution / Liter

500ml of TBE (1X) and 50ml of TBE (10X) stock solution was added to a final volume of 500ml dH_2O. Prepared 500ml of TBE (0.5X) and 25ml of TBE (10X) stock solution was added to a final volume of 500ml dH_2O

3-9-2 Gel Electrophoresis Protocol

1- gel-casting tray was placed in a plastic tray, after checking that the teeth of the comb were approximately 0.5 cm above the gel bottom.

2-Prepared 500ml of TBE (1X). and 50ml of TBE(10X) stock solution was added to a final volume of 500ml of deionized water.

3-A100ml of the buffer was placed into a 500ml flask and 0.8g of agarose was added. The agarose was melt by heating the solution on a hot plate for approximately 10 min. The agarose solution was carefully swirled to ensure that the agarose could be dissolved so that there would be no visible agarose particles.

4-The agarose solution was cooled to approximately 60^{o}C and add 2-3 µl of ethidium bromide stock solution. The agarose was slowly poured into the gel-casting tray. Any air bubbles had been removed by the yellow tip.

5- The comb was approximately 1.5 cm positioned from the edge of the gel. The agarose was let to solidify for approximately 20-30 minutes. After the agarose had solidified remove the comb was removed with a gentle back and forth motion, taking care not to tear the gel.

6-The gel-casting tray was removed and the tray was [laced on the central supporting platform of the gel box.

7-Electrophoresis buffer was added to the buffer chamber until it would reach a level of 0.5-1cm above the surface of the gel.

8-First load of 5µl of ladder molecular weight marker was added to each side of the gel (flanking the sample line) and 20µl of DNA specimen was added to the other well.

9- The lid was placed on the gel box, and the electrodes were connected setting the device at 75 volts. The power supply was turned on and electrophoresis continued until the tracking dye would move at least 10 cm of the gel length.

3-9-3 Photo Documentation

Agarose gel was visualized in a UV transilluminator provided with a gel documentation unit: the agarose gel was placed above the UV transilluminator device; the gel was exposed to UV light and the photos were captured using Canon digital camera.

3-10 Single-Strand Conformation Polymorphism (SSCP) for AT1R receptor

3-10-1 Polyacrylamide-Bisacrylamide Stock Solution Preparation (Sambrook and Rushell, 2001).

The stock solution was prepared as in the following:

1-A 30% w/v of 29:1 of acrylamide – bisacrylamide was prepared by weighing 29gm of acrylamide and 1gm of bisacrylamide then it was dissolved in ultra-pure distill water to complete the volume to 100 ml.

2-A 40% w/v of 29:1 of acrylamide – bisacrylamide was prepared by weighing 38.66gm of acrylamide and 1.33gm of bisacrylamide then it was dissolved in ultra-pure distill water to complete the volume to 100 ml.

The acrylamide – bisacrylamide was dissolved in ultra-pure distill water where the reaction temperature dropped down to be extremely chilled. This was because the reaction was energy absorbance and this could be a good indicator of the purity of the materials used. The stock solution was wrapped with aluminum foil and stored at 4^0C.

3-10-2 Resolved Gel Preparation and Poured

The gel concentration used in these experiments was 8% with a continuous phase.

An 8ml of 0.5x TBE buffer was taken and mixed with 2.8 of glycerol (100%), then 20.8 deionized distilled water was added; 40 µl of TEMED, and 400 µl of freshly prepared ammonium persulfate (10%) were mixed with the acrylamide – bisacrylamide gel stock solution (40%). The contents were mixed briefly, depending on the quality of ammonium persulfate and TEMED used.

3-10-3 Prepared of SSCP Loading Dye

A 0.025 g of bromophenol blue and 0.025 g of xylene cyanol was dissolved in 9.5 ml formamide then 100µl sodium hydroxide was added, Making up to 10 ml (Hongyo *et al*.,1993)

3-10-4 Steps for SSCP

1- A10 µl of PCR product and 10 µl of 2x SSCP gel loading dye were added to a microcentrifuge tube and the contents were mixed gently.

2- The tubes were placed into a 95^0C water bath for 10 minutes and then on ice for about 5 minutes.

3- A10 µl of the samples were loaded into wells of an 8% acrylamide /bisacrylamide gel by specialized gel loading tips.

4- Electrophoresis took place in mini-slab gel provided by Cleaver Scientific –UK and performed at room temperature using pre-cold electrophoresis buffer (stored in the refrigerator) and the whole tank was placed in a big container that was loaded with an ice pack and cooled water. Finally, the power supply was set up at 125V for about 3.5 hours.

5- Then polyacrylamide gel was photographed after staining it with silver nitrate dye.

- Fixing

A 50 ml of absolute ethanol was supplemented with 500 ml of distilled water with a concentration of 10%; 2.5 acetic acids of 0.5% concentration was added to it and a solution of silver nitrate consisting of 0.5 g of silver nitrate was added to 250 ml of water. The distilled concentration was 0.2% and the volume was supplemented with distilled water to 500 ml

The gel was placed in the silver nitrate solution for 20 minutes, while gently stirring.

- Rinsing

the gel was placed in distilled water for half a minute.

- Developing

The solution was transferred to a container containing 7.5 mg sodium hydroxide completed with 250 ml of distilled water, and then 250 microliters of formaldehyde were freshly added to it (Byun *et al* .,2009).

3-11 Statical Analysis

All the statistical analysis was performed by using SPSS version 23, while all data were excited as (mean±SE) by using the one-way ANOVA test, Chi-square test, Duncan's, and Pearson correlation analysis used to determine significant differences between groups ($P \leq 0.05$ and $P \leq 0.01$).

Chapter four

Results and Discussion

4- Results and Discussion

4-1 Demographic Distribution

The present samples were divided into three groups: control group, covid-19 patients (C), and covid with DM patients (CDM2) control Female 3, male 28, Covid-19 female 16, male 14, Covid-19+DM female 17, and male 14 according to age, duration gender, infection level, and body mass index (BMI) as listed in table 4-1. The results showed that there were significant differences regarding the age, duration, gender, and Infection level categories among study groups while BMI showed no significant changes.

There were significant differences of gender at ($p \leq 0.01$) between males and females' groups, where the male in CDM2 was (46.67 %) while in the C group it was (43.3%). The female was (56.7%) in the C group and was (53.33 %) in the CDM2 groups (Figure 4-3). Several reports point to the sex differences in COVID-19 outcomes, though the current evidence suggested no gender difference in Covid-19 infection (Alon *et al*., 2020, Gebhard *et al*., 2020, Chang, 2020). Mukherjee and Pahan propose that these disparities may be due to higher levels of ACE2 and TMPRSS2 in males, as well as hormonal influences on the immune response, while gender differences in behavior can also be contributed to the greater severity and fatality in COVID-19 (Mukherjee and Pahan, 2021).

The infection level was classified into three-level included (Mild, moderate and severe); the more percentage of infection level was the severe level in the CDM2 (48.30%) than in C group (26.66%); the moderate level was high in the CDM2 (38.70%) than in the C group (30%). Finally, the simple level was low in the CDM2 (12.9%) than in the C group (43.33%) and the severity of infection increased more in the CDM2 than in the C group (Figure 4-2).

According to Age, there were significant differences ($p \leq 0.01$) among the three candidate groups the distribution categories were depended on in the current study including (> 30, 30-50, and <50 years). The group less than 30 years

category was more in the C group (36.7%) than CDM2 (6.5%), while the 30-50 category in C and CDM2 groups was approximately (26.7%, 22.6%); in the <50 categories a high percent was observed in the CDM2 (71%) than in C group (36.7%). Monod *et al.*(2021) state that individuals aging 20 to 49 are the only age groups sustaining resurgent SARS-CoV-2 transmission with reproduction numbers well above one and that at least 65 of 100 COVID-19 infections originate from individuals aging 20 to 49 in the United States where those groups are the most infected group. They also state that adults aging 20 to 50 years suffering from diabetes mellitus be more vulnerable to Covid-19 with covid-19 symptoms of acute infection.

According to BMI there were three categories depended in the current study including (normal, overweight, and obese) there were non-significant differences among these groups mention that the severity of COVID-19 was found to have a significant burden on intensive care resources in hospitals worldwide and specifically in lower-and-middle income countries due to a lack of health finance and resources. Hence, the patients having higher BMI with other comorbidities should be given special attention to reduce morbidity and mortality associated with COVID-19 infection as a result of the deterioration of the health standards of obese people and the low insulin index (Malik *et al*., 2020). In current study, BMI was not associated with Covid disease due to random sample collection

The duration periods were classified into 3 sub-groups including (<7, 7-14 and >14 days). The <7 days in the C group was (73.3%) while in CDM2 it was (51.61%); the 7-14 days in the C group was (13.33%) and it was (32.25%) in the CDM2 group, finally the >14 days was (13.33%) in the CDM2 group and it was (16.12%) in C group. The differences among groups were significantly (p 0.0311) at ($p \leq 0.05$).

Table (4-1): The Socio-Demographic Distribution of The Study Subject's Categories.

Categories	Mean±SE			Sig.
	Control	COVID-19	COVID+DM	
Male	28(90.3%)	14(43.3%)	14 (46.67 %)	0.0001**
Female	3(9.7%)	16(56.7%)	17 (53.33 %)	
Infection level				
Mild		13(43.33%)	4(12.9%)	0.0258*
Moderate		9(30%)	12(38.70%)	
Sever		8(26.66%)	15(48.30%)	
Age categories (years)				
>30	17(54.8%)	11(36.7%)	2(6.5%)	0.0000**
30-50	11(35.5%)	8(26.7%)	7(22.6%)	
<50	3(9.7%)	11 (36.7%)	22(71%)	
BMI (kg/m^2)				
Normal	13(40.9%)	13(43.3%)	9(29.0%)	0.7065
Overweight	10(32.3%)	9(30%)	14(45.2%)	
Obese	8(25.8%)	8(26.7%)	8 (25.8%)	
Duration (days)				
<7		22(73.3%)	16(51.61%)	0.0311*
7-14		4(13.33%)	10(32.25%)	
>14		4(13.33%)	5(16.12%)	

*Significant at P-value ≤ 0.05 ** significant at P-value ≤ 0.01.

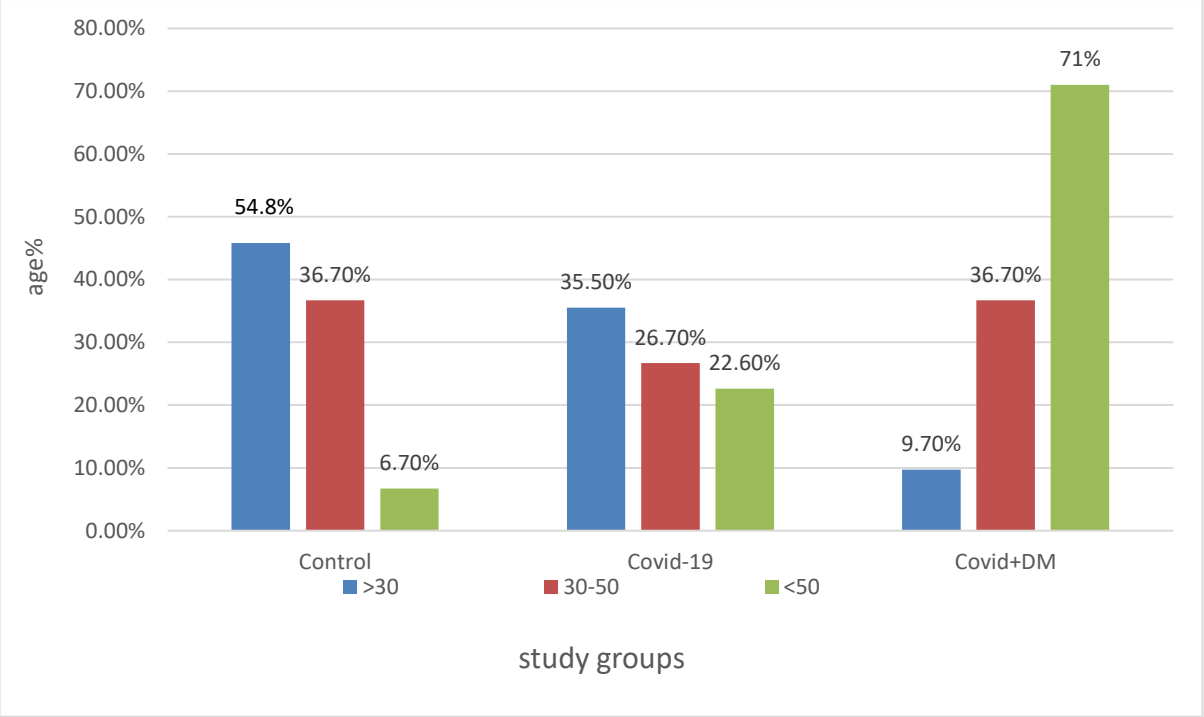


Figure (4-1): Distribution of Study Population by Age Categories.

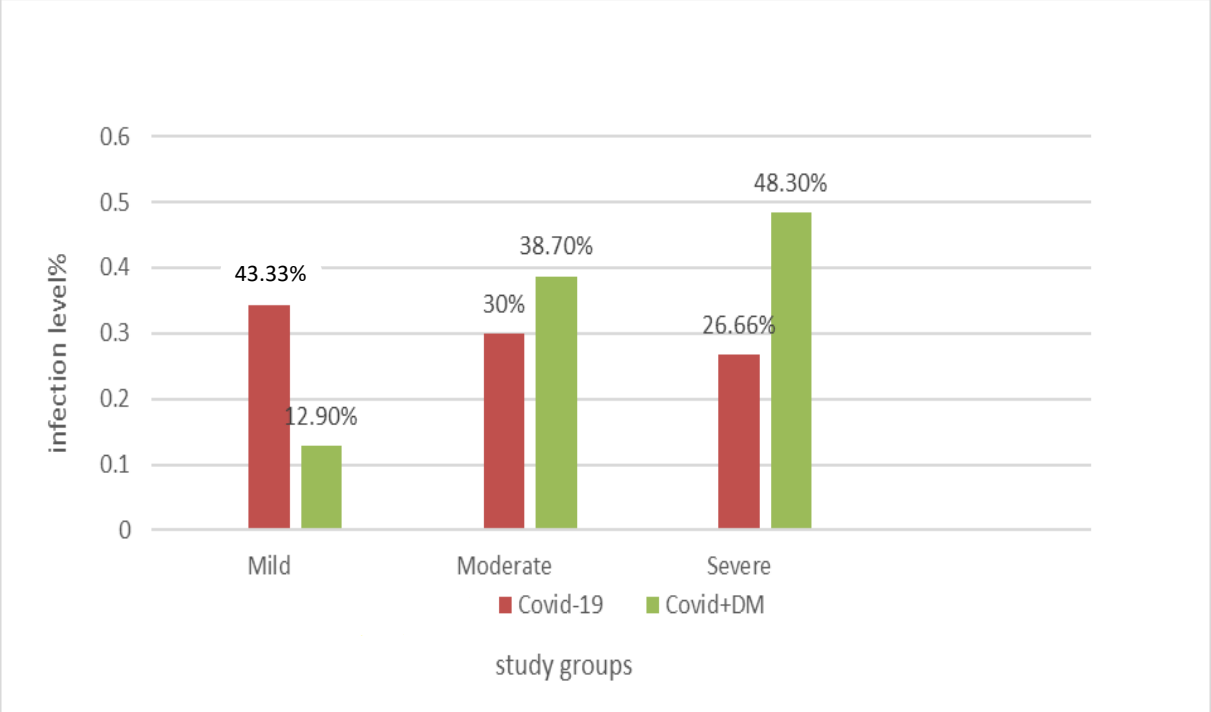


Figure (4-2): Distribution of Study Population According to Infection Level.

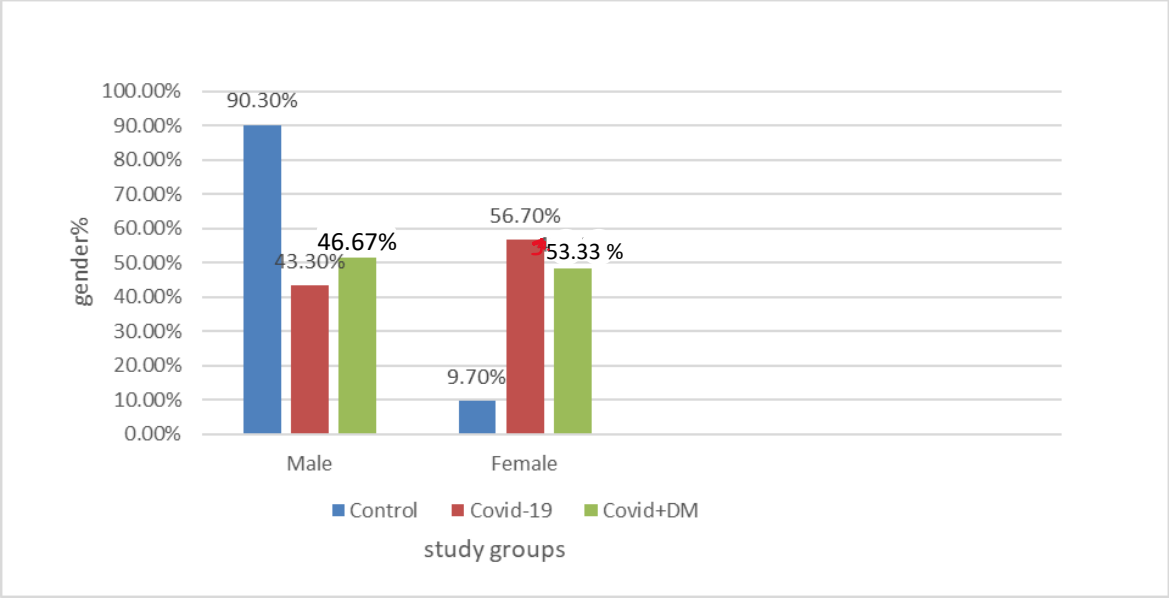


Figure (4-3): Distribution of Study Population by Sex (Gender).

4-2 Biochemistry Parameters Associated with the Covid-19 Infection

In the current study, eight parameters were implemented for the study groups including Fasting Blood Glucose (FBG), Glycated Hemoglobin (HBA1C), Insulin level (IN), insulin Resistance (IR), Insulin Sensitivity (IS), C-Reactive Protein (CRP), D-dimer and Ferritin. Generally, significant differences were observed in the study variables except for INS and IN which did not show significant differences.

The main significant difference among all three groups (control, Covid-19, and covid-19+DM) was the Ferritin parameters with 108.59±18.4, 322.28±30.1 and 583.10±44.6 at ($P \leq 0.01$) (Table 4-2). These findings were similar to Vargas and Cortés who found that individuals with severe and very severe Covid-19 exhibited increased serum ferritin level, where the serum ferritin in the very severe COVID-19 group was significantly higher than that the moderate Covid-19 group (Vargas and Cortés, 2020).

The FBG, HbA1c, and INR parameters showed significant differences only in the patients' group of Covid-19 with DM (253.09±16.2, 9.15±2.0,1 and 17.51±4.1, respectively, while there were no significant differences between

control and patient with Covid-19 groups. Wang and colleagues have found a strong relationship between FBG, glycosylated hemoglobin (HbA1c) level, inflammation, and prognosis of severe Covid-19 patients (Wang *et al*., 2020). INR values have been significantly associated with Covid-19 severity and mortality. Both INR and D-dimer elevations can be useful in diagnosing COVID-19-associated coagulopathy (Zinellu *et al*., 2021).

The CRP and D-dimer parameters showed elevated values in both groups of Covid-19 patients and Covid-19+DM patients compared to the control group (43.72±5.3, 61.18±5.3, 1731.41±114.5 and 1282.97±221.3 respectively. Ali and colleagues have found that Troponin, D-dimer, and CRP levels have been significantly higher in Covid-19 died patients than in Covid-19 survivors in comparison with the unrelated Covid-19 patient control (Ali *et al*., 2022).

While both INS and IN parameters showed no significant differences among the three groups, Chen and his colleagues have oppositely provided the first evidence that Covid-19 may increase the risk of insulin resistance in patients without diabetes (Chen *et al*., 2021). Santos and a coworker have found an increase in the severity of the Covid-19 disease with the effects of drugs used to treat insulin resistance and diabetes in patients with Covid-19 (Santos *et al*., 2021).

Table (4- 2): The Mean Differences of Study Parameters in Study Groups.

Variables	Control	COVID-19	COVID-19+DM	Sig
FBG	94.83±23.4b	119.37±43.9b	253.09±16.2a	0.000**
HbA1c	5.16±0.5b	5.37±0.7b	9.15±2.01a	0.000**
INR	5.41±1.2b	7.88±2.3b	17.51±4.1a	0.021*
INS	0.33±0.05 a	0.31±0.01a	0.30±0.02 a	0.293
IN	22.76±1.6 a	29.11±5.3 a	24.95±4.7 a	0.685
CRP	2.44±0.2b	43.72±5.3a	61.18±5.3a	0.000**
D-dimer	140.37±19.4b	1731.41±114.5a	1282.97±221.3a	0.008**
Ferritin	108.59±18.4c	322.28±30.1b	583.10±44.6a	0.000**

*Significant at P-value ≤ 0.05, ** significant at P-value ≤ 0.01, a, b and c refer to differences among groups according to Duncan's statistical test.

4-2-1 Biochemistry Parameters Vary According to Age Categories

Table 4-3 showed the eight parameters of the significant differences with the age divided into three groups (>30, 30-50, and <50 years). D-dimer and Ferritin were the most significantly changed parameters among three groups of age (>30, 30-50, and <50 years) D-dimer Mean±SE: 425.00±24.1, 708.54±50.1, 1543.75±22.7, Ferritin Mean±SE: 105.46±17.4, 258.98±12.6, 729.65±36.8, respectively. Ferritin levels will increase in the second week after affecting with Covid-19, and the other parameters will be changed according to the normal range including as D-Dimer, ESR, and C.R protein. These levels can be increased with age but Covid-19 infection elevates the level of these parameters increment (Ali *et al*., 2022).

who discovered a link between a high ferritin level and severe symptoms along with a bad prognosis for the condition It is proposed that ferritin is utilized as a biomarker for infections since its circulation rises during viral infections. In critically sick Covid-19 patients, increased ferritin levels due to cytokine storm and subsequent hemophagocytic lymphohistiocytosis have been detected (Velavan and Meyer, 2020).

The results of the current study variables according to the age categories showed non-significant differences in the CDM2 group for the parameters (FBG, HBA1C, INR, INS) except that for in (IN, CRP, D-dimer, ferritin level) there were significant differences among them as far as age was concerned (Table 4-3). The IN, CR, D-dimer, and ferritin were elevated with increased age.

Table (4-3): Mean Differences of Study Parameters According to Age Categories in the Diabetic Patients with COVID-19 Infection.

Subjects	COVID+DM			Sig.
	>30	30-50	<50	
FBG	237.75±12.9 a	219.31±33.3 a	265.24±14.5 a	0.734
HbA1c	9.92±4.5 a	9.19±1.7 a	9.06±1.6 a	0.856
INR	11.32±3.9 a	12.65±4.8 a	19.62±2.6 a	0.820
INS	0.30±0.07 a	0.34±0.1 a	0.29±0.03 a	0.301
IN	15.06±3.1a	24.02±8.6 b	26.14±3.4 b	0.042*
CRP	34.00±9.7a	36.11±6.4 a	71.63±10.7 b	0.036*
D-dimer	425.00±24.1a	708.54±50.1b	1543.75±22.7c	0.032*
Ferritin	105.46±17.4a	258.98±12.6 b	729.65±36.8 c	0.049*

*Significant at P-value ≤ 0.05, a, b and c refer to significant differences among groups according to Duncan's statistical test.

In the C group there were non-significant differences among age categories for the parameters (FBG, HBA1C, INR, and INS) except that for in (IN, CRP, D-dimer, ferritin level) where significant differences were observed (Table 4-4).

Table (4-4): Mean Differences of Study Parameters According to Age Categories in COVID-19 Infection.

Subjects	COVID only			Sig.
	>30	30-50	<50	
FBG	97.14±14.2 a	125.69±18.6 a	137.00±43.5 a	0.082
HbA1c	5.22±0.72 a	5.3±0.52 a	5.52±0.9 a	0.654
INR	9.82±3.4 a	5.28±1.9 a	7.8±1.3 a	0.597
INS	0.31±0.05 a	0.32±0.05 a	0.31±0.03 a	0.894
IN	39.00±4.2c	16.80±3.7 a	28.19±3.4b	0.034*
CRP	29.74±4.6a	36.57±3.8a	62.912±6.4b	0.023*
D-dimer	1327.16±132.1b	557.46±46.2 a	2989.45±136.2c	0.019*
Ferritin	82.97±17.2 a	254.34±14.7 b	611.01±55.6 c	0.009*

*Significant at P-value ≤ 0.05 ** significant at P-value ≤ 0.01, a, b and c refer to significant differences among groups according to Duncan's statistical test.

4-2-2 Biochemistry Parameters Vary According to BMI Categories

The results of the current study as listed in tables 4-5 showed no significant differences for both HBA1c and INS among patients with Covid-19+DM (CDM2) and among three groups of body mass index which were divided into (normal:18.5-24.9 kg/m2, overweight: 25-29.9 kg/m2 and obese: <30kg/m2), while the FBG showed significant differences only in the obese group as more than 30kg/m2 with 307.12 compared to normal and over wight groups (215.83 and 246.17) respectively. This goes in conformity with Smith and colleagues who have found high increasing of fasting plasma glucose FBG in Covid-19 with DM suffering from obesity (Smith *et al*., 2021).

High significant differences were found among the INR, IN, CRP, D-dimer, and Ferritin parameters. All of these parameters increased more in <30 (obese) than the normal and overweight groups as listed in table 4-5. Many recent studies have referred to the direct relationship between obesity the and severity of Covid-19 infection levels such as increasing levels of CRP, D-dimer, and Ferritin (Deng *et al*., 2020, Wang *et al*., 2020), but for Insulin IN and Insulin resistance INR in Covid-19 with diabetes Mellitus IN and INR can play a crucial role when

associated with obesity. Finucane and Davenport have approved the association between Covid-19 and Covid-19+DM infection level, especially for Covid-19 patients with diabetes mellitus when obesity is directly associated with Insulin Index which is one of the most of obesity causes. So, obesity is directly correlated with the severity and infection level of Covid-19 and Covid-19+DM (Finucane and Davenport, 2020; Lockhart and Rahilly, 2020).

Table (4-5): Mean Differences of Study Parameters According to BMI Categories in Diabetic Patients with Covid-19 Infection.

Subjects	COVID+DM			Sig.
	Normal 18.5-24.9) kg\m^2	Over weight (25-29.9) kg\m^2	Obese <30 kg\m^2	
FBG	215.83±18.8 a	246.17±19.8 a	307.12±17.4 b	0.025*
HbA1c	8.39±1.9 a	9.11±1.8 a	10.06±2.3 a	0.714
INR	6.79±1.8 a	14.82±3.2b	34.27±5.6 c	0.025*
INS	0.32±0.04 a	0.31±0.07 a	0.27±0.02 a	0.671
IN	14.38±4.1a	23.27±5.2b	39.77±3.3c	0.035*
CRP	37.56±6.2 a	63.57±5.1b	83.58±6.9c	0.029*
D-dimer	550.28±36.1a	1102.60±138.7b	2422.92±261.4c	0.017*
Ferritin	336.44±37.2 a	542.99±47.9 b	930.78±45.2c	0.031*

*Significant at P-value ≤ 0.05, a, b and c refer to differences among groups according to Duncan's statistical test.

Table 4-6 showed the same eight parameters above but in association with Covid-19 patients only without DM, and the results showed dramatic significance between three groups of BMI for D-dimer and Ferritin parameters with Sig. 0.029 0.018 respectively, at (P≤ 0.05). Qeadan and colleagues have found that both Ferritin and D-dimer levels have been higher in non-survivors and acute Covid-19 patients. They have also found the Covid-19 patient has not been significantly affected by gender, age, hypertension, cardiovascular disease and diabetes (Qeadan *et al*., 2021). This was exactly what the study found, where each of FBG, HbA1c, INR and INS showed no significant change among all the three groups of BMI except that for IN and CRP which showed significant differences just

for those group with <30kg/m2 (Obese group) with 0.037 and 0.040 of significance at ($P \leq 0.05$).

Table (4-6): Mean Differences of Study Parameters According to BMI Categories in Patients with Covid-19 Infection.

Subjects	COVID only			Sig.
	Normal (18.5-24.9) kg\m^2	Overweight (25-29.9) kg\m^2	Obese <30 kg\m^2	
FBG	113.12±24.7a	103.37±17.4a	147.52±44.7a	0.082
HbA1c	5.16±±0.7 a	5.25±0.6 a	5.86±0.82 a	0.092
INR	8.90±1.9 a	9.09±1.6 a	4.86±1.4 a	0.585
INS	0.30±0.04 a	0.32±0.07 a	0.31±0.03 a	0.644
IN	31.18±5.5 a	38.66±5.2 a	15.02±3.9b	0.037*
CRP	30.06±6.3 a	36.67±3.8 a	73.87±4.7b	0.040*
D-dimer	1141.15±126.3a	1904.74±137.6b	2495.60±314.5c	0.029*
Ferritin	159.09±25.4 a	321.10±15.8b	588.80±46.6c	0.018*

*Significant at P-value ≤ 0.05, a, b and c refer to differences among groups according to Duncan's statistical test.

4-2-3 Biochemistry Parameters Vary According to the Infection level

The statistical analysis of this study showed non-significant differences in FBG, HBA1C, INR, and INS, while it showed that significant differences were documented for the IN, CRP, D-dimer, and ferritin levels in the CDM2 group. IN, CRP and D-dimer were elevated in the mild level than the other categories. It was seen that the ferritin was higher in the moderate and severe subgroups (Table 4-7).

Table (4-7): Mean Differences of Study Parameters According to the Severity of COVID-19 Infection in Diabetic Patients with Covid-19 Infection.

Subjects	COVID+DM			Sig.
	Mild	Moderate	Sever	
FBG	265.23±17.2a	223.54±35.3 a	273.36±5.9 a	0.863
HbA1c	9.06±1.9 a	9.07±2.3 a	9.57±1.9 a	0.114
INR	22.52±8.1a	14.69±2.3a	7.11±1.1a	0.495
INS	0.29±0.03 a	0.28±0.04 a	0.38±0.1a	0.636
IN	28.00±2.7 a	26.91±2.3 a	11.27±2.3b	0.041*
CRP	71.48±7.1 a	55.71±4.6 a	39.19±8.6b	0.044*
D-dimer	1573.75±260.4a	961.91±109.8b	994.62±49.6b	0.046*
Ferritin	392.79±44.6a	819.13±70.5b	720.04±78.5b	0.000**

*Significant at P-value ≤ 0.05, ** significant at P-value ≤ 0.01, a, b and c refer to differences among groups according to Duncan's statistical test.

In the C group, the statistical analysis showed non-significant differences in FBG, HBA1C, INS, and IN, while other parameters including INR, CRP, D-dimer, and Ferritin were significantly different. It increased in the severity infection compared with other infections level (Table 4-8).

Table (4-8): Mean Differences of Study Parameters According to the Severity of COVID-19 Infection in Covid-19 Infection Patients.

Subjects	COVID			Sig.
	Mild	Moderate	Sever	
FBG	114.95±44.6a	108.81±10.7a	154.25±5.4b	0.031*
HbA1c	5.37±0.8a	5.30±0.4 a	5.46±0.2a	0.216
INR	8.08±1.7a	11.22±2.4a	3.44±4.9b	0.041*
INS	0.31±0.04 a	0.29±0.04 a	0.35±0.07 a	0.503
IN	30.98±7.8a	40.28±3.9b	7.70±1.8c	0.132
CRP	39.78±4.1 a	38.97±7.5 a	70.16±9.1 b	0.012*
D-dimer	1523.36±136.2a	704.00±46.2b	3903.13±111.9c	0.024*
Ferritin	220.58±13.5a	120.93±10.3a	1083.03±13.8b	0.000**

*Significant at P-value ≤ 0.05, ** significant at P-value ≤ 0.01, a, b and c refer to differences among groups according to Duncan's statistical test

4-3 Association between Covid-19 Patients and Patients with Diabetes Mellitus Infected Covid-19 According to Some Blood Parameters

Table (4-9) showed the association between the diabetes mellitus patients infected with Covid-19 and patients with covid-19 only for mild, moderate, and severe symptoms according to our markers understudy

Where the mean of fasting blood glucose (FBG), hemoglobin A1c, insulin sensitivity (INS), and ferritin exhibited significant differences between the two groups at (0.000, 0.000, 0.017, 0.000), respectively, but Insulin resistance (INR), Insulin (IN), C-reactive protein, and D-dimer did not significant. (p 0.01 and p 0.05) were used in the statistical analysis**.**

Table (4-9): Association Between Covid-19 and Covid -19 +Diabetes Mellitus Groups.

Parameter	COVID+DM			COVID			Sig.
	Mild	Moderate	Sever	Mild	Moderate	Sever	
FBG	265.23± 10.6*	223.54± 15.3	273.36 ± 16.4	114.95± 9.7*	108.81± 10.2	154.25± 11.2	0.000**
HbA1c	9.06± 1.9*	9.07± 2.3**	9.57± 1.9***	5.37± 0.8*	5.30± 0.4**	5.46± 0.2***	0.000**
INR	22.52± 1.5	14.69± 2.4	7.11± 1.6	8.08± 0.7	11.22± 1.4	3.44± 0.6	0.371
INS	0.29± 0.03	0.28± 0.04	0.38± 0.04	0.31± 0.04	0.29± 0.04	0.35± 0.07	0.017*
IN	28.00± 5.9	26.91± 2.3	11.27± 1.5	30.98± 7.8	40.28± 9.1	7.70± 1.8	0.532
CRP	71.48± 7.2	55.71± 6.5	39.19± 2.6	39.78± 4.9	38.97± 1.5	70.16± 9.1	0.392
D-dimer	1573.75 ± 22.4	961.91± 18. 3	994.62 ± 24.5	1523.36 ± 212.2	704.00± 86.2	3903.13 ± 12.9	0.438
Ferritin	392.79± 46.3	819.13± 70.5**	720.04 ± 28.5	220.58± 14.5	120.93± 20.0**	1083.03 ± 137.8	0.000**

*Significant at P-value ≤ 0.05, ** significant at P-value ≤ 0.01.

The COVID-19 has displayed a broad spectrum of severity, ranging from mild-moderate to severe illness leading to death. Many factors influence disease

outcome, including age and gender. Severe outcomes have been associated with preexisting chronic illnesses, such as hypertension and diabetes (Alshukry *et al*.,2021). In general, it has been found that patients with diabetes had more severe COVID-19 outcomes than patients without diabetes as it was represented by the mean of blood markers (Table 4-9). The results have been in agreement with the findings obtained from a Chinese COVID-19 cohort, in which COVID-19 diabetic patients had a 7.3% increased risk of morbidity (Wu *et al*.,2020). Moreover, a British cohort showed that COVID-19 patients with uncontrolled diabetes had a higher risk of death than the other patients did (Williamson *et al*.,2020).

There was significantly more higher levels of blood parameters in all COVID-19 diabetic patients than in non-diabetic COVID-19 patients (Table 4-9). Previous studies by (Chen *et al*.,2020) have found an increase in many blood markers, including hemoglobin, C-reactive proteins, dimer, ferritin, and other factors. This is consistent with the results obtained from the current study which showed an increase in these parameters for diabetic patients with covid-19 compared to non-diabetics. The reason for such differences in severity outcomes in diabetic patients with COVID-19 compared to patients with covid-19 only was likely due to the multifactorial syndromic nature of diabetes. In the cohort, for instance, we found that diabetic patients had a higher percentage of FBG and HbA1c, and INR. Furthermore other markers were noticed (273.36± 16.4,9.57± 1.,9 and 22.52± 1.5) respectively, compared with the non-diabetic patients (154.25± 11.2, 5.46± 0.2, and 11.22± 1.4) respectively (Table 4-9).

4-4 The Association between Patients Diabetes Mellitus Infected Covid-19 and Infection Duration According to Some Blood Parameters

4-4-1 The Association between Patients Diabetes Mellitus Infected Covid-19 and Infection Duration

Table (4-10) showed the association between patients with Diabetes Mellitus infected with Covid-19 and infection duration at >7,7-14 and <14 days according to the parameters under study. Where the Mean of INR, INS, IN, CRP, D-dimer and ferritin showed significant differences for all categories of days at (0.046, 0.041,0.023,0.030, 0.031, and 0.034) respectively, while the FBG and HbA1c did not show any significant differences for all categories, since the statistical analysis was made at ($p \leq 0.05$).

Table (4-10): The Association between Patients with Diabetes Mellitus Infected with Covid-19 and Infection Duration.

Parameters	COVID+DM (Infection duration)			Sig.
	>7 days	7-14	<14	
FBG	265.23±17.2	223.54±15.3	273.36±58.5	**0.707**
HbA1c	9.06±1.9 a	9.07±2.3 a	9.57±1.9a	**0.885**
INR	22.52±3.8 a	14.69±2.2 a	7.11±1.1b	**0.046***
INS	0.29±0.03 a	0.28±0.04a	0.38±0.10b	**0.041***
IN	28.00±2.7 a	26.91±2.2 a	11.27±1.2b	**0.023***
CRP	71.48±57.12 a	55.71±45.65 a	39.19±28.60b	**0.030***
D-dimer	1573.75±202.4 a	961.91±10.9b	994.62±96.5b	**0.031***
Ferritin	392.79±46.3 a	819.13±70.5 b	720.04±178.5 b	**0.034***

*Significant at P-value ≤ 0.05 level

Since diabetes mellitus is a chronic inflammatory disorder associated with high glucose levels, diabetic patients can show many changes in metabolism and blood vessels that weaken the body's defenses and prevent the immune system from responding properly to viral and bacterial infections, In addition, many

disorders occur in innate immunity can increase the risk of pneumonia and influenza (Cuschieri and Grech, 2020).

The results showed an increase in the mean of insulin (IN) and insulin resistance (INR) in the first 7 days of infection at (28.00±2.7 a, 22.52±3.8 a) respectively as it was explained by Muniyappa and Gubbi (2020) who stated that patients suffering from both types diabetes (1and 2) have an ACE-2 production increased due to frequent treatment with ACE-2 inhibitors and angiotensin II type 1 receptor blockers (ARB), which have antihypertensive and nephroprotective effects. Treatment with ACE-2 and angiotensin-receptor blockers inhibitors increases ACE-2 production, which facilitates COVID-19 infection consequently (Fang *et al*.,2020).

Many studies have mentioned that there is a significant association between diabetes and increased morbidity of COVID-19, as there is evidence that people with diabetes show an increased risk of infection and severe disease. some findings have highlighted that the prevalence of diabetes among those infected was 16% in patients with more severe forms of the disease and 5.7% in patients with milder forms of the disease (Ma RCW and Holt, 2020). These results are in agreement with the study results which showed that the percentage of inflammatory parameters, fasting blood sugar, and insulin sensitivity increased significantly for diabetic patients infected with Coronavirus (Table 4-10).

4-4-2 The Association between Covid-19 Patients and Infection Duration.

Table (4-11) showed the relationship between Covid-19 patients and infection duration at >7,7-14, and 14 days according to the parameters studied, with the Mean of INR, IN, D-dimer, and ferritin showing significant differences for all categories of days at (0.039, 0.026,0.022, and 0.000, respectively), whereas the FBG, HbA1c, INS, and CRP did not show any significant differences for all categories, since the statistical analysis was done at ($p \leq 0.01$ and $p \leq 0.05$).

Table (4-11): The Association between Covid-19 Patients and Infection Duration

Parameters	COVID only			Sig.
	>7 days	7-14	<14	
FBG	114.95±8.6 a	108.81±10.02 a	154.25±5.4 a	**0.220**
HbA1c	5.37±0.8 a	5.30±0.4 a	5.46±0.2 a	**0.961**
INR	8.08±1.7 a	11.22±1.4 a	3.44±1.4b	**0.039***
INS	0.31±0.04 a	0.29±0.04 a	0.35±0.07 a	**0.182**
IN	30.98±7.3 a	40.28±3.4 a	7.70±1.7b	**0.026***
CRP	39.78±4.9 a	38.97±7.5 a	70.16±9.7 a	**0.471**
D-dimer	1523.36±252.27 a	704.00±86.2b	3903.13±41.9c	**0.022***
Ferritin	220.58±33.5a	120.93±24.0b	1083.03±137.8c	**0.000****

*Significant at P-value ≤ 0.05, ** significant at P-value ≤ 0.01.

Viral studies in which culture has been performed and where viral replication can be elicited are also better data for inferring the infectious period, relative to viral load estimates alone (Wölfel *et al.*, 2020). Therefore, the data include mean periods of 7-14 days after symptoms, Where some research indicates that the duration of detection of the virus can be during this period (Lavezzo *et al.*, 2020).

The mean of parameters increased with the infection duration except that for CRP, D-dimer and ferritin elevated in the first 7 days and, then decreased afterwards. This was due to the effect of these inflammatory markers being higher than in blood parameters at the beginning of the disease. After having assessed the association between infection duration with the severity of COVID-19 (Wu *et al.*,2020). It was indicated that the rate of infection with the covid-19 became more severe as the incubation period increased, and thus the proportions of some parameters in the blood such as hemoglobin (HbA1c), clotting factor (INR) and fasting blood sugar (FBG) levels in table (4-11).

4-4-3 Association between Covid-19 Patients and Patients with Diabetes Mellitus Infected Covid-19 According to Infection Duration

Table (4-12) revealed the relationship between patients with diabetes mellitus who were infected with covid-19 and patients who were only infected with covid-19 for >7,7-14, and 14 days, according to the parameters studied.

Whereas the Mean of IN and D-dimer revealed significant differences for all infection duration categories at (0.033 and 0.011), FBG, HbA1c, INR, INS, CRP, and ferritin did not show any significant differences for all days category, as the statistical analysis was done at (p 0.01 and 0.05).

Table (4-12): Association between Covid-19 Patients and Patients with Diabetes Mellitus Infected covid-19 According to Infection Duration.

Parameters	COVID+DM			COVID only			Sig.
	>7 days	7-14	<14	>7 days	7-14	<14	
FBG	265.23± 17.0	223.54± 18.3	273.36± 58.5	114.95± 14.6	108.81± 10.0	154.25± 5.4	**0.707**
HbA1c	9.06± 1.9	9.07± 2.3	9.57± 1.9	5.37± 0.8	5.30± 0.4	5.46± 0.2	**0.885**
INR	22.52± 8.0	14.69± 2.2	7.11± 1.0	8.08± 1.7	11.22± 1.4	3.44± 0.4	**0.546**
INS	0.29± 0.03	0.28± 0.042	0.38± 0.10	0.31± 0.04	0.29± 0.04	0.35± 0.07	**0.061**
IN	28.00± 2.3	26.91± 4. 3	11.27± 1.4	30.98± 7.8	40.28± 7.9	7.70± 1.8	**0.033***
CRP	71.48± 7. 2	55.71± 4.6	39.19± 8.6	39.78± 4.1	38.97± 7.5	70.16± 9.1	**0.430**
D-dimer	1573.75 ± 202.4	961.91± 98.0	994.62± 46.5	1523.36±2 52.2	704.00± 86. 3	3903.13± 112. 5	**0.011***
Ferritin	392.79± 46.3	819.13± 70.5	720.04± 27.3	220.58± 34.5	120.93± 14. 3	1083.03± 137.8	**0.114**

*Significant at P-value ≤ 0.05, ** significant at P-value ≤ 0.01.

In the current study, there was a highly significant difference in the parameters in diabetes patients infected with covid-19 in contrast to the covid-19 patients only, where its high levels in the blood was explained by the high

association between, diabetes and SARS-CoV-2 infection following a two-way model as SARS-CoV-2 worsening pre-existing diabetes or predisposing non-diabetic people to diabetes. The mechanism that allows the entry of the virus into the cell involves ACE-2, which is highly expressed in the liver and pancreas, especially in beta cells that produce insulin hormones (Maddaloni and Buzzetti, 2020). Highly significant differences were found in INR at (22.52±8.0) in >7 days of infection because the infection by SARS-CoV-2 in pancreatic beta cells generated insulin resistance and decreased insulin secretion, worsening hyperglycemia in the acute phase of infection, whereas, in the chronic phase, it could trigger autoimmunity of these pancreatic cells in predisposed patients.

As a result of diabetes, hyperglycemia and insulin resistance induce increased synthesis of advanced glycation end products (AGEs) and pro-inflammatory cytokines like CRP that generate oxidative stress. Such findings are consistent with to referred to an increase in the percentage of CRP, ferritin, D-dimer (Cuschieri and Grech, 2020).

4-5 The Correlation Coefficients between Covid-19 Patients and Patients with and without Diabetes Mellitus

4-5-1 The Correlation Coefficients between Covid-19 Patients and blood parameters

Table (4-13) showed the correlation between Covid-19 patients and infection duration at (1-14 days) according to the parameters under study. Where the correlation coefficient (r) of FBG with HbA1c, CRP ferritin, and CT showed significant differences at (0.503, 0.495, 0.693 and 0.703) respectively, while with INR, INS, IN, D-dimer and infection duration no significant differences were signaled. HbA1c with CRP, d-dimer and CT showed significant differences at (0.699, 0.458 and 0.47) respectively, while INR, INS, IN, ferritin, CT and infection duration showed no significant differences. INR with INS and IN showed a significant correlation at (0.762 and 0.956) respectively, while with

CRP, D-dimer, ferritin and infection duration did not reveal any significant differences. INS with IN showed significant differences at (0.737), while with CRP, D-dimer, ferritin, CT, and infection duration did not disclose any significant differences. IN did not reveal any significant differences with any markers. CRP with D-dimer, ferritin and CT showed significant differences at (0.719 and 0.60, 0.774) respectively, while infection duration did not pinpoint any significant differences. The D- dimer with ferritin and CT showed a significant correlation at (0.566,0.530) while infection duration did not show any significant differences. Ferritin with infection duration showed significant differences at (0.587), since the statistical analysis was made at ($p \leq 0.01$and $p \leq 0.05$). It is necessary to measure some blood and immunological parameters to assess the severity of COVID-19, so one can easily assess patient severity and infection by monitoring these indicators. In the findings, some analyzed haematological parameters of COVID-19 patients and among these parameters, manifested a decrease in the level of HbA1c and increased levels of CRP, ferritin and d-dimers. These results are said to be consistent with (Rahman *et al*.,2021), who have found that Hematological parameters have been associated with COVID-19 severity. Some other studies have supported the present findings describing the evidence of the correlation between CRP, D-dimer and ferritin levels in Covid -19 patients (Deng *et al*.,2019; Ruan *et al*.,2020). Lippi *et al*.(2020) designed four different studies about the blood parameters in covid -19 patients where showed that there was a high correlation between HbA1c level and COVID that contributed to the good diagnosis of disease, especially the role of coronavirus in clot blood formation. The results showed that the correlation coefficient between ferritin and Covid-19 in the blood increased significantly since ferritin was a protein that stored iron. Therefore, the elevated concentration induced cytokine storms and severity of COVID-19 patients (Giamarellos *et al*.,2020; Velavan and Meyer, 2020). It is believed that ferritin levels can serve as a factor for monitoring COVID-19 severity (Henry *et al*.,2020). In the current study, the noticed highly significant

differences between inflammatory proteins and Covid-19 such as CRP, d-dimer and ferritin were consistent with a previous study that showed that COVID-19 patients had significantly increased levels of serum inflammation-related biomarkers, represented by interleukin-6, D-dimer and C-reactive protein, as well as biomarkers related to disease prognosis (Guan *et al*., 2019; Zhou *et al*.,2020).

Table (4-13): The Correlation Coefficients between Covid-19 Patients and Blood Parameters.

Parameters		HBA 1c	INR	INS	IN	CRP	D-dimer	Ferritin	CT-Scan	Covid duration
FBG	r	0.503**	0.057	0.121	0.191	0.495**	0.136	0.693**	0.703**	0.346
	p	0.005	0.765	0.522	0.312	0.005	0.473	0.000	0.000	0.061
hba1c	r	1	0.105	0.039	0.027	0.699**	0.458*	0.345	0.478**	0.026
	p		0.580	0.839	0.888	0.000	0.011	0.062	0.007	0.891
INR	r		1	0.762**	0.956**	0.165	0.228	0.117	0.182	0.025
	p			0.000	0.000	0.383	0.225	0.537	0.335	0.897
INS	r			1	0.737**	0.190	0.347	0.210	0.211	0.050
	p				0.000	0.314	0.060	0.265	0.263	0.793
IN	r				1	0.265	0.230	0.260	0.322	0.093
	p					0.158	0.222	0.166	0.083	0.626
CRP	r					1	0.719**	0.601**	0.774**	0.079
	p						0.000	0.000	0.000	0.679
D-dimer	r						1	0.566**	0.530**	0.147
	p							0.001	0.003	0.438
Ferritin	r							1	0.898**	0.587**
	p								0.000	0.001
CT-Scan	r								1	0.454*
	P									0.012

*** Significant at the 0.05 level (2-tailed), ** Significant at the 0.01 level (2-tailed).**

4-5-2 The Correlation Coefficient between Diabetes Mellitus Infected Covid-19 Patients and Blood Parameters

Table (4-14) showed the Correlation Coefficient between Mellitus Infected Covid-19 Patients and infection duration at 1-14 days according to the parameters under study. The correlation coefficient (r) of FBG with INR showed a significant correlation at (0.647), while with HbA1c, INS, IN, CRP, D-dimer, ferritin, CT and infection duration didn't show any significant correlation. HbA1c did not show any significant correlation with other markers.

INR with INS and IN revealed a significant correlation at (0.394 and 0.791) respectively, while with CRP, D-dimer, ferritin, CT and infection duration did not point to any significant differences. INS with IN and infection duration showed a significant correlation at (0.592 and 0.369) respectively, while CRP, D-dimer, CT and ferritin did not show any significant correlation. IN, CRP,and D-dimer didn't show any significant correlation with any markers, ferritin with CT scan in (0.576) and didn't significant with covid duration. Since the statistical analysis was made at ($p \leq 0.01$ and $p \leq 0.05$).

Individuals with diabetes are more prone to SARS-CoV-2 infection, and the severity of the disease is on the rise. Pathogenic links between DM and COVID-19 include raising fasting blood sugar and the release of inflammatory proteins that lead to the cytokine storm (Sen *et al*.,2021).

The severity of COVID 19 has been highly correlated with glycemic such as insulin, insulin resistance, insulin sensitivity, and fasting blood sugar, because of the ability of the Coronavirus to ACE 2 receptors of Beta cells in the pancreas, liver cells, and other organs (Gregory *et al*., 2022). Table (4-14) showed highly significant differences between these parameters in diabetic patients infected with covid-19.

Previous studies showed that diabetes was a risk factor for multiple viral infections and deaths, including 2009 A (H1N1) influenza, MERS-CoV, and SARS-CoV (Knapp, 2013), so the correlation coefficient was here tested between these two parameters. Besides the current results, we also analyzed the Ct-Scan data and found that diabetic patients had a higher incidence of covid-19 than non-diabetics, as these results indicated that diabetic patients with COVID-19 could have severe inflammatory responses and lung infiltration, which was the main cause of elevated inflammatory protein and other haematological parameters (Shang *et al*.,2021).

The concentration of serum D-dimer of diabetic patients was significantly higher than that of nondiabetic patients indicating that COVID-19 patients with diabetes were more likely to develop a hypercoagulable prothrombotic state, These results were consistent with the findings of (Le *et al*.,2015), who mentioned that the D-dimer was one of the main markers of coagulation activity and the higher concentration in serum was closely related to a variety of thrombotic diseases, including myocardial infarction, cerebral infarction, pulmonary embolism, and venous thrombosis.

Table (4-14): The Correlation Coefficient between Diabetes Mellitus Infected Covid-19 Patients and Blood Parameters.

Parameters		HbA1c	INR	INS	IN	CRP	D-dimer	Ferritin	CT-Scan	Covid duration
FBG	**r**	0.323	0.647**	-0.200	0.250	0.127	0.029	0.077	0.147	0.072
	p	0.076	0.000	0.280	0.175	0.497	0.875	0.680	0.430	0.699
HbA1c	**r**	1	0.169	-0.040	0.078	0.027	0.025	0.007	-0.168-	-0.027-
	p		0.364	0.830	0.675	0.885	0.892	0.971	0.365	0.887
INR	**r**		1	-0.394*	0.791**	-0.025-	-0.030	-0.102-	-0.100-	-0.177-
	p			0.028	0.000	0.894	0.871	0.585	0.594	0.341
INS	**r**			1	-0.592**	0.024	-0.120	-0.126-	0.058	0.369*
	p				.000	0.900	0.519	0.499	0.757	0.041
IN	**r**				1	-0.020	-0.096	-0.111-	-0.085-	-0.219-
	p					.913	0.606	0.551	0.648	0.237
CRP	**r**					1	0.287	0.206	0.272	-0.271-
	p						0.117	0.265	0.139	0.141
D-dimer	**r**						1	0.331	0.328	-0.154-
	p							0.069	0.072	0.408
Ferritin	**r**							1	0.576**	0.098
	p								0.001	0.598
CT-Scan	**r**								1	0.586**
	P									0.001

*** Significant at the 0.05 level (2-tailed), ** Significant at the 0.01 level (2-tailed).**

4-6 Results of Genetic Studies

4-6-1 Extraction of DNA

By using a specific DNA extraction kit (Fibrogene/USA), the genome was extracted, purified from the blood sample of the control, covid -19 patients, and diabetes patients infected with covid-19 then it was migrated by using agarose gel as a first step to ensure the presence of DNA samples after extraction. figure (4-4) showed the DNA band of these three groups.

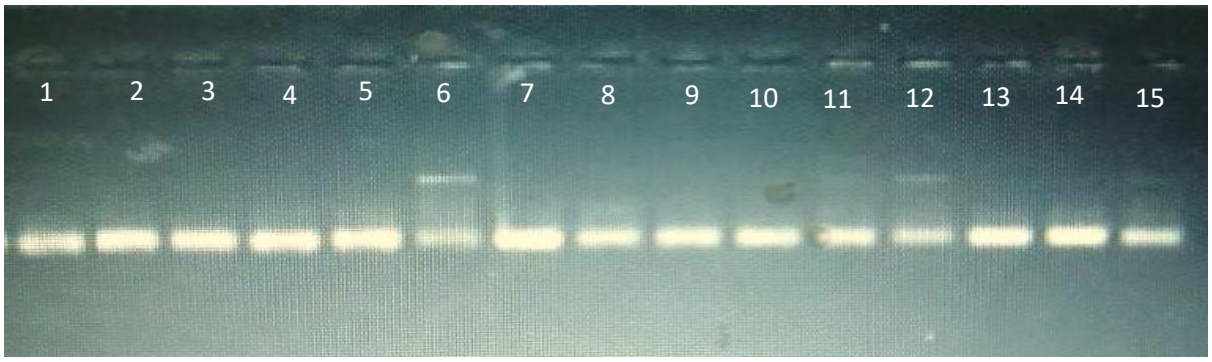


Figure 4.4: Extraction of genomic DNA from blood sample, 1% agarose gel electrophoresis, TBE 1X, at a voltage 75volt 20 mA for 1 hour (10 µl in each well). Lane 1-5 DNA for control, lane 6-10 DNA of covid-19 patients, lane 11-15 DNA for diabetes mellitus infected covid-19.

In the current study, a high infection rate of covid-19 in the patients with diabetes mellitus was marked, especially in those with severe diabetes when compared with the control group. This can be very important of covid-19 in the evolution and exacerbation of diabetes and body dysfunction (Lahera *et al*.,2017).

4-6-2 ACE2 Genotyping Polymorphism

PCR technique was used to allow DNA amplification. Variation (gene polymorphism) in the ACE2 gene was targeted using specific primers of 190 bp and 490 bp respectively. (figure 4-5) showed the targeted genome bands.

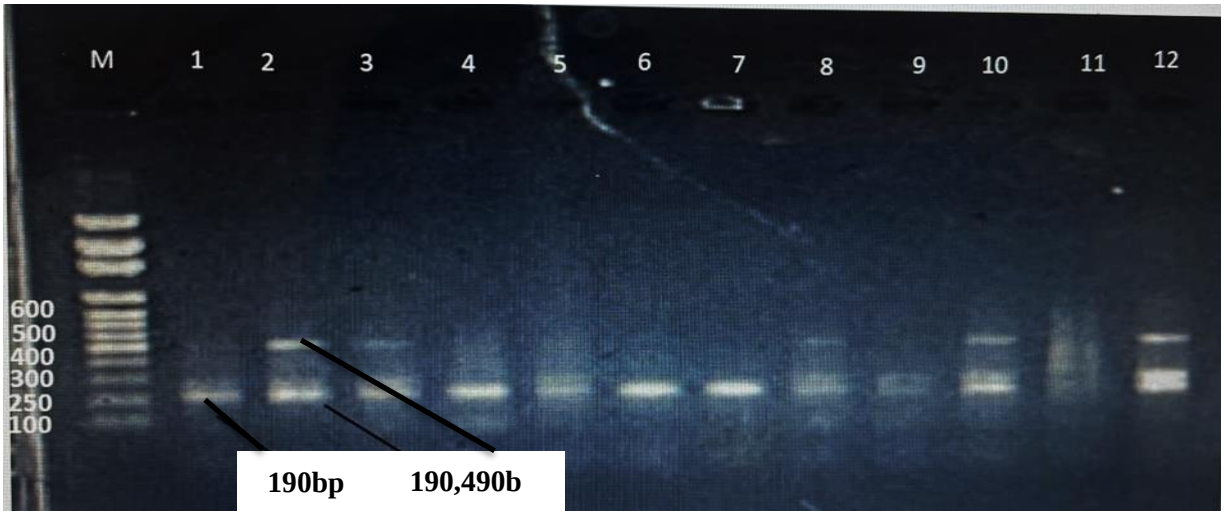


Figure (4-5): The electrophoreses pattern of PCR product for ACE2 gene, This amplification product two bands 490bp and 190bp of control, diabetes mellitus infected covid-19 and covid-19 patients under condition 1% agarose, annealing 57 C°, 75 V, 20 mA for 1h. M: Promega ladder, Lane 1-4 PCR product of control, Lane 5-8 for covid-19, and Lane 9-12 for covid+DM.

The differences in the size of DNA bands obtained from blood at the comparison of the DNA of patients with COVID-19, diabetic patients with Covid-19, and the control group could be related to the free radicals produced by proinflammatory cytokines, such as TNF-a, IFN-g, IL-1, IL-8, CRP, and ferritin released during severe COVID-19 infection (Li *et al*., 2013). These findings have supported the use of peripheral blood DNA as a biomarker for ACE2 genetic polymorphism study to determine the severity and prognosis of patients with COVID-19 (Scozzi *et al*.,2021).

The ACE allele has been reported to be significantly prevalent among hypertensive and diabetes Mellitus subjects having a parental history of CVD by Tiret *et al*.(2018) (0.68 versus 0.56), and Kario *et al*.(2013) (0.46 versus 0.37).

Yet, the study showed that there was no significant difference in the genotype distribution between Covid-19 and Covid-19 with diabetes ,where the subjects having diabetes had a higher frequency of D/D genotype (30.76 vs. 18.18%) and D allele (0.49 vs. 0.42%) than did the subjects having no family history.

4-6-3 The Genotype Distribution of ACE2 Gene Polymorphism in Patients and Control

Table (4-15) showed the distribution of ACE2 gene of control, covid-19 patients, and diabetes mellitus patients infected with covid-19, where the (ID; DD) group showed significant differences at (0.04) while the II and I groups manifested no significant differences.

Table (4-15): The Genotype Distribution of ACE2 Gene Polymorphism in Patients and Control Groups

Genotype	Control	Covid-19	Covid+DM	Qi square	Sig.
II	0	0	0		
ID	17(54.8%)	9(30.0%)	8(25.8%)	6.53	0.04*
DD	14(45.2%)	21(70.00%)	23(74.2%)		
I	17(27.4)	9(15.0)	8(12.9)	5.05	0.8
D	45(72.6)	51(85.0)	54(87.1)		

***Significant at P-value ≤ 0.05**

The present study focused on the percentage of ACE2 genotyping in three groups including control, covid-19, and diabetes with covid-19. The ACE D genotype was the dominant genotype (72.6%, 85%, and 87%) for the control, covid-19, and diabetes with covid-19 respectively, followed by DD, ID and I genotypes. The results of the current study were in line with the study that confirmed the frequency of ACE I/D genotype in a series of 26 COVID-19 cases finding that the ACE DD genotype was the dominant genotype in 73% of the patients (Annunziata *et al*.,2020).

Karagiannidis *et al*.(2020) found an association between ACE2 and risk of SARS-CoV-2 infection. The current study was in agreement with the studies that confirmed that genetic variations in the angiotensin receptors (ACE2) which could make some people more susceptible to recurrence of infection than others. In addition other scientists have hypothesized that polymorphisms in the genes encoding critical components of the angiotensin system such as ACE or ACE2 might have a role in infection risk for a variety of reasons (Novelli *et al*.,2020).

There were significant differences in (ID; DD) group at (0.04) in contrast to the (I; D) groups. This indicated that there could be some alleles related with increased ACE2 serum levels or even cellular ACE2 expression (Wu *et al*.,2017).

The coding region variants in the gene ACE2 seem to play an important role in the severity of infection, although they might be functional (Calcagnile *et al*.,2021) Furthermore, ACE2 genotype distribution referred to highly significant differences between diabetes mellitus infected covid-19, covid-19, and control group table (4-15). Qi square analysis confirmed that the DD-allele could have an independent risk factor for a COVID-19 infection besides the known risk factors male gender and chronic diseases. It has been postulated recently that genetic polymorphism in ACE2 may play a central role in COVID-19 (Sriram *et al*.,2020).

The results mentioned an increase in the percentage of D allele by (72.6%, 85%, and 87.1%) for each of the control groups, Covid-19 patients and diabetic patients with Covid-19 respectively compared to the other alleles DD, ID, I, which showed a small percentage according to the statistical analysis. Meaning: If D-allele carriers would actually produce more ACE2 of those with DD or ID genotypes, D-allele holders could be more susceptible to infection with Covid-19 disease compared to carriers of the other allele, which could cause Severe infection to organs such as the pancreas, lung, and heart (Lumbers *et al*.,2020).

4-6-4 The Genotype Distribution of ACE2 Gene Polymorphism in Covid-19 Patients

Table (4-16) showed the association between ACE2 gene polymorphism and covid-19 patients according to parameters understudy, where the Mean of D-dimer and ferritin showed significant differences at (0.037 and 0.030) respectively for two categories, while FBG, HbA1c, INR, INS, IN, and CRP did not show any significant differences for the two categories ($p \leq 0.05$).

Table (4-16): Association between the ACE2 Gene Polymorphism (DD, DI) Groups and Covid-19 Patients

Subjects	COVID only		Sig.
	DD	DI	
	Mean±S.E		
FBG	119.84±8.14	118.56±	**0.541**
HbA1c	5.36±0.16	5.41±0.26	**0.611**
INR	7.93±2.40	7.82±2.29	**0.441**
INS	0.32±0.01	0.31±0.01	**0.667**
IN	30.34±3.6	27.0±4.2	**0.124**
CRP	43.69±10.71	43.79±7.5	**0.537**
D-dimer	1788.0±68.1	1633.68±24.3	**0.037***
Ferritin	365.42±10.9	247.78±14.6	**0.030***

***significant at ($p \leq 0.05$)**

The primary finding of the current study revealed that there was increasing in the covid-19-associated hematological and diabetic parameters as for the expression of genotype frequencies DD in the moderate and severe COVID-19 cases as shown in table (4-16) , in which the concentration of each FBG, INR, CRP, D-dimer, and ferritin was more than a normal value.

The ACE2 genotypes are linked to hypertension and chronic disease such as diabetes, cardiovascular disease, and the inflammatory parameters are the leading risk factors for disease severity (Patnaik *et al*.,2014). This could be due to a pathophysiological causal link between ACE2 genotyping alleles (DD, DI) and disease severity.

The association of elevated CRP, D-dimer, ferritin and COVID-19 disease severity in this study reflected the ongoing activation of the hemostatic system and potentially of chronic disease complications (Luo *et al*.,2019). This may be linked to a genetic basis as the increasing of these parameters was also significantly associated with the ACE2 (DD + DI) SNP since this genotype was linked to potentially chronic diseases. These results were consistent with the results obtained by (Srivastava *et al*.,2020).

Infection with COVID-19 has been linked to higher levels of inflammatory indicators such CRP, LDH, and ferritin, according to many investigations (Salvamani *et al*.,2020). Such a finding was consistent with the data which revealed that COVID-19 illness severity was linked to ferritin, CRP, D-dimers, and HbA1c levels (Badawi ,2020). It was found that genetic variants within an individual's ACE2-positive cell population were evidently associated to hypercytokinaemia and hence their response to COVID-19 illness.

4-6-5 The Genotype Distribution of ACE2 Gene Polymorphism in Diabetes Mellitus Covid-19 Patients

Table (4-17) showed the association between ACE2 gene polymorphism and diabetes mellitus Covid-19 patients according to the parameters under study, where the Mean of FBG, INR, IN, CRP, and ferritin showed significant differences at (0.036, 0.041, 0.022, 0.032 and 0.033) respectively for two categories, while HbA1c, INS and d-dimer did not show any significant differences for two categories.

Table (4-17): Association between the ACE2 Gene Polymorphism (DD, DI) Groups and Diabetes Mellitus Covid-19 Patients

Subjects	COVID +DM		Sig.
	DD	DI	
	Mean±S.E		
FBG	277.16±33.04	230.36±40.2	0.036*
HbA1c	9.58±0.5	8.69±0.4	0.127
INR	13.97±2.9	24.92±4.3	0.041*
INS	0.30±0.01	0.31±0.02	0.611
IN	21.08±3.4	33.41±9.6	0.022*
CRP	53.31±9.8	79.61±17.3	0.032*
D-dimer	1390.43±57.6	1275.42±42.6	0.511
Ferritin	667.46±41.2	544.45±12.7	0.033*

*significant at ($p \leq 0.05$)

There was an effect of ACE DD/DI polymorphism on the natural course of COVID-19. This was consistent with a recent Spanish study by (Gomez *et al*.,2020). who reported an increased risk for an unfavorable COVID-19 outcome in ACE DD genotype carriers.

There was a significant association of the ACE2 (DD) allele with d-dimer, ferritin and ACE2 (DI) allele with CRP (table 4-17). These findings were associated with increased risk for an COVID-19 severity, therefore the clarification of these results stemmed from the association of some genes with inflammatory proteins such as D-dimer and ferritin, whereas some other genes were associated with CRP. This heterogeneity resulted from the samples obtained from diabetic patients infected with Covid-19, which effected the concentration of this proteins in blood.

The results showed a significant increase in blood markers and diabetes markers in the ACE 2 polymorphism of the (DD,DI) alleles for diabetic patients with covid-19 compared with covid-19 patients only. This increase may be related to an increase in the gene expression of these two alleles, and thus an increase in the ACE2 receptors in the body, allowing the Corona virus to bind with many cells in the body could have caused an increase in the concentration of these parameters . This explanation agreed with Abdelsattar *et al*.(2020) finding who also noticed an elevation in some of these parameters.

Due to the limitation of a relatively small sample size, there was a difficulty in making further speculations regarding links the between these genetic polymorphism in ACE2 (DD,DI) and other research indices. Nevertheless, the data that represented additional information about diabetes mellitus patients infected covid-19 demonstrated the ability of ACE2 (DD) and (DI) gene polymorphisms to predict COVID-19-related illness severity explained 86 percent of the severity when combined with conventional cell and blood indicators.

Under normal physiological settings, angiotensin (1-7) and angiotensin (1-9) are produced. The anti-inflammatory and antioxidant effects of the pulmonary ACE2/Ang (1-7) system have been shown, and ACE2 has been proven to protect against the lethal H5N1 avian influenza infection (Zou *et al*.,2020). As a consequence, the decreased ACE2 expression in DM might explain the higher occurrence of severe lung damage and ARDS linked with COVID-19 (Tikellis *et al*., 2011).

4-6-6 The Correlation between Genotyping, and Groups of Control, Covid-19 , Diabetes Mellitus Patients Infected Covid-19

Table (4-18) showed the correlation between diabetes mellitus patients infected with covid-19 and the control group according to the parameters under study. Where the Mean of FBG, HbA1c INR, CRP and ferritin was high in

diabetes mellitus patients infected with covid-19 compared to other groups with (DD) type at (277.16±33.04, 9.58±0.5, 13.97±2.9, 53.31±9.8 and 667.46±41.2) respectively, whereas with (DI) type it was at (230.36±40.2, 8.69±0.4, 24.92±4.3, 79.61±17.3 and 544.45±12.7) respectively. The Mean of INS and (DD) was high in covid-19 group at (0.32±0.01), while with (DI) type it was high in control group at (0.34±0.02). The Mean of IN and (DD) was high in the control group at (57.83 ± 8.2) while with (DI) type it was high in the diabetes mellitus infected covid-19 group at (33.41±9.6). Finally, the Mean of d-dimer was high with both (DD) and (DI) types in covid-19 group compared to other groups at (1788.0±68.1 and 1633.68±24.3) respectively.

Table (4-18): The Correlation between Control, Diabetes Mellitus Patients Infected Covid-19, and Covid-19 Patients with Blood Parameters

Subjects	Genotype	Control	Covid	Covid+DM
		Mean±S.E		
FGS	DD	76.50±4.97 a	119.84±8.14 b	277.16±33.04 c
	DI	80.50±7.77 a	118.56±b	230.36±40.2 c
HbA1c	DD	4.88±0.27 a	5.36±0.16 a	9.58±0.5 b
	DI	5.23±0.31a	5.41±0.26 a	8.69±0.4 b
INR	DD	10.67±3.34 b	7.93±2.40 a	13.97±2.9 c
	DI	4.00±2.63 a	7.82±2.29 b	24.92±4.3 c
INS	DD	0.27±0.01 a	0.32±0.01a	0.30±0.01 a
	DI	0.34±0.02 a	0.31±0.01a	0.31±0.02 a
IN	DD	57.83±8.2 c	30.34±3.6 b	21.08±3.4 a
	DI	18.03±6.7 a	27.0±4.2 b	33.41±9.6 c
CRP	DD	1.71±0.01 a	43.69±10.71 b	53.31±9.8 c
	DI	3.40±0.52 a	43.79±7.5 b	79.61±17.3 c
D-dimer	DD	164.00±11.40 a	1788.0±68.1 c	1390.43±57.6 b
	DI	140.00±17.5 a	1633.68±24.3 c	1275.42±42.6 b
Ferritin	DD	78.54±9.8 a	365.42±10.9 b	667.46±41.2 c
	DI	119.88±8.77 a	247.78±14.6 b	544.45±12.7 c

****Significant at P-value ≤ 0.01, a, b and c refer to differences among groups according to Duncan's statistical test.**

The reason for comparing the ACE2 genotyping alleles (DD, DI) in diabetic patients with Covid -19 and Covid-19 patients was that the alleles were the dominant alleles causing incidence of pneumonia . This was also confirmed by Itoyama *et al.*(2004) who showed that the incidence of pneumonia was higher in patients carrying D allele in SARS-CoV-1 infection in addition to the variants of ACE-2 and the receptor of SARS-CoV-2, caused by gene polymorphism which

were thought to result from differences in disease susceptibility and disease severity (Gómez *et al*.,2020).

The data showed the correlation between ACE2 genotyping (DD,DI) and some parameters in control group, diabetes infected COVID-19, and COVID-19 patients. For example, the DD genotype caused higher levels of blood parameters associated with covid and diabetes in comparison to DI genotype as shown in the result due to the increase of the expression of ACE2 receptor on the surface of cell causing more invasion of virus to body. This finding complied with (Chaudhary, 2020) who explained the effect of ACE2 gene expression in viral pathogenesis.

Considering the impact of ACE2 expression on the parameters under study ,the findings in table (4-18), suggested that ACE 2 polymorphism may have a role in different reactions to COVID-19 infection. Previous studies showed that the majority of the ACE 2 gene polymorphism (47%) was related to insertion/deletion (I/D) polymorphism (Sayed-Tabatabaei *et al*.,2006). The correlation between I/D polymorphism and cardiovascular disease, blood pressure, diabetes was previously shown the variety of DD, II, and DI genetic polymorphism in patients. For example, those with DD genotype had the highest and those with II genotype had the lowest ACE activity (Mohaghegh *et al*.,2021).

The results revealed that the study groups (control group, diabetes with covid-19, and covid-19) suffered from elevated FBG concentration in DD allele, while those with (DI) allele had an elevated level concentration of HbA1c, INR, INS and IN. The patients with DI allele were shown to have increases in activity of ACE-2 level in Pancreas which acted as a receptor for SARS-CoV-2 that produced more invasion of virus for beta cell in charge of producing insulin (Elifcan *et al*.,2021).

4-7 AT1R Genes Polymorphism.

The AT1R genotyping, the genomic DNA was amplified by using specific primers and accomplished by the Thermo-cycler apparatus under the optimal conditions. The results revealed that the presence of a single band (200 bp) of the target sequence of *AT1R* gene in agarose gel (figure 4-6).

Figure (4-7) showed the SSCP analysis which detected the sequence variations (single-point mutations) through electrophoretic mobility differences. Where A pattern showed haplotype (one band) for control specimens, while Pattern B had two bands of them to COVID patients, and diabetes patients infected with covid-19.

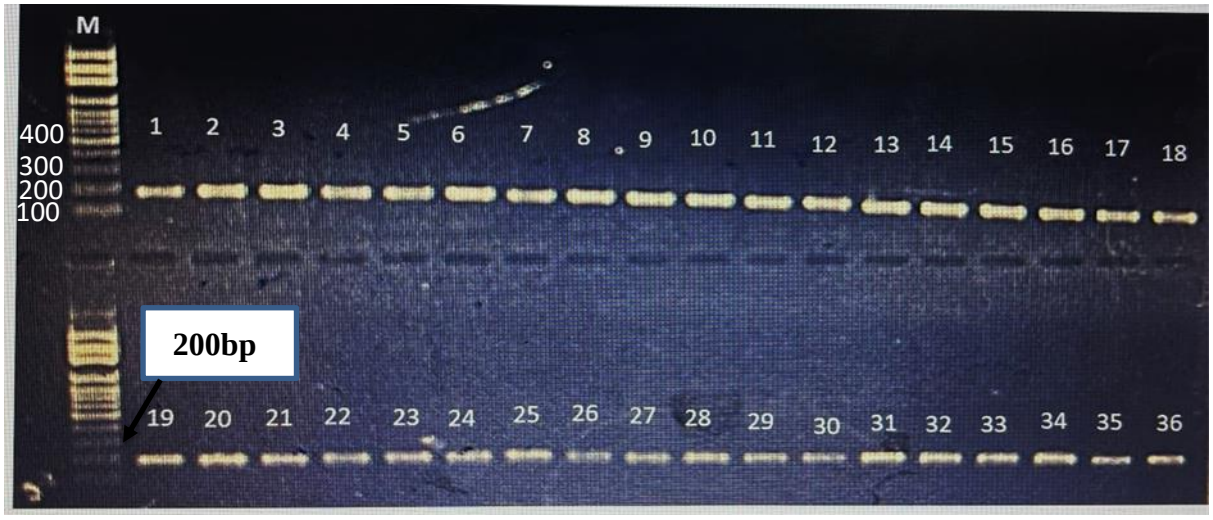


Figure (4-6): Agarose gel electrophoresis of amplified product patterns of AT1R with specific primer. M: refers to DNA size marker (100bp). lanes 1 - 36 refer to PCR products of AT1R (200bp) of patients and healthy control groups. Electrophoresis conditions: 1% agarose concentration 1%; 75 V, 20 mA.

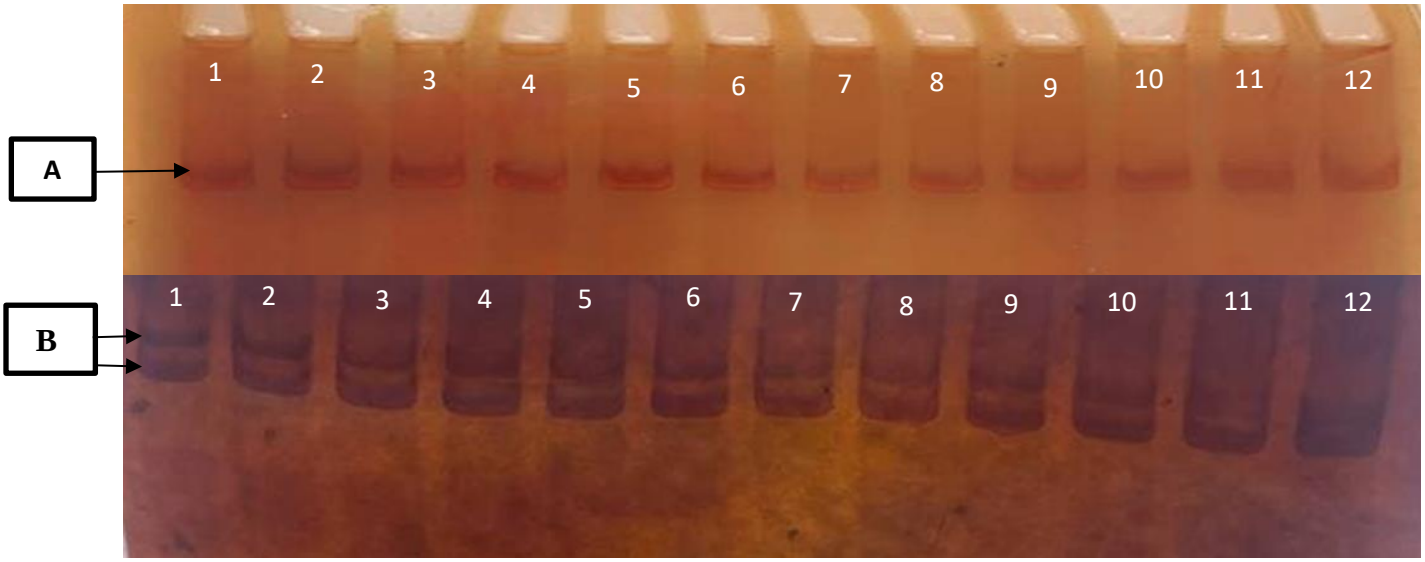


figure (4-7): SSCP electrophoreses pattern of AT1R receptor for samples control, COVID-19, and COVID-19+DM, 15% gel polyacrylamide, 100V, 20mA, for 3h, was showed haplotype (Pattern A: one band) to control and (Pattern B: two bands) in COVID and COVID+DM.

4-7-1 the SSCP Distribution in the Patients and Control Groups.

The results revealed that DNA polymorphism distributions were 96.77% and 3.23 % in the control group respectively ;DNA polymorphism distributions were 6.67% and 93.33 % in COVID only, while it was 12.9% and 87.1 % in the COVID-DM group. The results demonstrated that there was no association between DNA polymorphisms according to the number of bands with patients as compared with the control groups (Table 4-19).

There were significant differences ($P \leq 0.01$) in the frequencies of AT1R polymorphism between patients with COVID-19, Covid-19 with diabetes and control subjects. The patients with diabetes mellites infection with covid-19 and diabetic nephropathy had the same pattern of genotype frequencies.

Generally, the increased activation of angiotensin 1 receptors (AT1R) at the expense of the ACE2/Ang 1–7-driven pathway resulted in fibrosis, hypertrophy, increased reactive oxygen species (ROS), vasoconstriction, hyperglycemia, and gut dysbiosis. (Bourgonje *et al*., 2020; Devaux *et al*., 2020; Groß *et al*., 2020; La Vignera *et al*., 2020). In addition, TNF- (tumor necrosis factor-) levels rose, and the tumor necrosis factor receptor (TNFR) was activated. These factors, when combined with infected people' comorbidities like as diabetic mellitus (T2DM) and hypertension, can trigger a cytokine storm with deadly consequences. (Coperchini *et al*., 2020; Gubernatorova *et al*., 2020; Nile *et al*., 2020).

The finding showed that AT1R autoantibodies may play a role in Sars-CoV-2 infection by decreasing the effect of AngII accumulation following the virus occupation of the ACE2 receptor. Because AT1Rab was present in healthy controls without any clinical signs, it may be explained as a result of a possible condition of tolerance to AT1Rab's continuous stimulation on its receptor. Because of the rise in AngII caused by Sars-CoV-2 occupying ACE2, patients with antiAT1R autoantibodies having an immune system that was "refractory" to acute activation, whereas subjects without anti.AT1R autoantibodies could have a cytokine storm (Papola *et al*.,2022)

The results of the current study showed that the SARS-CoV-2 virus targeted RAS and produced hyperactivation of the characteristic detrimental axis (Ang II–AT1R), which was characterized by vasoconstriction, hyper inflammation, oxidative stress, and cell death. Ang II, a key component of RAS, largely coordinates its endocrine, autocrine, paracrine, and intracrine actions through AT1R, which promotes NF-B, ROS, tyrosine kinase, and other signaling pathways can problems in numerous organs and tissues, including the lungs, heart, and kidneys skin, gonads, and the brain Finally, RAS inhibitors (particularly ARBs) may be crucial factors in treating COVID-19 affecting renal,

pulmonary, cardiovascular, immunological, and brain functioning, according to various research studies completed so far. (El-Arif *et al*.,2022).

Table (4-19) The SSCP (one band, and two) Distribution in the Study Group

SSCP	Control	COVID only	COVID-DM	Qi square	P
	No. (%)				
A	30(96.77%)	2(6.67%)	4(12.90%)	**40.667**	**0.0001****
B	1(3.23%)	28(93.33%)	27(87.10%)	**25.107**	**0.0001****

**** significant at P-value ≤ 0.01.**

4-7-2 The association of PCR-SSCP Polymorphisms of AT1R Gene in COVID Disease Groups with the Physiological Parameter

Table (4-20) refered to the present significant differences ($P \leq 0.05$) in the serum levels of FBG, IN, CRP, D-dimer, and Ferritin among PCR-SSCP polymorphisms of the AT1R gene in the COVID group, while there were no significant differences in the concentrations of HbA1c, INR, INS in COVID group (Ceolotto *et al*., 2001).

Table (4-20): The Association of PCR-SSCP Polymorphisms of AT1R Gene in COVID Disease Groups with the Physiological Parameter

Subjects	COVID only		Sig.
	1	2	
	Mean±S.E		
FBS	183.0±78.0	114.83±6.6	**0.041***
HbA1c	5.35±0.05	5.38±0.14	**0.678**
INR	8.68±0.7	7.83±1.8	**0.222**
INS	0.31±0.04	0.32±0.01	**0.671**
IN	15.20±2.9	30.11±6.8	**0.013***
CRP	59.65±14.6	42.59±8.8	**0.049***
D-dimer	1379.59±20.4	1756.55±54.2	**0.016***
Ferritin	604.08±35.6	302.16±77.0	**0.034***

*** significant at P-value ≤ 0.05.**

In general, it was found that patients with diabetes had more severe COVID-19 outcomes than patients without diabetes, represented by the mean of blood markers (Table 4-20). These results were in agreement with the findings obtained from a Chinese COVID-19 cohort, in which COVID-19 diabetic patients had a 7.3% increased risk of morbidity (Wu *et al*.,2020). Moreover, a British cohort showed that COVID-19 patients with uncontrolled diabetes had a higher risk of death than other patients did (Williamson *et al*.,2020).

4-7-3 The Association of PCR-SSCP Polymorphisms of AT1R Gene in Diabetes and Covid Patients Groups with the Physiological Parameter

Table (4-21): referred to the present significant differences ($P \leq 0.05$) in the serum levels of INR, IN, CRP, and D-dimer among PCR-SSCP polymorphisms of the AT1R gene in the COVID group, while there were no significant differences in the concentrations of HbA1c, FBG, INS, Ferritin in COVID group (Ashavaid *et al*., 2000). There were Significant differences ($P \leq 0.05$) in the serum levels of INR, IN, CRP, and D-dimer among PCR-SSCP polymorphisms of the AT1R gene.

Table (4-21): The Association of PCR-SSCP Polymorphisms of AT1R Gene in Diabetes and Covid Patients groups with the Physiological Parameter

Subjects	COVID +DM		Sig.
	1	2	
	Mean±S.E		
FBS	228.08±66.9	256.80±26.1	0.433
HbA1c	9.78±1.3	9.06±0.3	0.691
INR	11.31±1.9	18.43±5.7	0.023*
INS	0.33±0.03	0.30±0.01	0.429
IN	15.10±0.8	26.41±4.7	0.011*
CRP	36.18±8.9	64.89±10.0	0.005**
D-dimer	806.0±113.5	1353.64±40.4	0.0006**
Ferritin	657.70±46.1	572.05±92.3	0.056

*** significant at P-value ≤ 0.05 ** significant at P-value ≤0.01**

The severity of COVID 19 is highly correlated with glycemic such as insulin, insulin resistance, insulin sensitivity, and fasting blood sugar, because of the ability of the Coronavirus to get into ACE 2 receptors of Beta cells in the pancreas, liver cells and other organs (Gregory *et al*., 2022). Table (4-21) showed highly significant differences between these parameters in diabetic patients infected with covid-19.

Lippi *et al*(2020) studied four different cases about the blood parameters in covid -19 patients which showed that there was a high correlation between HbA1c level and COVID contributing to the good diagnosis of disease, especially the role of coronavirus in clot blood formation.

Hyperglycemia and insulin resistance resulting from diabetes, induce increased synthesis of advanced glycation end products (AGEs) and pro-inflammatory cytokines like CRP that generate oxidative stress (Cuschieri and Grech, 2020). Such a finding went in line with our findings where an increase in the percentage of CRP, ferritin, D-dimer was found.

4.8 rs657152 SNPs Gene Polymorphisms

For rs657152 genotyping, the genomic DNA was amplified using specific primers and accomplished by the Thermo-cycler apparatus under the optimal conditions The results revealed the presence of a single band (300 bp) of the target sequence of rs657152 gene in agarose gel (figure 4-8).

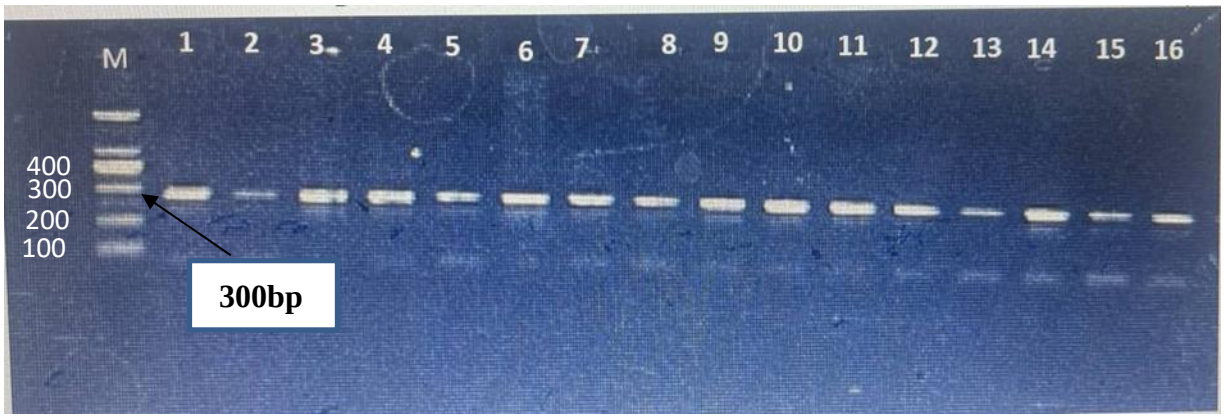


Figure (4-8) the electrophoreses pattern of PCR product for rs657152, this amplification product one band 300 bp, M: DNA ladder, lane (1-5) PCR product of COVID only, (6-10) PCR product for COVID+DM, (11-16) for Control groups. 1% agarose, 75V, 20 mA for 1 hour (5 µl in each well) annealing temperature 58 C^{o}.

Deep-vein thrombosis and pulmonary embolism are common in individuals with severe Covid-19, and the variation rs657152-A has been linked to these disorders. Correlated polymorphisms are also linked to the increased levels of the blood-clotting proteins von Willebrand factor and factor VIII, as well as interleukin-6, both of which are typically elevated in Covid-19 patients. (Hu *et al* ., 2021).

A recent study found that those with blood types A or B, regardless of Covid-19 status, have a greater risk of thromboembolic events than people with type O. (picchiotti *et al* ., 2020) have shown that greater levels of the soluble lectin CD209 are related with the single-nucleotide polymorphism rs505922-C.

4-9 Sequencing

Figure (4-9) showed the results of gene polymorphism sequencing, where the Single Nucleotide Polymorphism SNP was in two locations as shown in figure 4-9, first location A deletion and second location A insertion**.**

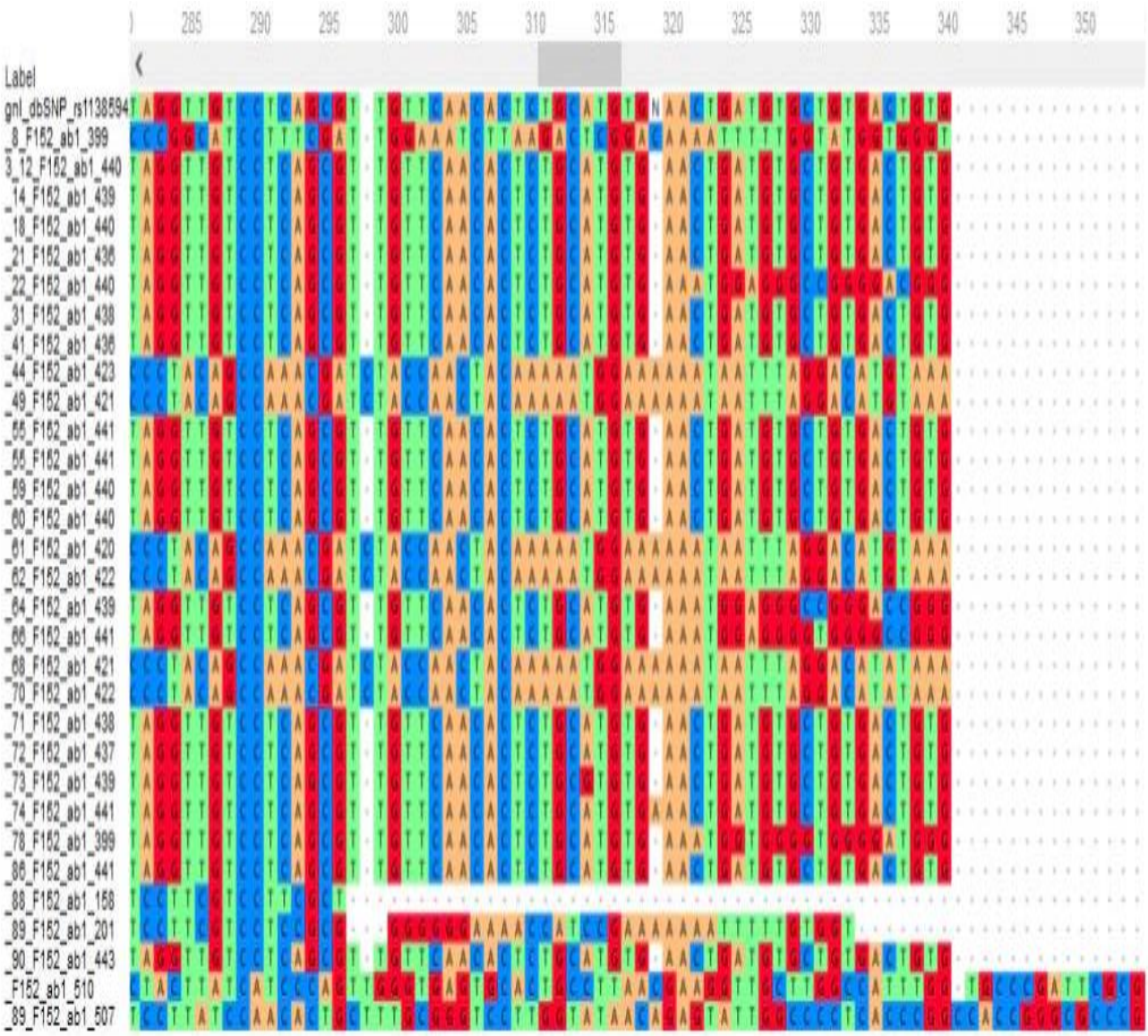


Figure (4-8) Multiple ailments of study sample ABO Transferase rs657152 SNPs.

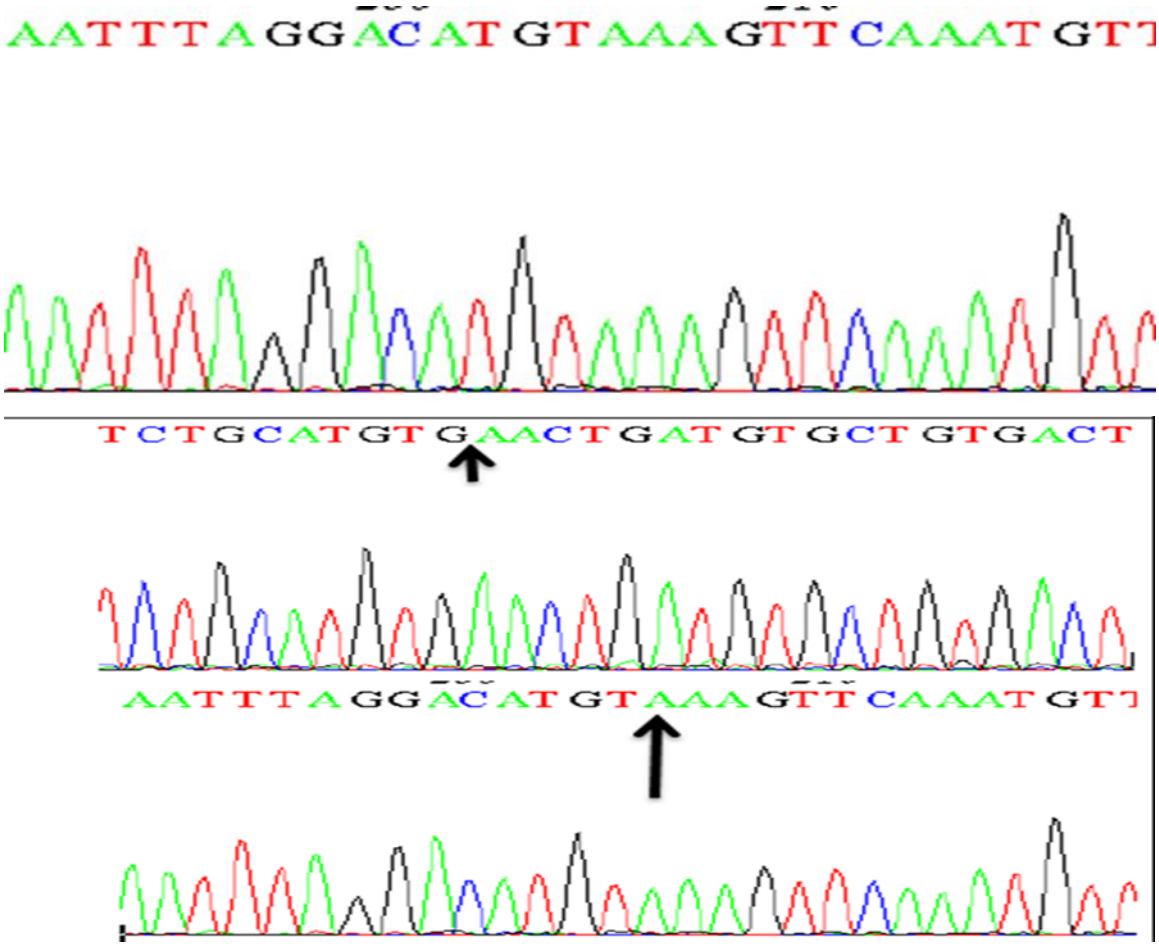


Figure (4-9): Element of Detected rs657152 SNPs Pattern within the DNA Chromatogram of the Targeted 300 bp Sequencing Distribution I (A), A in the Study Group

4-9-1 The Sequence Distribution of Adenine Allele in Patients with Diabetes Mellitus Infected Covid-19, Covid-19 only, and Control Group

Table (4-22) showed the association between the adenine allele sequencing (A/I= Addition and A/D=deletion) and the patients in the groups under study, where the A/D showed a high value at (2.667) for diabetes mellitus infected covid-19, covid-19, and control group respectively. The A/I showed a low value at (1.300) for the same group. In contrast, table (4-23) showed the association between the adenine allele sequencing (A/I= Addition and A/D=deletion) and the patients in the groups under study where the A/I and A/D showed high value at (0.433).

Table (4-22): The Sequence Distribution of Adenine Alleles in Patients with Covid-19, Covid-19 with Diabetes and Control Groups.

Sequences	Control	Covid-19	COVID-DM	Qi square	P
	No. (%)				
A/I*	9(64.29%)	5(83.33%)	6(66.67%)	**1.300**	**0.522**
A/D*	5(35.71%)	1(16.67%)	3(33.33%)	**2.667**	**0.264**

***A/I refer to Adenine insertion, A/D refer to Adenine deletion**

The ABO and Rh(D) blood types were not linked to an increased or decreased risk of SARS-CoV-2 infection according to the findings. These findings contradicted those of prior research, which had a significant influence on the media. Other explanations for the inconsistent results existed, including samples looked to be healthy and adult having with no obvious comorbidities. Only severe instances of COVID-19 may be linked to ABO blood types. Another study postulated that, whereas ABO blood type and/or cardiovascular disease were predictive of the severity of COVID-19 in patients, they were not characteristics predisposed to SARS-CoV-2 infection. 5 This notion was supported by a pathophysiological mechanism: people with the blood type A were more likely to acquire diabetes (Paré *et al*., 2008)

Lehrer and Rheinstein (2020) found no evidence that the ABO blood group affected COVID-19 infection risk or outcome. More research will be needed to confirm ABO's participation in COVID-19. The current study showed that SNP rs657152 and its link to illness complications was related to Covid-19th as regards the Iraqi population ,although this did not mean it was a severe risk factor.

The present study revealed that polymorphisms SNPs could cause a considerable alteration in RNA secondary structure. These changes might cause

dysregulations in ACE2 transcription/translation or protein stability, altering COVID-19 binding to the ACE2 receptor and influencing SARS-CoV-2 pathogenesis. By exposing the sensitive amino acid sequence or changing its appropriate folding, the secondary structure of mRNA generated by the described polymorphisms may make a protein more susceptible to proteases (Pouladi and Abdolahi,2021). ACE2 is involved in the balance of a system which its malfunctioning has been linked to a number of conditions including hypertension, heart failure, and diabetes mellitus (Gheblawi *et al*.,2020). In humans, several studies have shown a strong association of alanine polymorphisms with diabetes. Thus, together with the blood parameters, they can play an important role in COVID-19 infection and the patients with these changes are more susceptible to get the disease (Luo *et al*.,2019).

According to a study about diabetes mellitus, Ig A nephropathy, the DD genotype, or D allele of this polymorphism has been associated with elevated circulating and tissue ACE activity as well as increased risk for hypertension and diabetic renal (Li *et al*., 2007).

The current study evaluated the association between sequence distribution of adenine alleles (A/D, A/I) in covid-19 patients and diabetes patients with covid-19 These results were consistent with the findings of the study by (Walls *et al*.,2020) demonstrating that some of SNPs in ACE2 were associated with COVID-19 disease and the genotype frequencies of rs2074192 and rs1978124 SNPs, In addition the relation between ACE2 SNPs with the severity of the disease was increasing.

4-9-2 The Sequence Distribution of Adenine Allele in Patients with Diabetes Mellitus Infected Covid-19, Covid-19, and Control Group

Tables (4-23, 4-24, and 4-25) showed the Allele frequency between Covid-19 patients and control group, diabetes patients infected with covid-19 with a control group, and diabetes patients infected with covid-19 and covid-19 patients.

The results of the first group (Covid-19 patients and the control group) did not showed significant differences at the probability value (0.406) and the individual ratio was 0.360 at the limits of about 0.032 - 4.006 as shown in table (4-23), while in the second group (diabetes patients infected with covid-19 and control group) did not also show any significant differences at the p-value (0.906) and the individual percentage was 0.900 with a range of about 0.154 - 5.258 listed in the table (4-24). Finally, the third group (diabetes patients infected covid-19 and covid-19 patients) did not show any significant differences at the p-value (0.482) and the individual percentage was 2.500 with a range of about 0.194-32.195 (table 4-25).

Table (4-23): Odd ratio of adenine alleles sequence between Covid-19 patients and Control group.

Sequences	Control	COVID only	Odd ratio	P
A/I*	9(64.29%)	5(83.33%)	**0.360**	**0.406**
A/D*	5(35.71%)	1(16.67%)	**(0.032-4.006)**	

Table (4-24): Odd ratio of Adenine Alleles Sequence between Diabetes Patients Infected with Covid-19 and Control Group.

Sequences	Control	COVID-DM	Odd ratio	P
A/I*	9(64.29%)	6(66.67%)	**0.900**	**0.906**
A/D*	5(35.71%)	3(33.33%)	**(0.154- 5.258)**	

Table (4-25): Odd ratio of Adenine Alleles Sequence between Diabetes Patients Infected Covid-19 and Covid-19 Patients.

Sequences	Covid-19	COVID-DM	Odd ratio	P
A/I*	5(83.33%)	6(66.67%)	**2.500 (0.194-32.195)**	**0.482**
A/D*	1(16.67%)	3(33.33%)		

***A/I refer to Adenine insertion, A/D refer to Adenine deletion**

Sieńko and colleagues (2020) suggested that Some single nucleotide polymorphisms (SNPs) in the ACE2 gene were a risk factor for COVID-19 infection. ACE2 structure, serum concentration, and circulating angiotensin levels were all affected by (1-7) genotypes. Furthermore, there was evidence that the ACE genotype could influence the outcomes of ARDS therapy, the most serious complication of SARS-CoV-2 infection. The frequency of the ACE D allele may influence COVID-19 morbidity, infection, and death. In light of the COVID-19 pandemic, the goal of this narrative review was to evaluate and determine the mechanisms of ACE-I and ARBs, with a focus on angiotensin receptors and their polymorphism. These drugs have been often recommended to aged people.

Yamamoto *et al*.(2021) studied the ACE1 gene (a homolog of ACE2) which was linked to COVID-19 phenotypic expression, particularly in terms of severity. The apparent disparity in mortality between West and East Asia appeared to be explained by ethnic differences in the ACE1 insertion (I)/deletion (D) polymorphism, suggesting that the ACE1 II genotype was a risk factor. This conflicting conclusion may be true in some geographic locations, particularly among specific patient populations. It might also be due to interactions with other genes or biochemical pathways that are still unknown.

4-9-3 The Association between Adenine Allele Sequence in Covid-19 patients and the Blood Parameters

Table (4-26) showed the association between the adenine allele sequencing in COVID-19 patients according to blood parameters. where the mean of FGB, HbA1C,INR,IN,CRP,D-dimer and ferritin showed significant differences at (0.031,0.024,0.011, 0.0001, 0.0001, 0.0001 and 0.0001) respectively, while the INS, did not show any significant differences at ($p \leq 0.01$ and ≤ 0.05).

Table (4-26) The Association between Adenine Allele Sequence in Covid-19 Patient and Blood Parameters.

Subjects	COVID only		Sig.
	A/D*	A/I*	
	Mean±S.E		
FBS	180.0±10.2	107.68±13.7	**0.031***
HbA1c	7.13±1.6	5.01±0.3	**0.024***
INR	1.78±0.3	5.92±2.3	**0.011***
INS	0.35±0.01	0.32±0.02	**0.614**
IN	4.0±0.28	28.87±14.4	**0.0001****
CRP	175.0±11.9	20.51±13.8	**0.0001****
D-dimer	10000±100.6	1289.16±52.9	**0.0001****
Ferritin	1200±54.7	250.40±18.7	**0.0001****

***Significant at P-value ≤ 0.05 ** significant at P-value ≤ 0.01.**

***A/I refer to Adenine insertion, A/D refer to Adenine deletion**

The current results showed how addition and deletion in the adenine allele sequence and blood parameters could influence an individual's susceptibility to SARS-CoV-2 infection and discussed some marker associated the diabetes (table 4-27). In addition, Residue changes of ACE2 receptor in the binding interface would influence its expression and affinity with SARS-CoV-2 (Chen *et al.*,2021).

Yi *et al*.(2006) studied the DNA sequencing technology to comprehensively define the degree of inter-individual variations in susceptibility and subsequent immune responses determined by gene polymorphisms. As such, we compared the adenine allele frequencies of expressions of 30 covid-19 and some inflammatory proteins such as CRP, D-dimer, and ferritin that showed highly significant differences at (0.0001) and found that they could partially account for the differences in COVID-19 prevalence and morbidity rates in the region under study.

4-9-4 The Association between Adenine Allele Sequence in Diabetes Patients with Covid-19 and the Blood Parameters

Table (4-27) showed the association between the adenine allele sequencing in COVID-19 patients according to blood parameters, where the mean of FBS, CRP, D-dimer, and ferritin showed significant differences at (0.023,0.049,0.011, and 0.0001) respectively, while the HbA1c, INR, INS, and IN didn’t show any significant differences.

Table (4-27): The Association between Adenine Allele Sequence in Diabetes Patients with Covid-19 and Blood Parameters

Subjects	COVID +DM		Sig.
	A/D*	A/I*	
	Mean±S.E		
FBS	374.50±14.5	243.20±44.2	**0.023***
HbA1c	9.30±1.9	8.49±0.7	**0.415**
INR	22.06±8.2	18.26±5.5	**0.06**
INS	0.30±0.03	0.29±0.01	**0.122**
IN	19.11±7.5	34.50±10.7	**0.034**
CRP	53.32±12.4	44.58±3.6	**0.049***
D-dimer	343.25±17.8	853.01±23.1	**0.011***
Ferritin	183.31±28.6	1092.27±22.26	**0.0001****

***Significant at P-value ≤ 0.05 ** significant at P-value ≤ 0.01.**

***A/I refer to Adenine insertion, A/D refer to Adenine deletion**

The current study described some potential mechanisms underlying the increased susceptibility of patients with diabetes to a more severe COVID-19 disease, leading to higher morbidity and mortality. This mechanism involved comparisons according to blood parameters, SSCP assay, and sequencing to explain a possible increased susceptibility to infection with SARS-CoV-2 in patients with diabetes.

According to above findings, the increase in the parameters associated with diabetes such as FBG,HbA1c,INR, INS, and IN in A/D group compared A/I group explained the susceptibility of diabetes patients to be infected by severe covid-19 more than the other people . These results agreed with the study of (Azar *et al*.,2020) who explained a possible increased susceptibility to infection diabetes patients.

Several reports showed a consensus on the role of ACE2 between diabetes and COVID-19, since diabetic patients have high heterogeneity in ACE2 receptor-specific alleles. Thus, this would facilitate the viral entry and subsequent replication while other infected people showed low levels of ACE2 and an apparent increase in ACE2 due to other factors such as treatment with ACE/ARBs and hyperglycemia (Fang *et al*.,2020). Some reports mentioned that the ACE2 genetic polymorphisms rs2106809 and rs2074192 in diabetics patients may cause increasing in the levels of cytokines such as IL1-β and IL-6 and other inflammatory proteins such as CRP, dimer, and ferritin so with a state of chronic inflammation this is called a "cytokine storm". They also suggested to change AMPK/mTOR signaling pathway in patients with diabetes so as to aggravate insulin resistance IN,(INR) and diabetes-induced complications (Suleiman *et al*.,2021).

Conclusion

1- COVID-19 has a strong relationship with Diabetes Mellitus (DM). It is discovered that not only diabetes is a key risk factor for developing severe COVID-19, but also the virus might trigger DM in some cases. They show the bidirectional relationship between COVID-19 and DM.

2- The greater the age and duration of infection, the greater the impact of the Covid virus on patients

3- The levels of IN, C-Reactive protein, D-dimer, and ferritin are markedly in COVID-19 and COVID+DM increase in the severe covid patients.

4- Levels of FBG, HBA1C, and IN increase sometimes in covid and markedly Covid+ Diabetes.

5- Gene polymorphism of ACE2 plays a role in the pathogenesis Covid-19.

6- Disorder in levels of ACE2, and AT1R lead to increase progressive of disease in Covid19 patients.

Recommendation

1- Increasing the sample size of Covid patients of different severity, as well as including other Iraqi governorates

2- Studying the covid-19 with type 1 diabetes and The relationship of covid-19 with other diseases such as pressure, kidney failure, heart and stroke,attack.

3- Detecting the effective other blood markers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and higher lactate dehydrogenase (LDH), higher creatine kinase (CK), and immunological side IL-6, and IL-8.

4- Investigating, ACE2 antagonists may be considered as potential therapeutic strategies to alleviate the complications associated with COVID.19 disease.

5- Studying of other genes associated with covid-19 and diabetes diseases such as PRKRA and LAPTM4B and other SNP in ABO blood groups such as rs505922, rs8176719, and rs8176746.

6- Studying of gene expression using real-time PCR.

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APPENDIX

Questionnaire

Name
Age
Sex
weight
duration of disease
place of injury
do you have other diseases?
do you have diabetes?
covid-19 treatment type
diabetes treatment type + dose quantity
CT scan
Does he have injured or deceased relatives with covid-19?
Dose he has injured or deceased from diabetes

بالمقارنة مع الإصابات الوبائية السابقة ، فإن coronavirus لديه أعلى معدل انتقال وخطر الوفاة. من ناحية أخرى ، يعد مرض السكري من أكثر الأمراض انتشارًا. وبالتالي ، فإن مرضى السكر المصابين بفيروس COVID-19 هم الأكثر إصابة. كان الهدف من هذا البحث هو معرفة ما إذا كانت هناك علاقة بين الإصابة بفيروس COVID-19 ومرضى السكري من النوع 2. تتعلق استنتاجات هذه الدراسة بالعلاقة بين مرض السكري ومرضى كوفيد -19

أجريت الدراسة الحالية في المختبر DNA في كلية العلوم ، جامعة بابل. تم جمع العينات واكتمل الجزء العملي من هذه الدراسة في أكتوبر (2021-2022). تم الحصول على العينات من مرضى Covid-19 و Covid-19 ومرض السكري والأشخاص الأصحاء. حيث جمعت العينات من محافظة بابل من مستشفى مرجان الطبية ومستشفى الصادق التعليمي حيث بلغ العدد الإجمالي للعينات الكلي 92 منها 31 عينة دم من اشخاص اصحاء (3 إناث و 28 ذكور) و 30 عينة دم من مرضى مصابين بفيروس كورونا. (16 أناث ، 14 ذكور) و 31 عينة دم من المرضى المصابين بـ COVID-19 + السكري (17أناث ، 14 ذكور).

قسمت الدراسة الحالية إلى ثلاثة أجزاء: الدراسة الأولى تناولت الجانب الديموغرافية ، اما الثانية تناولت قياس معايير الدم ، والثالثة تناولت الجانب الجزيئي. تضمن الجزء الأول التوزيع الاجتماعي والديموغرافي من ناحيه الجنس ، ومستوى الإصابة ، والعمر ، ومدة الإصابة ، وكتلة الجسم. تضمن الجزء الثاني تقدير المعلمات الفسلجية (HBA1C، FBG ، الأنسولين ، مقاومة الأنسولين ، حساسية الأنسولين ، CRP، D-dimer، Ferritin) والجزء الثالث تناول تأثيرات بعض الجينات (ACE2، AT1R، rs657152) بواسطة الـ PCR.

أظهرت نتائج هذه الدراسة بخصوص الجنس أظهر فروق معنوية ($p\leq0.01$) بين مجموعتي الذكور والإناث ، حيث كان الذكور اكثر معدلات اصابة في المجموعة CDM2 (46.67 %) بينما في المجموعة C كانت (43.3%) اما الاناث (56.7٪) في المجموعة C وكانت (53.33 %) في المجموعة CDM2. تم تصنيف مستوى الإصابة إلى ثلاثة مستويات: خفيف ، متوسط ، وشديد. كانت النسبة المئوية لمستوى الإصابة في CDM2 (48.30٪) أعلى من المجموعة C (26.66٪) ، اما شدة الاصابة فقد كانت مرتفعه في المجموعة CDM2 (38.70٪) مما كان عليه من C (30%) و كان المستوى البسيط منخفضا الاصابة في CDM2 (12.9٪) منه في المجموعة C (43.33٪) ، وزادت شدة الإصابة في CDM2 عنها في المجموعة C.

أضهرت للفئات العمرية فروقا للفئات الثلاث المعتمدة في الدراسة الحالية (> 30 ، 30-50 ، <50 سنة). كانت المجموعة الأقل من 30 عامًا أكثر في C (36.7٪) منها في فئة CDM2 (6.5٪) ، بينما كانت النتائج متقاربه في 30-50 في المجموعتين C و CDM2 (26.7٪ ، 22.6٪). و لوحظ ارتفاع النسبة المئوية في الفئات اكثر من 50 (71٪) CDM2 من مجموعة C (36.7٪). اما فيما يتعلق في الفئات الثلاث لمؤشر كتلة الجسم (BMI) (طبيعي ، الوزن الزائد ، والسمنة) ، فلم تكن هناك فروق ذات دلالة إحصائية بين هذه المجموعات.

أما لفترة الإصابة ، فقد تم تصنيف الفترات إلى 3 مجموعات فرعية (<7 ، 7-14> ، 14 يوما). فأن نسبه 7 أيام في المجموعة (73.3٪) C بينما كانت (51.61٪) في CDM2 ، ونسبه 7-14 يومًا في المجموعة (13.33٪) C وكانت (32.25٪) في المجموعة CDM2 ، وأخيرا كانت اكثر بالنسبه الـ 14 يوما (13.33٪) في مجموعة CDM2 و(16.12٪) في المجموعة C. كانت الفروق بين المجموعات معنوية بقيمة ($P\leq 0.05$).

أظهرت نتائج الدراسة الفسلجية وجود فروق معنوية بين الأشخاص الأصحاء ومرضى كوفيد بالإضافة إلى مرضى كوفيد + داء السكري في الـ ferritin (583.10±44.6a 322.28±30.1b 108.5 ±18.4c)على التوالي عند ($p. \leq 0.01$) ، أظهرت فروقً معنوية في FBS ، HbA1C و INR فقط في مجموعة covid-19 + السكري (253.09±16.2a 9.15±2.01a 17.51±4.1a) على التوالي عند ($p\leq 0.01$) ($p\leq 0.05$) وفي الوقت نفسه لم يكن هناك فرق بين مجموعه الاصحاء ومجموعه كوفيد-19. اما D-dimer , CRP قيمًا عالية في مجموعة covid-19 + مرضى السكري (61.18±5.3a , 1282.97±221.3a)عند ($p\leq 0.01$) على التوالي. واخيرا لم تظهر فروق معنويه في IN, INS

بخصوص الدراسة الجزيئية ، تم اكتشاف تعدد أشكال النوكليوتيدات المفردة (SNPs) لجين ACE2 باستخدام تقنية PCR في المرضى مقارنة مع اشخاص الاصحاء فقد أظهرالتركيب الوراثي (DD؛ ID) ذات نسب عاليه في مرضى covid-19 و covid-19+ مرضى السكري ذات فروقا معنويه عند (0.04) ، بينما لم تظهر I و II أي علاقة معنويه و كان النمط الجيني ACE D سائدًا في ثلاث مجموعات: (72.6٪ ، 85٪ ، 54٪) في مجموعة الاصحاء ، ومجموعة COVID-19 ، ومجموعة COVID-19 + السكري على التوالي ، تليها الأليلات (I ,DD,ID). اما في AT1R وجود فروقا معنويه بين الاصحاء و المرضى باستخدام تقنيات تفاعل البوليميراز المتسلسل PCR-SSCP ، حيث وجد أن توزيع تعدد أشكال الحمض النووي كان (96.77٪ ، 3.23٪) في الاصحاء و (6.67٪ ، 93.33٪) في مجموعة covid-19 و (12.9٪ ، 87.1) covid-19+ مرضى السكري ، أظهرت النتائج عدم وجود ارتباط بين تعدد أشكال الحمض النووي وفقًا لعدد الباندات مع المرضى مقارنة بالاصحاء. وفي الوقت نفسه ، كانت هناك اختلافات

كبيرة في تعدد الأشكال AT1R covid-19 و Covid -19 +مرضى السكري و الأصحاء عند ($P \leq 0.01$). اما بالنسبة لـ rs567152 SNP فلم يضهر اي فرق معنوي في التركيب الجيني بين الاشخاص الاصحاء والمرضى covid-19 و مرضى covid-19 + مرضى السكري

أخيرًا ، لوحظ أن مرضى السكري الذين أصيبوا بـ Covid-19 يبدو أن لديهم مستويات أعلى بشكل ملحوظ من (CRP و D-dimer و Ferritin) والمعايير المتعلقة بمرض السكري مقارنة بالمرضى الذين عانوا من Covid-19 وحده ، وكذلك مستويات عاليه من جين ACE2 ، مستقبلات AT1R على هذا النحو تجعلهم أكثر عرضة للإصابة بالمرض والوفاة.

جمهورية العراق
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دراسه تعدد الأشكال الجيني في الانسان المصاحب للأصابة بكوفيد-19 في مرضى السكري النوع 2

رسالة

مقدمة الى مجلس كلية العلوم/جامعة بابل

كجزء من متطلبات نيل درجة الماجستير في العلوم/علوم الحياة

من قبل

مريم علي حسين علوان

بكالوريوس علوم حياة

جامعة بابل 2016

بإشراف

أ. د. علي حمود السعدي

1444هـ 2022م