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Department of Biology

Gene Polymorphisms and Levels of some Immunological Markers in Patients with COVID-19 Pneumonia Associated with Adenovirus as Coinfection

Dissertation

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بسم الله الرحمن الرحيم

﴿ وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي

وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا ﴾

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Dedication

*To **my father's** soul who gave me passion, tenderness and overflowed me with care and love ... God bless him, and to the one who carries the true meaning of love, my dear **mother**.*

*To my lovely wife **Sahar** for constantly being on my side, and for all his support, care and love, to my lovely daughter and son (**Zahraa & Mohammed**).*

*To All **my wonderful brothers** and **sisters**, and to all of **my friends** who supported me.*

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Summary

Coronavirus 2019 is distinguished by the occurrence of a cytokines storm and excessive immune response through high secretion of cytokines and chemokines that there exists a deficit in the regulation of immune-associated tissue destruction when compared with the wound recovery as well as tissue restoration process that considered one of the most prominent methods used in virus, which determines the intensity of the illness.

One hundred fifty patients were diagnosed with infection related to the viral strain SARS-CoV-2 participated in this study, as their infection with the virus was confirmed through Real Time-Polymerase Chain Reaction (RT-PCR) of specific nasopharyngeal swab samples along with the rapid antigen, and the participants received health care in Imam Hussein Medical City from March until July 2022.

The average age was observed to be (44.04, 62.16 and 61.14 years) for three patients groups (Mild, Moderate and Severe) and the age range was between 20 to 79 years to control and mild groups, in comparison, 20 -89 years for moderate and severe groups. and the numbers of participants included 23 men and 27 women for control group, with 36 men and 14 women for mild group. Nonetheless, there were 31 men and 19 women within the moderate as well as severe groups . the preliminary results of this study imply that men participants of elderly status have had a greater probability of risk.

There were certainly exists a association amongst particular factors and the onset of symptoms brought on by COVID-19 (For human Spike protein COVID-19, Correlation=0.591, p =<0.001; and Adenovirus viral load, Correlation=0.457, p = <0.001; as well as the COVID-19 IgM the Correlation=0.249, p =0.021; while COVID-19 IgG is Correlation=0.347, p = <0.001 in addition to Interleukin 6 receptor (IL-6R) Correlation=0.695, p = <0.001 and monocyte–macrophage chemotactic receptor2 (CCR2) Correlation=0.564, p =<0.001). But Angiotensin-converting enzyme 2 (ACE2) and

Summary

Coxsackievirus and adenovirus receptor (CAR) It has been proven that there truly exists an opposite relationship connecting certain factors with the apparition of symptoms tied to COVID-19. (Correlation of Angiotensin-converting enzyme 2 receptor equal to = - 0.34, and *p* =<0.001; Correlation Coxsackievirus and adenovirus receptor equal to = - 0.071, *p* =0.046 respectively). Each previous tests performed by enzyme-linked immunosorbent assay (ELISA) and enzyme-linked fluorescence assay (Vidas).

All COVID-19 patients groups and healthy control undergo experimental two SNPs for *IL-6R* gene and one SNP for *CCR2* gene polymorphisms (rs4845374, rs2228145 and rs1799864 respectively), *IL-6R* genotypes in all patients groups compared with healthy control for two SNPs (rs4845374 and rs2228145) significantly positive correlation (p=0.0097, Correlation= 0.160 and p=0.0001, Correlation= 0.125) respectively with raise COVID-19 symptoms. and *CCR2* genotypes in all patients groups compared with healthy control for SNP (rs1799864) had a significantly positive correlation (*p* =0.0001, Correlation= 0.564) with raised COVID-19 symptoms. All study SNPs performed by Sanger Sequencing and Allele specific primers.

Mutant **TA** genotype (rs4845374) SNP of *IL-6R* gene was more significantly frequent in mild, moderate and severe groups when comparing the groups participating in the experiment to the control group, (*p*=0.0183, 0.0364 and 0.0243) respectively; therefore, it was considered a protective factor, it demonstrated an odds ratio of (0.3375, 0.3816 and 0.3472) respectively in three patients groups. While Genotype **AA** mutant type was more frequent significantly in the severe group in comparison with other groups, (p =0.0324); therefore, with an odds ratio of (0.0420), it was regarded as a protective factor for the severe group. Allele **A** was more significantly frequent in mild, moderate and severe groups compared with the control group, (p =0.0044, 0.0157 and 0.0003) respectively;

therefore, it was considered a protective factor, it demonstrated an odds ratio of (0.3162, 0.3720 and 0.2361) respectively in three groups.

Mutant **AC** genotype (rs2228145) SNP of *IL-6R* gene was more significantly frequent in mild, moderate and severe groups compared with the control group, (p =0.0001, 0.0103 and 0.0052) respectively; therefore, it was considered a protective factor, it demonstrated an odds ratio of (0.0952, 0.3333 and 0.2667) respectively in three groups. While Genotype **CC** mutant type was more significantly frequent in mild, moderate and severe groups compared with the control group, (p =0.0017, 0.0417 and 0.0024) respectively; therefore, with an odds ratio of (0.0086, 0.0462 and 0.0111) respectively in three groups, it was regarded as a protective factor for the severe group. Allele **C** was more significantly frequent in mild, moderate and the severe groups comparing with control group, (p =0.0001, 0.0024 and 0.0001) respectively; therefore, it was considered a protective factor, it demonstrated an odds ratio of (0.2045, 0.3750 and 0.2045) respectively in three groups.

Mutant **GA** genotype (rs1799864) SNP of *CCR2* gene was more frequent in moderate and the severe groups in compared with the control group (p = 0.0573, 0.0051) respectively; therefore, it was odds ratio of (2.5455, 5.4118) respectively, therefore, it was regarded considered a risk factor for those with moderate and severe conditions. While the opposite occurs in the mild group in comparison with control group. Genotype **AA** mutant type was absent in patients group in comparison with control group. Allele **A** was more frequent in patients with moderate and the severe group (p= 0.3096,0.1140) respectively; therefore, it had an odds ratio of (1.3448,1.5820) respectively, in comparison with group control, which was regarded as simply a risk factor for both patient groups.

In conclusion, all viral parameters studied and immunological markers (except sACE2 and CAR receptor) the amounts of spike protein concentration, COVID-19 IgG,

adenovirus viral load, IL-6R and CCR2 were raised in individuals suffered from COVID-19. and our concluded about gene polymorphisms that SNPs of *IL-6R* gene Considered as protective factors while Risk factors in SNP of *CCR2* gene. nevertheless, it is essential to keep in mind that not all alterations in biological processes could be exclusively referred to these levels being elevated.

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List of abbreviations

Abbreviation	Term
ACE2	Angiotensin-Converting Enzyme 2
Ad2	Adenovirus types 2
Ad5	Adenovirus types 5
ADAM17	A Disintegrin And Metalloproteinase 17
ANG-II	Angiotensin II
APCs	Antigen-Presenting Cells
ARDS	Acute Respiratory Distress Syndrome
ARMS	Amplified Refractory Mutation System
AS-PCR	Allele-Specific Polymerase Chain Reaction
AT2	Alveolar cells of Type II
B cells	B Lymphocyte cells
BMI	Body Mass Index
C	Cytosine

Abbreviation	Term
C3	Complement 3
C5	Complement 5
CAD	Coronary Artery Disease
CAP	Community Acquired Pneumonia
CAR	Coxsackievirus and Adenovirus Receptor
CCL2	Monocyte Chemoattractant Protein-1
CCL5	C-C Motif Chemokine Ligand 5
CCL9	C-C Motif Chemokine Ligand 9
CCR	Monocyte–Macrophage Chemotactic Receptor
CD147	Cluster of Differentiation 147
CD4	Cluster of Differentiation 4
CD8	Cytotoxic T cell
cDNA	complementary DNA
CF	Complement Fixation
CI	Confidence Interval
CLIA	Chemiluminescence Immunoassay
COPD	Chronic Obstructive Pulmonary Disease
COVID-19	Coronavirus-2019 disease
CPAP	Continuous Positive Airway Pressure
CPE	Cytopathic effect
CRS	Cytokine Release Syndrome
CSF	Cerebrospinal Fluid
CSG	Coronavirus Study Group
CT	Computerized Tomography

Abbreviation	Term
Ct values	Threshold cycles values
CVD	Cardiovascular disease
CXCL10	Ligand CXC-chemokine 10
CXCL3	C-X-C Motif Chemokine Ligand 3
CXCL8	C-X-C Motif Chemokine Ligand 8
CXCR	CXC chemokine receptor
DAMPs	Damage-Associated Molecular Patterns
DCM	Dilated Cardiomyopathy
DCs	Dendritic cells
DCs	Plasmacytoid DCs
df	Degree of Freedom
DM	Diabetes mellitus
E	Envelope (E) protein
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-Linked ImmunoSorbent Assay
ER	Endoplasmic reticulum
ERGIC	Endoplasmic reticulum Golgi apparatus intermediate compartment
ERK	Extracellular signal-regulated kinase
EtBr	Ethidium bromide
EVs	Enteroviruses
FCoV	Feline Coronavirus
Fcs	Crystallizable zone

Abbreviation	Term
FDA	Food and Drug Administration
GAGs	Glycosaminoglycans
G-CSF	Granulocyte colony-stimulating factor
GIT	Cardiovascular gastrointestinal tract
GM-CSF	Granulocyte-macrophage colony-stimulating factor
gp130	Glycoprotein 130
HAdV	Human adenovirus
HCoVs	Human coronaviruses
HDU	High Dependency Unit
HE	Hemagglutinin-esterase
HEK	Human embryonic kidney
HLA	Human leukocyte antigens
HR1	Heptad repeat regions 1
HR2	Heptad repeat regions 2
IBV	Infectious Bronchitis Virus
ICTV	International Committee on Taxonomy of Viruses
ICU	Intensive therapy unit
IF	Immunofluorescence
IFN-γ	Interferon gamma
IgA	Immunoglobulin alpha
IgG	Immunoglobulin Gamma
IgM	Immunoglobulin Mu
IL-1	Interlukin 1
IL-10	Interlukin 10

Abbreviation	Term
IL-2	Interlukin 2
IL-6	Interlukin 6
IL-6R	Interleukin 6 receptor
IP-10	Interferon γ-induced protein
IPA	Ingenuity Pathway Analysis
IRF	Interferon-regulatory factor
IRFs	Interferon regulatory factors
JAK/STAT	Janus kinase-signal transducer and activator of transcription
LFI	Lateral flow immunoassay
LPS	Lipopolysaccharides
LRT	Lower respiratory tract
M	Membrane (M) protein
MACs	Membrane attack complex
MCP-1	Monocyte Chemoattractant Protein-1
MERS-CoV	Middle East Respiratory Syndrome
MHC	Major histocompatibility complex
MHV	Mouse hepatitis virus
MMP	Matrix metalloproteinase
N	Nucleocapsid
n	Number of cases
NCBI	National Center for Biotechnology Information
NF-B	Nuclear factor-kappa B
NK	Natural killer cells

Abbreviation	Term
NP	Nasopharyngeal
NRP1	Neuropilin protein
OR	Odds ratio
ORF1	Open reading frames in gene 1
P	P value
PAMPs	Pathogen related molecular patterns
PI3K	Phosphatidylinositol 3-kinase
PRRs	Pattern recognition receptors
R	Correlation
RA	Rheumatoid arthritis
RAAS	Renin-angiotensin-aldosterone system
RAS	Renin angiotensin system
RBD	Receptor-binding domains
RdRp	RNA dependent RNA polymerase
RIA	Radioimmunoassay
Rs	Reference SNP
RSV	Respiratory syncytial virus
RTC	Reverse transcriptase proteins
RT-PCR	Reverse transcription polymerase chain reaction
S*	Significant
S	Spike protein
SABC	HRP-Streptavidin Conjugate
SARS-CoV	Coronavirus Severe acute respiratory syndrome
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2

Abbreviation	Term
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
sIL-6R	Soluble interleukin 6 receptor
SIRS	Systemic Inflammatory Response Syndrome
SNP	Single-nucleotide polymorphism
SPSS	Statistical package for social sciences
ssRNA	Single stranded RNA
T	Thymine
T cells	T lymphocyte
TACE	TNF-α converting enzyme
TAE	Tris acetate EDTA
TB	Tuberculosis
TBE	Tris borate EDTA
TEM	Transmission electron microscopy
TFH	Follicular helper T
TGFβ	Transforming growth factor beta
Th17	T helper 17 cell
TJ	Tight junctions
TLRs	Toll-like receptors
TMB	Tetramethylbenzidine
TMPRSS2	Transmembrane protease serine 2
TNF-α	Tumors necrosis factor α
Treg	Regulatory T cells
URT	Upper respiratory tract

Abbreviation	Term
UTR	Untranslated region
UV	Ultraviolet
VCAM	Vascular cell adhesion molecule
WBCs	White blood cells
WHO	World Health Organization
$\chi 2$	Chi-squared

CHAPTER ONE

Introduction

1.1 Introduction

Pulmonary infection can be caused via coronaviruses infection, which is classified inside the family Coronaviridae, viral infections among individuals tend to appear in the forms of silent symptomatic (Asymptomatic) or presenting with symptoms, particularly the latter featuring problems with breathing, a raised body temperature, cough, in addition digestive problems. In cases of extreme severity, bronchitis can arise, ultimately resulting in fatality for the person who has been infected (Sharma *et al.,* 2021).

Major ways to transmission from person to person tend to involve touching one another. The most prevalent mechanisms for communication involve the evaporation of droplets through wheezing, coughing and sneezing that subsequently come to touching the mucous membranes that line the nasal cavities, the mouth, as well as eyes, The release of viruses is noticed to take place within the airways, salivary glands, urination and stool, consequently contributing to the dissemination of the infectious agent via other routes. Participants suffering from serious SARS-CoV-2 infection experience more powerful along to more prolonged virus responsibilities that can be related with cytokine storm (Umakanthan *et al.*,2020).

SARS-CoV-2 virus with SARS-CoV exhibit a pattern of sequence similarity of 79.5%, indicating an important relationship among both of these viruses, the two viruses utilize angiotensin convert enzyme 2 as the receptor of the virus facilitates its entrance into its target cells, consequently, ACE2 emerges as an essential target for the development of therapies towards viruses (Bao *et al.*,2021), Raised concentrations of interleukin-6 have been detected in individuals suffering from SARS-CoV-2, but these raised amounts have been linked to adverse clinical consequences. The involvement of ACE2 as well as immune-related regulators has paramount importance throughout the infection and development of viral infection (Aziz *et al.*,2020).

Participants have raised levels of ACE2 in their lung tissue exhibit enhanced triggering of humoral immune responses that become more susceptible to the development of a cytokine-induced inflammatory storm, primarily characterized by an overabundance of IL6. Furthermore, these patients have a considerable decline in cell-mediated immunity (Bao *et al.*,2021).

The concept of "cytokine storm" denotes an overactive immune system provoked via an external motivating factor, the etiologic process for this cytokine outbreak was intricate, which includes an accelerated development of the disease as well as an extensive rate of fatalities. Based on the current evidence at hand, it can be noticed that persons who suffer from this viral infection could be experiencing an overabundance of cytokines that are pro-inflammatory, usually referred to as the "cytokine storm." This occurrence is characterized via a rise in the amount of several crucial cytokines, such as interleukin-1a, interleukin-2, interleukin-6, TNF-α, IFN-γ, IP-10, GM-CSF, MCP-1, and IL-10. Particularly, certain cytokines within this group have been found to exhibit an association with the seriousness of the viral infection, the term "cytokine storm" describes to a circumstance known as hyperinflammation which takes place as an outcome of an overabundant release of mediators via a persons immune system which is not properly controlled. This medical condition (SARS-CoV-2 infections) can be amplified by the dysfunction of various organs as well as coagulation diseases, and at the most extreme instances, it may outcome in fatalities (Ye *et al.*,2020; Zanza *et al.*, 2022).

The causal relationships between SARS-CoV-2 infections, the viral load with humoral reaction as well as degree of severity of viral infection were noticed by way of empirical investigations, the prognostic capacity of this viral load, as assessed within the nasopharynx, remains ambiguous , although several research indicate that there is an association that exists between viral load and the degree of severity of

disease, The viral load of this virus within the circulation, which is termed viremia, has been linked to the degree of severity of an illness as well as its probability of fatality, it may be utilized as an indicator of risk for the outcome of the illness among patients who have been hospitalized to healthcare facilities due to COVID-19 disease. In addition, the accumulation of SARS-CoV-2 through the circulation of blood has been linked to a raised level of proteome markers that suggest unusual causes inflammation response as well as injury within the airways and various tissues in the body, along with the beginning of fibrosis of the tissues and phases of healing, A vital variable that influences a contagious agent's clearance from the human tissues is its humoral reaction towards invasion viral infection (Bauer *et al.*,2023).

The primary clinical presentations of infections caused by SARS-CoV-2 encompass bronchitis accompanied by damage to the lungs characterized by edema of the lungs and widespread pulmonary injury, eventually which results in the emergence of a sickness referred to as an acute respiratory distress syndrome (ARDS) condition, hypoxia as well as potentially fatal hypoxemia (Xu *et al.*,2020a). Additional problems that affect various organs in the body include coagulation diseases, neurological disorders, with digestive and renal disorders, The mentioned issues can frequently be triggered because of the condition known as the syndrome of cytokines and chemokines being released and unregulated generalized inflammation, The pathological mechanism of this virus encompasses the reaction of the immune system as well as its underlying factors, having a particular emphasis on inflammation-related cytokines and chemokines (Wu *et al.*,2020a).

In inflammation locations, many different kinds of cells can trigger the creation of CC chemokine receptor 2 (CCR2), however it is mainly produced via particular cell types likely monocytes, also dendritic cell types (DCs), macrophages and

Natural killer (NK) cells as well, T lymphocytes (Fantuzzi *et al.*,2019). Regarding the crucial role of the CCL2/CCR2 pathway for encouraging monocytes infiltrate through the respiratory region along with eventual tissue harm, it appears that targeting the CCL2/CCR2 pathway holds promise as an expected medical therapy for controlling as well as immunomodulating individuals afflicted (Rahmati and Moosavi,2020).

In addition to CCR2 expression by COVID-19 disease also can be creation of IL-6 encompassing the interaction of cytokines together attached to the membrane IL-6R along with gp130 that co-receptor, which is recognized as possessing a role in the regulation of tissue homeostasis alongside repair mechanisms, nevertheless, it has been perceived that certain non-immune cell, especially stromal with epithelial cells, are able to elicit substantial reactions of inflammation through a process known as trans-signaling, whereupon soluble IL-6R/IL-6 binds to membranes that are gp130. This participation effectively triggers reactions of inflammation (McGonagle *et al.*,2020).

It is well known that host genetic polymorphisms play a key role in the susceptibility or resistance to different viral infections, taking into account the main role of host genes in the entry and replication of SARS-CoV-2 in cells and in mounting the immune response, it seems that a combination of multiple genes might be involved in COVID-19 pathogenesis, accordingly, to date, numerous studies have been conducted on the association between genetic polymorphisms and COVID-19. Some studies have indicated that polymorphisms in genes related to innate and adaptive immune response [toll-like receptors (TLRs), human leukocyte antigen (HLA) and cytokines/chemokines] and in genes involved in viral binding and entry into host cells (angiotensin converting enzyme-2) are associated with COVID-19 development and/or severity (Dieter *et al.*,2022).

In an investigation comparing 1217 sequential airway specimens obtained from individuals with SARS-CoV-2 infection, the authors reached their decision that there was a possibility to have higher co-infection levels between this infection along with other airborne pathogens (Tadolini *et al.,*2020). The preceding investigation reported that a subset of patients admitted to the hospital for the reason of SARS-CoV-2 exhibited coinfection with an additional pulmonary virus, among these concurrent infections, Respiratory Syncytial Virus (RSV) as well as the influenza virus A were identified as the predominant viral infections, while adenovirus concurrent infections was less frequently reported (Lansbury *et al.,*2020).

1.2 The Aims of the study:

The aim of the current study was to evaluate some molecular and immunological parameters of COVID-19 patients with their correlation with the development of symptoms of COVID 19 infection. These aims were determined through the following objectives

1. Confirming COVID-19 infection by used RT-PCR and rapid strip antigen test.
2. Determining serum levels of human COVID-19 Spike protein, Adenovirus viral load, COVID-19 Antibody IgM, IgG, IL-6R, CCR2, ACE2 and CAR receptors in COVID-19 patients and control groups by ELISA and Vidas device.
3. Evaluating the possible correlation between the parameters in patients with different COVID-19 symptoms.
4. Detecting the polymorphisms of *IL-6R* gene and *CCR2* gene correlations with different COVID-19 symptoms by Sanger Sequencing and PCR technique (ARMS) in patients and control.

CHAPTER Two

Literature Review

2.1 Coronavirus Historical Overview

In 1960, there was the first appearance of human coronaviruses (HCoVs), during which two types of viruses were from people isolated with cold: HCoV-OC43 and type HCoV-229E. Since then, until now, seven types have appeared that have been discovered, as three of them are characterized by their severe pathogens, and perhaps the most prominent types are the Coronavirus that causes Middle East Respiratory Syndrome (MERS-CoV), Coronavirus Severe Acute Respiratory Syndrome (SARS-CoV) and Coronavirus Severe acute respiratory syndrome 2 (SARS-CoV-2) (Hu *et al.*,2015; McIntosh *et al.*,1967).

Where coronaviruses caused global devastation in 2002 due to SARS-CoV, that causes acute respiratory syndrome, which is considered one of the highly pathogenic diseases in the world, and the well-known belief that SARS-CoV is virus that infects animals and have inherited capacity to pass the species wall and transmit to infect humans, in 2012 in Saudi Arabia, another outbreak of a respiratory syndrome alike to the outbreak the SARS-CoV virus, called MERS-CoV, appeared, which infected 2,494 people and caused 858 deaths (Yang *et al.*,2020b ; Wang N *et al.*,2013).

In late year 2019 a new strain of the Coronavirus appeared, and it first appeared in the Wuhan Chinese city, where the virus spread to all parts of the world, Although some local studies found that the source of infection came from a market selling fish and wild animals, this confirms the hypothesis of transmission virus from animal-to-human, as well as the potential for virus SARS-CoV-2 transference from the someone to another, In either direction during direct contact or by droplets via the surroundings air or touching surfaces contaminated with the virus. During February 2020, the World Health Organization (WHO) launched a to epidemic illness brought on by 2019-nCoV new name: Coronavirus Disease (COVID-19, (Then the International

Committee reclassified viruses with another name known as (SARS-CoV-2) associated with severe acute respiratory syndrome (Cheng *et al.*,2020; Delahay *et al.*,2021; Gorbalenya *et al.*,2020).

However, the evidence that indicates the cause of the spread of SARS-CoV-2 rapidly is the result of the possibility of its transmission between humans without distinct symptoms, which makes it an epidemic of spread, and because travel and movement between countries and continents, it has become a simple matter, increase to the efficient transmission of SARS-CoV-2 virus, with increasing the risk spreading of the virus around the world. In 2020, the World Health Organization declared the outbreak of 2019 coronavirus disease, declaring an international emergency as the World Health Organization had previously declared an emergency due to: H1N1 (2009), Ebola in West Africa (2014), polio (2014) and Zika (2016) and Ebola in the Democratic Republic of the Congo (2019) (Syangtan *et al.*,2021; Kimball *et al.*,2020; Wilder *et al.*,2020).

2.2 Transmission of SARS-CoV-2

SARS-CoV-2 virus transmission were reported between people within the same geographical area, then the matter became more severe after international viral spread that is happening rapidly and the occurrence of an epidemic, scientists were doing their best to determine the reservoir of the virus or the carrier host, which could be the one that caused the virus transfer from animals to human beings, and based on the diagnostic cases of people infected with the virus, it was found that they were present in the Wuhan seafood market. It is the origin of the SARS-CoV-2 virus. So that can be divided transmission of SARS-CoV-2 in to: Aerosols and droplets transmission (Li *et al.*, 2020a; Guo *et al.*, 2020a) and Transmission by Fomite with

Other Body Fluids or person to person (Agrahari *et al.*, 2021; He *et al.*, 2020b; Pan *et al.*, 2020).

2.3 Epidemiology of SARS-CoV-2

In early December 2019, a group of health workers in Hubei Province, China, in Wuhan, near the seafood market, reported severe pneumonia-like symptoms of unknown cause (She *et al.*,2020). Initially, it was called the contagious, 2019 Coronavirus (2019-nCoV), thus the name of the pathogen was later reformulated as SARS-CoV-2 by the Coronavirus Study Group (CSG) of the International Committee on Taxonomy of Viruses (ICTV) (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses,2020), In February 2020, the illness resulting from the SARS-CoV-2 virus has been officially designated as COVID-19. In the beginning of 2020, the World Health Organization (WHO) declared the SARS-CoV-2 outbreak as its most recent global health emergency, following previous emergencies such as H1N1 through the year 2009, polio within 2014, the Ebola virus in West Africa in 2014, which was Zika in 2016, and the Ebola virus in the Congo's Democratic Republic of the Congo in 2019 (Chen *et al.*, 2020). At the time of the 2019-nCoV-pandemic, the pathogenicity of 2019-nCoV-nCoV was not fully known, but it is life-threatening and has caused many deaths through its ability to adapt to the host and cause severe infections, both in humans and other organisms that commonly cause systemic diseases. Digestive or respiratory, because the two systems have nearly the same ACE-2 receptor (Li *et al.*, 2020b)

In addition to the transmission of the Coronavirus from animals to humans, what caused its spread around the world made The declaration of an international crisis by the World Health Organization is in response to the worldwide epidemic of the SARS-CoV-2 virus among people through the possibility of transmission from one

person to another, which caused it to spread very widely among Different countries of the world in all continents, Asia, Europe, America, and some Africa, The severity of infection with the virus varies depending on several factors, such as comorbidities, age, Sex, living conditions, and the environment (Khalili *et al.,* 2020 ; Mantovani *et al.*, 2020).

2.4 Pathogenesis of SARS-CoV-2

The adhesion of the SARS-CoV-2 virus to the ACE2 receptor is observed primarily in the epithelial cell membranes of the cavities of the nose and mouth, the conjunctiva of a person's eye, and the inner ear channel, especially in alveolar cells of type II (AT2), despite the presence of this receptor in other sites of the human body such as Cardiac muscle cells, epithelial cells, ileum, oral and esophageal cells, as well as urothelial cells in the bladder and cells of the proximal tubules of the kidneys (Zou *et al.,* 2020), ACE2/angiotensin system disruption explains specific clinical aspects of SARS-CoV-2 for instance, hypokalemia and vasoconstriction and the development of acute respiratory distress syndrome (Gheblawi *et al.*,2020). It is noteworthy which the quantity of ACE2 receptor expression in the lung, which is the most important target of the virus, and is considerably lower in the pancreas, cardiovascular gastrointestinal tract, and genitourinary systems (Zou *et al.*, 2020).

Furthermore, ACE2 plays a crucial role not only in viral infection but also in RAAS by regulating the levels of angiotensin II (ANG-II), The primary source of cardiac complications in COVID-19 appears to be the disruption of the ACE2 receptor mechanism (Yalcin *et al*.,2021).

Additionally, a spike protein of COVID-19 is composed of two distinct regions, namely an S1-containing area and a receptor-binding domain (RBD), the RBD

specifically interacts with the angiotensin-converting enzyme 2 (ACE2) receptor, While the S2 region plays a role in the integration of membranes and viral entry, this area is responsible for mediating the fusion of the viral to the cellular membrane (Hoffmann *et al.*, 2020). The process about the fusion of membranes with cells in the host and pathogenic virus particles is initiated via the cells host proteases, namely transmembrane protease serine 2 and B cathepsin (Walls *et al.*,2020).

The process of infection occurs in two ways, the delayed path mediated by the endosomal cell and the path mediated by the cell surface, where TMPRSS2 cleaves the viral S protein and thus facilitates the process of virus fusion with the cellular membrane and the integration of nucleic acid within the recipient cell (Jackson *et al.*,2022). Following the internalization of the virus through ACE2 receptor, proteases enzyme like cathepsin are activated, leading to structural modifications in the spike, these modifications are causes the decrease in endosomal pH (Yang *et al.*, 2020a).

Furthermore, it is important to take note of the ACE2 receptor as one of the most important receptors for virus attachment and integration into the cell, therefore, the human body contains many other molecules that represent alternative receptors for SARS-CoV-2, including L-SIGN, C-type lectins, and DC-SIGN (Amraei *et al.*, 2021), The role of lectins appears important by distinguishing a wide range of pathogens and ensuring their adhesion between cells, and by distinguishing glycans on the surface of the virion, this contributes to facilitating adhesion and viral entry into the target cell (Wang *et al.*, 2021) . It has been found that cluster of differentiation 147, is the attaching receptor that recognizes the viral protein S as well plays a crucial role in the entry of the virus. This type of receptor was recently linked to the spread of the SARS-CoV-2 infection that causes severe acute respiratory syndrome (Chu *et al.*, 2018).

Moreover, it has been discovered that CD147 is responsible for the synthesis of various pro-inflammatory cytokines, such as interferon-gamma (INF-), interleukin-6 (IL-6), monocyte chemoattractant protein (MCP-1), and tumor necrosis factor (TNF), also contributes to inflammation (Zhai *et al.*, 2016). In addition to facilitating virus entry and causing a cytokine storm, CD147 has a novel role that contributes to fibroblast activation in COVID-19 pulmonary fibrosis (Wu *et al.*, 2022). The initial stage of viral penetration into cells of interest involves the interaction between the S protein and ACE2. This process is facilitated by the proteolytic breakdown and subsequent fusion of the membranes of the cell and the virus, there is speculation the fact that SARS-CoV-2 could trigger damage to lung tissue (Kuba *et al.*, 2005). One aspect to consider is that upon infecting cells in the lungs, SARS-CoV-2 has been observed to interact through the S1 to ACE2 transmembrane domains, leading to an observed decrease in ACE2 levels (Beyerstedt *et al.*, 2021). The synthesis of Ang II is significantly or comparatively increased, leading to the infiltration of macrophages and subsequent elevation of cytokines and endothelial attachment molecules such as IL-6, MCP1, VCAM-1, selectin E, and various other factors (Li *et al.*, 2018).

Furthermore, the decrease in ACE2 receptor expression leads to a reduction of the defensive mechanisms against acute pulmonary injury (Imai *et al.*, 2005), Nevertheless, the role of unbound soluble angiotensin-converting enzyme 2 (sACE2) in the process of inflammatory processes and tissue damage may be significant (Haga *et al.*, 2008). Furthermore, the recognition of intracellular signaling by the host cells very significant furin proteases and TMPRSS2 in the S proteins splitting at the S1 / and S2 junctions to help ACE2 linking as well as inside of S2 to trigger the fusion of membranes can be further facilitated by the viruses attaching to ACE2. SARS-CoV-2 additionally adopted an alternative mechanism for infiltrating cells in the host, involving the process of endocytosis followed by fusion mediated by the S protein

between the endosome and the virus, the spike protein of the pathogenic virus plays a crucial role in facilitating invasion by controlling the fusion of membranes between the cells in the host, thereby enabling direct transmission of the infection (Papa *et al.,* 2021; Walls *et al.,* 2020).

The global pandemic that caused by the SARS-CoV-2 has led to significant morbidity and mortality on an international level. In the context of identifying SARS-CoV-2 infection at an early stage, host cells employ various pattern recognition receptors (PRRs), the swiftly release of interferon has been shown to be highly efficacious in combating infection with SARS-CoV-2, over time, the virus has developed various strategies to impede the timely release of interferon and undermine the defenses of cells by disrupting multiple stages within interferon-associated signaling pathway, Consequently, certain individuals suffering with COVID-19 exhibited a considerably greater risk to infection with SARS-CoV-2, while others showed either moderate or no symptoms , according to one hypothesis, such variation could be explained by functional changes in innate immune integrity (Lee *et al.,* 2014a ; Liu *et al.,* 2022).

The functions of dendritic cells (DC) encompass the regulation of inflammatory reactions, facilitating the development of tolerance, the infiltration of immune system cells, and the production of antiviral inflammatory mediators, Dendritic cells (DCs) have the potential to play a role in the bodies defenses against COVID-19 (Xiong *et al.,* 2020). This includes facilitating the spread of SARS-CoV-2 to the lymph nodes, as well as contributing to the development of impaired interferon responses and immune responses by T cells in those with the condition. (Alamri *et al.,* 2021).

Dendritic cells play a crucial role in triggering and modulation of the adaptive immune systems response. These cells have a significant impact on the activation of

the body defenses via its IL-17 pathway, primarily by promoting the activation of CD4+ Th17 cell populations by encouraging the monocytes as well as neutrophils (Hunter and Jones, 2015). Plasmacytoid dendritic cells (pDCs) are present in various lung tissues, such as the air passages, parenchyma, as well as the alveolar septa, these are known to generate type I interferon as an immune response to infection by viruses (Li *et al.,* 2019).

Type I interferon generation is critical to the suppression of infection with SARS-CoV-2, as evidenced by the more severe COVID-19 infection in individuals exhibiting auto-antibodies toward IFN or genetic defects of the type I interferon reaction, plasmacytoid dendritic cells represent a unique subset of immune cell types that possess specific abilities in the recognition and controlling of infectious viruses by the strong secretion of type I interferons (IFNs), this illustrates the important role of pDCs in determining the severity of COVID-19 disease (Cervantes-Barragan *et al.*, 2021). Immature DCs first locate and capture the antigen, developing into mature DCs that process and deliver the antigen to class I and class II MHC molecules, activating and differentiating naive T cells into cytotoxic T lymphocytes (CD8+T lymphocytes) and helper T lymphocytes (CD4+T lymphocytes), and ultimately maturing B cells in the lymph node (Yadava and Marsland, 2013).

Mature dendritic cells regulate the expression of CD80, CD86, and CCR7 (C-C Motif Chemokine Receptor 7) for functional properties, thus, in addition to their high ability to stimulate T lymphocyte responses (Costela-Ruiz *et al.*, 2020).

Additionally, dendritic cells (DCs) play a significant role in the production IL-6 , This cytokine promotes the triggering of an alternative macrophage pathway, and potentially resulting in the development of acute fibrosis of the lungs (Page *et al.*, 2012). Research upon plasmacytoid dendritic cells (pDCs) in individuals infected via

SARS-CoV-2 has demonstrated their capacity to generate type I interferons (IFN I), which play a crucial role in inhibiting the replication of the virus (Cervantes-Barragan *et al.*,2007).

Elevated mature DCs in bronchoalveolar lavage may signal the presence of these powerful cells in the lungs and could indicate their response to SARS-CoV-2 infection. DCs, which have moderate expressions of ACE2, are more effector than lymphocytes and less than endothelial/epithelial cells and broadly produce the pro-inflammatory cytokines TNF-α and IL-6. (Xiong *et al.*, 2020).

In addition to the foregoing, macrophage cells are among the main producers of inflammation associated with COVID-19 and excessive inflammation (Bost *et al.*,2020), and they have a high ability to develop a wide range of membrane receptors that act as sensors for microorganisms and mediating factors, it is soluble and has an important role in specific recognition, signaling, activation, and migration pathways (Guilliams *et al.*, 2018). Moreover, macrophages are recognized to be expert phagocytes when it comes to utilizing complement, Toll-like receptors, Fcs (crystalllizable zone), as well as capture receptors for consuming, effective elimination of pathogens and tissue debris from the body, the oscillation of macrophages is recognized in both cellular as well humoral immune response aspects of the immune system, encompassing both adaptive and innate immunity, and also in antimicrobial defenses and infections Furthermore, macrophages are known to be professional phagocytes in the use of complement, non-actin-like receptors, Toll-like receptors, Fcs (crystallizable zone), and scavenger receptors for ingestion and effective cleansing of the body from harmful agents and tissue debris, it is also known that the macrophage oscillates in the cellular and humoral; Innate and adaptive immunity, antimicrobial defenses, and infections (Gordon and Plüddemann , 2017). It is a double-edged sword, on the one hand, it participates in the mechanisms of

tissue repair and homeostasis, and on the other hand, it has the ability to increase tissue damage, by interaction with different cells as well as release enzymes, cytokines and chemokines, and the activation of complement cascades and plasma coagulation (Teuwen *et al.,* 2020). It was classified into two types: pro-inflammatory, denoted by M1, and anti-inflammatory, compensatory, denoted by M2 (Martinez and Gordon, 2014).

2.5. Factors associated with SARS-CoV-2 infection

SARS-CoV-2 can cause diverse clinical outcomes, and several studies indicated that many risk factors may increase the disease severity and its associated complications.

2.5.1 Demographic factors

The First of these factors is the patients age, as surveys showed that ages ≥ 65 years showed higher rates of mortalities (Wu *et al.,* 2020a). As the incidence in men was shown that it is more prevalent than among women. The observed phenomenon can be ascribed to disparities in hormone levels of steroids throughout men and women, which exert an impact on several facets of the immune system, furthermore, it should be noted that the X chromosome contains certain immunological gene regulators, like as TLR7, which have the ability to reduce levels of viruses and inflammatory in women (Conti and Younes, 2020). Obesity is recognized as a factor of danger, and individuals having an obese body mass index (BMI) have a worse prognosis due to the presence of persistent inflammatory which contributes to an enhanced storm of cytokines (Bhaskar *et al.,* 2020).

2.5.2 Pathological status

The presence of medical histories, like high blood pressure and a history of CVD, is associated with a greater probability of contracting COVID-19, this is because the illness has been found to cause damage to the heart muscle due to a lack of oxygen, and immediate harm to heart cells in addition to widespread inflammation throughout the body (Babapoor-Farrokhran *et al.*, 2020). Moreover, individuals diagnosed with diabetes mellitus, also known as DM, experience more severe consequences in comparison to those without diabetes, this is possibly related to an unbalance in the expression pathways of ACE2, leading to a response of inflammation that might potentially disrupt the functioning of pancreatic beta cells (Wang *et al.,* 2020a). Predisposing variables were found as persistent respiratory conditions due to the presence of lung epithelial damage in individuals, patients who have persistent obstructive pulmonary disease (COPD) commonly demonstrate elevated levels of ACE2 receptors (Henrot *et al.*, 2019). This eventually contributes to the spreading and invasion of the airway epithelium by SARS-CoV-2 (Hasanagic and Serdarevic, 2020). Conversely, in COVID-19 patients, insufficient levels may increase inflammatory reactions induced by interferon-γ (IFN-γ) as well as interleukin-6 (IL-6) (McCartney and Byrne, 2020).

2.5.3 Human genetic factors

Daily, physician provide care for individuals have contracted the COVID-19 virus present clinical quandaries. The capability to discern each persons vulnerability to SARS-CoV-2 serious complications according to variations in the owner defensive as well as inflammatory mechanisms, especially because of variation in genetics, could lead to considerable benefits in controlling the spread of infections. Multiple studies have been conducted to examine the inherited risk factors associated with

genetic diversity throughout there are three primary loci comprising the main histocompatibility complex (MHC) group I classification. The sets of genes producing the proteins known as human leukocyte antigenic (HLA) types A, B, and C have the potential for impact an individual's vulnerability to an individual's contraction of SARS-CoV-2 as well as consequent degree of severity the illness experienced (Dieter *et al.*,2022; Nguyen *et al.*,2020). Consequently, they have an increased susceptibility to the SARS-CoV-2 infections. The estimation of infection severity might be facilitated with the early identification of a persons HLA genes (Nguyen *et al.*,2020; Thierry, 2020). A recent investigation discovered genetic variations in a gene called angiotensin-converting enzyme within the host, and these plays an important part in viral entering to cell, these genetic differences could potentially indicate the worldwide epidemiology of the illness, as well as suggesting that there might be a connection between the geographic distribution of ACE2 modifications in addition the distribution of COVID-19, the existence of multiple alleles that make up ACE2 protein had a possibility to have an effect on the protein's affinity for the infectious agent alongside eventual cellular infiltration (Godri Pollitt *et al.,* 2020; Delanghe *et al.,* 2020). A number of multivariable-adjusted studies using COVID-19 patients revealed a correlation between demographic characteristics and increased disease severity (Az *et al.,* 2021)

1- Sex and age

2- Hypertension, Asthma, Allergies, Obesity and Diabetes

3- Factors that Cause Chronic Obstructive Pulmonary Disease and Raise the Severity of Interstitial Pulmonary Dysfunction.

4- Chronic Liver Conditions

5- Individuals additionally suffering from chronic kidney disease, also People with hematologic and cancerous conditions

6- Pregnancy
7- Viral Load
8- Genetic factors

2.6. Immune Responses to SARS-CoV-2 Infection

The initial site where infection occurs within the upper region of respiratory tract is the cells of the epithelium that line the nasal cavity by inhalation of coronavirus particles and early non-specific antigen limit viral replication and eliminate from Upper Respiratory Tract by Innate immune responses, simultaneously Innate immunity is recognized as a regulatory mechanism for following immune responses that are adaptive. These reactions encompass the production of antibodies by the plasma cells, which are derived from B lymphocytes generated in the bone marrow, as well as the activation of the effector T lymphocytes, which are found in local lymph node tissue. This coordinated immune reaction occurs a few days following infection and aids in the elimination of the infectious agent (Gómez-Carballa *et al.*, 2022; Ramasamy, 2021).

In addition to inducing the antiviral response in both infected and adjacent epithelium cells by impeding the production of proteins and damaging the messenger RNA, thus indirectly eliminating the virus in addition to inducing the active role of antigen presenting dendritic cells and production of interferon 1 and 3 that need to recognize and associate pathogen-associated molecular patterns (PAMPs) and cellular pattern recognition receptors (PRRs). The triggering of macrophages and also dendritic cells results in the synthesis of cytokines that including IL-1, IL-6, IL-12, which IL-18, and TNF. These cytokines play a crucial role in augmenting both local and systemic inflammatory reactions. Additionally, they stimulate natural killer cells, to destroy cells infected by viruses and improve the adaptive immune reaction. The

T helper cells, which identify damage-associated molecular patterns (DAMPs) in affected epithelium cells, respond by releasing IFN γ. This further promotes the proliferation of the natural killer cells, macrophages, and dendritic cells, and the adaptive response of the immune system (Ramasamy, 2021; Sette and Crotty, 2021).

Notably, SARS-CoV-2 triggers either immune response that are innate and adaptive. CD8+ T lymphocytes are responsible for the elimination of infectious cells, while CD4+ T lymphocytes play a crucial role in triggering the proliferation of B lymphocytes, leading to the production of antibodies specific to the virus such as immunoglobulin G and immunoglobulin M (Traggiai *et al.,* 2004). Furthermore, T helper cells produce a wide variety of the mediators, such as cytokines pro-inflammatory, that serve to support immunity and facilitate interactions with other types of immune cells. The secretion of antibodies together with complement components (C3a and C5a) by host immunity cells plays a crucial role in antiviral defenses (Lu *et al.*, 2011; Niu *et al.*, 2018).

2.6.1 Cellular Immune Responses to SARS-CoV-2 Infection

The virus has the ability to enter its target cells through two distinct pathways: an endocytic way that finishes in the endosomal region, in addition a membrane called the plasma membrane pathway located on the outermost layer of the cell. The integration between the viral and plasma membranes was an essential step for the two techniques, as it facilitated the passage of viral genetic material toward the cytoplasm (Shang *et al.*,2020). Similarly, SARS-CoV-2 infection causes the production of fusogenic S glycoprotein on the host cell membrane, which promotes cell-cell fusion by interacting with ACE2 on adjacent cells (Buchrieser *et al.*, 2020).

During the infection caused by SARS-CoV-2, host cells release several different kinds of inflammatory mediators. These molecules play a crucial role in encouraging

cellular inflammation in addition facilitating natural immune responses. this occurs when tissue-resident immune system cells detect the presence of the virus, leading to the initiation of a centralized innate response (Schultze and Aschenbrenner , 2021).

The complement pathway, which plays a role in bringing together both adaptive and innate immune responses, is also considered to be part of the immune system natural reaction for SARS-CoV-2 (Ricklin *et al.,* 2010). The activation of the complement pathway in individuals with COVID-19 can be linked to various physiological responses, including coagulation in the vascular system, malfunction of endothelial cells, with the presence of both chronic as well as acute inflammatory disorders (Jin *et al.,* 2020).

Moreover, T and B lymphocytes cooperate in combating infection by viruses, though with distinct functions. B cells are responsible for triggering the creation of antibodies, which possess the ability to directly recognize viral protein molecules. This recognition mechanism serves the purpose of impeding viral infection of specific target cells. T cell lymphocytes specifically recognize Major Histocompatibility Complex (MHC) classes I and II molecules solely when they are bound to viruses. T and B cells work together to fight viral infections, but they do not do the same thing. B cells produce antibodies that can directly identify viral proteins in order to prevent viruses from infecting the desired cells. T cells identify Major Histocompatibility Complex (MHC) classes I and II molecules only in association with viral proteins, not directly. Every nucleated cell in the body has a different amount of MHC-class I molecules on their surface, and these molecules transport viral proteins generated inside the cells. As a result, T lymphocytes (CD8 cytotoxic T cells) that detect MHC-class I molecules complexed with viral peptides target cells where viruses are reproducing specifically. CD8 T lymphocytes play a direct role in viral production inhibition and infection control by lysing virus-infected cells or

secreting antiviral cytokines (Hangartner *et al.*, 2006; McMahan *et al.,* 2021). A separate function is performed by CD4 helper T cells, which are T cells that identify viral proteins associated to MHC-class II. MHC-class II is expressed only by professional antigen-presenting cells, such as dendritic cells, monocytes, and macrophages, which are not infected yet present viral antigen ingested from the environment. T-helper cells (or CD4 T cells) release a number of cytokines (IL-2, IL-21, Interferons, and Tumor Necrosis Factor (TNF)-alpha) after recognizing viral antigen presented by expert antigen-presenting cells, which essentially aid in the creation and proliferation of CD8 T and B cells (McMahan *et al.,* 2021).

Follicular helper T (TFH) cells, a type of the helpers B cell with specific functions, along with the proinflammatory TH17 cell subset, and regulatory T (TReg) cells, which have crucial role in preventing overactive immune reaction and corresponding immunopathology (Lund *et al.,* 2008). If SARS-CoV-2 is abnormally effective, it can escape early innate immune responses, and consequently the occurrence of the disease (Sette and Crotty, 2021).

2.6.2 Humoral Immune Responses to SARS-CoV-2 Infection

When foreign pathogens invade the human body, the immune system initiates and induces a cascade of immune responses to clear the pathogens. Adaptive immunity, including humoral and T cell-mediated immunity, plays a critical role in the elimination of pathogens, including SARS-CoV-2. Cytotoxic lymphocytes (primarily cytotoxic CD8+ T cells) can eliminate infected cells, and specific antibodies against SARS-CoV-2 in the humoral immune response have the potential to neutralize this virus or even help cytotoxic T cells eliminate virus-infected cells to control disease progression (Dörner and Radbruch,2007; Liu *et al.,* 2017).

Under stimulation by SARS-CoV-2 antigens, B cells from germinal centers can proliferate and differentiate into plasma cells, producing and secreting specific antibodies to control viral replication, Virions may also directly modulate host-specific immunity by infecting immune cells expressing the viral receptor ACE2, such as pulmonary monocytes and macrophages. These antibodies can be present in the blood or produced de novo by memory B cells and plasma cells upon re-exposure to viral antigens. Thus, SARS-CoV-2-specific humoral immunity plays a critical role in antiviral defense by providing newly produced antibodies from activated plasma cells (Palm and Henry,2019).

Similar to other respiratory infections, SARS-CoV-2 infection stimulates rapid production of IgM, IgG and IgA antibodies, which are measurable in the sera as early as a week post-symptom onset, which are especially directed against nucleocapsid (N) and spike (S) proteins (Tan *et al.,* 2004). N is the virus nucleocapsid protein, is highly immunogenic and abundantly expressed in vivo after the virus infects human being. S is responsible for binding the virus to the human angiotensin converting enzyme 2 (ACE2) and its subsequent cellular uptake (Meyer *et al.,* 2014). The stability of IgG levels persisted over an extended duration, whereas a decline in IgM levels commenced at an earlier stage (Li *et al.,* 2008).

SARS-CoV infection induces seroconversion as early as day4 after onset of disease and was found in most patients by 14 days. Long lasting specific IgG and neutralizing Antibody are reported as long as 2 years after infection (Zhou *et al.,* 2020a). For MERS-CoV infection, seroconversion is seen at the second or third week of disease onset. For both types of coronavirus infections, delayed and weak Antibody response are associated with severe outcome (Liu *et al.,* 2017). In a preliminary study, one patient showed peak specific IgM at day after disease onset and the switching to IgG by week 2.25. Furthermore, all sera from patients were able to neutralize SARS-CoV-

2 in an in vitro plaque assay, suggesting a possible successful mounting of the humoral responses (Liu *et al.,* 2006).

Increased both IgM and IgG titers of antibodies which found in serum patients infected with severe illness, the available data suggests that an effective Antibody reaction is associated with increased severity of sickness (Tan *et al.,* 2020). Apart from antibodies that neutralize, that has protective and advantageous properties, the system has a large number of non-neutralizing antibodies, which aid in immune system cells and APCs that are becoming infected (Iwasaki and Yang ,2020). Additionally, a number of sources of evidence demonstrate the impact of severe respiratory disease in COVID-19 patients coincident with antiviral IgGs (Zhou *et al.,* 2020a).

2.6.3 Cytokines Production and Cytokines storm with SARS-CoV-2

Cytokines constitute a group of signaling molecules that are consist of peptides with play an essential function in modulating multiple biological functions through their interaction to receptors on the cell surface (Bartee and McFadden,2013). As a defense reaction to stress-generating endogenous events such as cancer or microbial infection (O'Neill, 2015; Vabret *et al.,* 2020). The essential function of the macrophages as being guardians part of the natural immune, facilitating the transformation coming from natural toward adaptive immune, is contingent upon an assortment of cytokines (Arango Duque and Descoteaux,2014), During COVID-19 infection can attack alveolar macrophages and epithelial cells in the alveoli which stimulates cells infected to release chemokines and cytokines (Fehr and Perlman,2015).

Other studies also reported that the COVID-19 severity is corresponding with an increased level of secreted chemokines and cytokines such as interleukin (IL)-2, IL-7, IL-10, tumor necrosis factor (TNF), granulocyte colony-stimulating factor (G-

CSF), protein monocyte chemoattractant 1 (MCP1; also known as CCL2), macrophage inflammatory protein 1 alpha (MIP1α; also known as CCL3), ligand CXC-chemokine 10 (CXCL10), C-reactive protein, ferritin, and D-dimers in blood when infected with SARS-CoV-2 (Liu *et al.,* 2020b ; Zhu *et al.,* 2020).

Multiple studies described that a weak and inappropriate response immunity to SARS-CoV-2 appears more frequently in the person infected by other diseases, therefore this may lead to the multiplication of the virus and the occurrence of complications that lead the patient towards severe cases of the disease by excessive recruitment of the human immunological response (Blanco-Melo *et al.,* 2020).

Although SARS-CoV-2 led to a pandemic of coronavirus disease in 2019, so the world became in a fierce confrontation against the virus through the development of new treatment methods (Huang *et al.,* 2020a). One of the most prominent methods used in confronting the virus, which determines the severity of the disease, is the excessive immune response through excessive secretion of the cytokines in addition chemokines, which referred to as "cytokine storm syndrome."(CSS) (Mehta *et al.,* 2020).

Also, cytokine production in patients with COVID-19 was found to be imbalanced with regard to controlling immune-mediated tissue damage versus wound healing and tissue repair response (Gu *et al.,*2019). Secondary hemophagocytic lymphohistiocytosis (macrophage activation syndrome), a hyperinflammatory syndrome characterized by the release of cytokines, cytopenia, and multi-organ failure, may develop in addition to high levels of cytokines (Tseng *et al.,*2005).

Although this response may occur across different clinical scenarios, like the syndrome of cytokine release noted among individuals participating in medical care (Frey and Porter, 2019), HLH tied to carcinomas, as well as autoimmune

disease (Karakike and Giamarellos-Bourboulis, 2019), it is also pertinent to highlight its relevance with regard to The term systemic The inflammation Responding Syndrome (SIRS) along with ARDS with regards to diseases that are infectious, they are all participating in the expression of the mentioned immune system reaction (Gu *et al.*,2019), However, in the case of COVID-19, there is a notable occurrence of a storm of cytokines syndrome that features rapid progression within a few days following the initiation of the pathological condition, which leads to raised rate of inpatient complications and deaths (Zhang *et al.,* 2020a).

This shows that acute respiratory distress syndrome results from the occurrence of a cytokine storm through the release by effector immune cells of large amounts of pro-inflammatory cytokines (IFNγ, IFNα, IL-1β, IL-6, IL-18, IL-33, IL-12, TNFα, TGFβ) and chemokines (CXCL10, CXCL8, CXCL3, CCL2, CCL9, CCL5) that lead the immune system to begin attacking the body and cause major damage and failure of many organs and may lead to death (Huang *et al.,* 2020b).

Serious COVID-19-related deaths are closely associated with cytokine release syndrome, and the scientific reason because that all cells and tissues in the body are affected by this excessive cellular immune response (Montazersaheb *et al.,* 2020).

The cytokine storm inflammatory is accompanied by immunopathological alterations in the pulmonary system, locally the excessive production of inflammatory mediators is the decisive factor that induces this pathological change and clinical manifestation. The initiation and developmentally of acute respiratory distress syndrome through viral infection is strongly linked with the occurrence of cytokine storm. Patients diagnosed with acute respiratory of the distress syndrome exhibit notable elevation the quantity of inflammatory mediators within their serum. Furthermore, there exists a positive association between the extent of this elevation

and the mortality rate observed in these patients (Douda *et al.*, 2011; Parsons *et al.*, 2005).

2.7. Human receptor of coronavirus and Adenovirus

2.7.1 Interleukin 6 receptors

Because severe symptoms emerged in many patients late following infected, when the SARS CoV-2 viral load was falling, localized inflammation played an early role of COVID-19 infections. Interleukin-6, a cytokine released by macrophages that triggers a proinflammatory response and is frequently high in COVID-19 patients, has emerged as one of the leading possibilities for mediating inflammation in COVID-19 (Rubin *et al.*, 2021).

Interleukin-6 Proinflammatory cytokine is produced in response to infection and tissue damage and exhibits a complex biology as it can signal via different modes of action (Garbers *et al.*, 2018). In classic signaling, IL-6 binds to the membrane-bound IL-6R, which is mainly expressed on hepatocytes and immune cells, and induces homodimerization of glycoprotein 130 (gp130). In the trans-signaling mode, IL-6 uses the soluble form of the IL-6R (sIL-6R). The IL-6: sIL-6R complex also induces gp130 homodimerization. IL-6 trans-signaling enables cells lacking the membrane-bound IL-6R to be stimulated by IL-6. This mode of signaling is considered to cause proinflammatory properties (Hunter and Jones, 2015)

The Interleukin-6-receptor mediates the biological activities associated with IL-6, which consists of two parts, the first being the type I transmembrane signal transducer protein gp130 (CD130) and the other type I transmembrane glycoprotein termed IL-6R (CD126 or gp80) and the latter binds to IL-6 and the two with the first part of the receptor, thus this IL-6/IL-6R complex leads to IL-6 signal transduction,

which includes activation of the signaling transduction pathways (JAK/STAT, ERK and PI3K) (Scheller *et al.,* 2011).

Active IL-6 trans-signaling influences T-cell recruitment, activation, and apoptosis. According to Dominitzki *et al.* (2007), It can boost differentiation of Th17 cells and at the same time suppress the development of regulatory T cells (21). IL-6 trans-signaling is the major route of IL-6 signaling to microvascular endothelial cells (EC), leading to EC activation, dysfunction, and deregulated inflammatory cell infiltration as published by Schmidt-Arras and Rose-John (2016). It has been implicated in the endotheliopathy in COVID-19, with a central role in the pathogenesis of ARDS and multi-organ failure (Teuwen *et al.,* 2020)

Numerous readily soluble receptors are currently recognized for many different chemokines and cytokines, and they play a crucial role in controlling inflammatory reactions by working as antagonists or agonists of inflammatory mediator signaling. These soluble receptors operate as antagonists through impeding the biological function of ligands (Reddy *et al.,* 2000). On the other hand, the soluble IL-6 receptor (sIL-6R) enhances the signaling process of IL-6 through the stimulation of populations of cells which possess the signaling transduction protein CD130 nevertheless do not exhibit mbIL-6R expression. It is noteworthy that a limited number of cellular types exhibit the expression of IL-6R on their cell membrane, thereby enabling them to exclusively react to IL-6 stimulation. The aforementioned cells include neutrophils, macrophages, and certain subsets of T lymphocytes, along with hepatocytes. In contrast to the IL-6R, CD130 exhibits ubiquitous expression (Scheller and Rose-John, 2006).

2.7.2 C-C chemokine receptor type 2

Chemokines are small molecules that are crucial in the development of inflammatory illnesses in a manner stimulated by several types of immune cells. Chemokines are known to exert a crucial function in the immune system's reaction associated with inflammation, as they facilitate the recruitment of white blood cells to specific locations where an infection has occurred (Rotondi *et al.,* 2007). Presently, a total of 50 chemokines and 20 receptors for chemokine. Chemokines are named after the most recent nomenclature, which categorizes them into the C, CC, CXC, and CX3C families based on their chemical structure. the chemical attraction of immunological cells at the location of the infection requires the binding of chemokines to their receptors (Bachelerie *et al.,* 2013). As well as other functions performed by chemokines in the immune response, inflammatory and neoplastic diseases, and the regulation of angiogenesis (Coperchini *et al.,* 2019). Recently, a variety of studies have examined the role of chemokines in infectious diseases related to SARS-CoV-2 infections. It appeared that specific chemokines could they have the emergence role about symptoms linked with COVID-19 is severity influenced (Li *et al.*, 2020c).

The biological and viral the characteristics of SARS-CoV-2 exhibit resemblances to those observed in two previous pandemic coronaviruses, so it is assumed that the chemical messenger signature of COVID-19 patients contains analogous mediators of inflammation. In contrast, SARS-CoV-2 has a greater incidence of transmission as well as a mortality rate that is lower compared to MERS and SARS-CoV (Jafarzadeh *et al.,* 2020).

Chemokine receptors are crucial in regulating leukocyte trafficking and thereby orchestrating immune responses. Thus, chemokine receptors are critical in all aspects

of immune responses including adaptive immunity in lymphoid organs, early influx of innate immune cells and migration of cells in inflamed tissues. Their expression is tightly regulated and depends on the immune milieu. Imbalance or perturbations in the homeostasis of chemokine and chemokine receptor expression are associated with inflammatory (Oo *et al.*, 2010).

In myeloid cells, T lymphocyte, and NK cells, a pro-inflammatory of chemokine receptors for many chemoattracts types especially CCR2 are expressed. These chemoattracts receptors coordinate the immune system's responses occurring inside the airways, such as allergies, TB, and influenza. CCR4, CCR5, and CXCR6 on the other hand, the predominant expression of these kinds of receptors is observed for both naïve as well as adaptive T cells, and they have a crucial role in regulating the passage of lymphoid cells towards the respiratory alongside mucosal regions. Immune cell homing to the skin requires CCR4, whereas CCR9 controls cell migration to mucosal tissues (Liechti *et al.*, 2022).

Thus, chemokine receptors can induce nonredundant, tissue-specific cell migration. Severe COVID-19 has been associated with perturbations in chemokine levels as well as expression of chemokine receptors highlighting their importance in immunopathology (Brownlie *et al.*, 2022).

Moreover, it is interesting to note that hyperinflammation generated by numerous pathogenic viruses, like H1N1 and H5N1, could potentially aid in the elucidation of the impact of chemokines involved in the development of inflammatory reactions in opposition for COVID-19 (Hayney *et al.*, 2017).

Therefore, recognizing the SARS-CoV-2 chemokine signature and distinguishing it from non-COVID-19 pathogenic microbial ARDS would minimize complications and decrease mortality. Although resident alveolar macrophages are beneficial in the

early stages of the disease, infiltrating monocytes and macrophages are essential in the progression of COVID-19 (Khalil *et al.*, 2021), Infiltrated monocytes as the major leukocytes migrated into the infected lungs, and excessive secretion of cytokines and chemokines by these immune cells can lead to remarkable severe lung inflammation in SARS-CoV-2 patients (Swiecki and Colonna ,2011).

Although various chemoattractant mediators induce monocyte migration, CCL2 is often expressed quickly by stromal and immune cells upon activation of pattern recognition receptors (PRR) or cytokine secretion (Palomino and Marti, 2015). CCL2 is primarily produced in the lungs by alveolar macrophages, T cells, and endothelial cells, while CCR2 is mainly upregulated on the surface of monocytes, macrophages, and T cells in an inflammatory condition (Henrot *et al.,* 2019). According to (Xu *et al.*, 2020b), the expression of CCR2 in extracellular matrix (ECM) glycosaminoglycans (GAGs), CCL2 can stimulate monocyte recruitment into the infected lungs, where they trigger calcium influx, produce oxygen radicals, and express integrin in COVID-19 patients. Elevated levels of CCL2 and CCL7, two chemokines potent at the recruitment of CCR2 monocytes, have also been found in BALF from patients with severe COVID-19 (Zhou *et al.*, 2020b). In COVID-19, the inflammatory cytokine storm is closely related to the development and progression of ARDS. The serum levels of cytokines are significantly increased in patients with ARDS, and the degree of increase is positively correlated with mortality rate (Douda *et al.,* 2011).

2.7.3 Angiotensin Converting Enzyme-2 Receptor

The angiotensin conversion enzyme 2 receptor is a transmembrane polypeptide which is abundantly synthesized located on the extracellular surface of various kinds of cells. The enzyme within controversy is responsible for the production smaller

proteins through the cleavage of the bigger protein angiotensinogen. These small proteins subsequently play a regulatory role in cellular functions, SARS-CoV-2 utilizes its spike-like protein for effectively attach with ACE2 receptors. This interaction is crucial for the subsequent entry and infection of cells in the host. Therefore, ACE2 serves as a receptor and cellular entryway for the viral infection responsible for COVID-19.

The ACE2 protein functions as the principal target of SARS-CoV-2, facilitating its cellular infectious. The angiotensin converting enzyme 2 (ACE2) is expressed in a wide range of different kinds of cells as well as tissues, which includes the heart, pulmonary system, blood vessels, kidneys, the liver, and digestive system. The epithelial cells that line some tissues and form barriers to protection contain it (Sharma *et al.,* 2020).

2.7.4 Coxsackievirus and Adenovirus receptor (CAR)

Adenoviruses (AdVs) are common human pathogens that generally cause typical cold symptoms in healthy individuals, but infections can progress to acute respiratory distress syndrome (ARDS) and disseminated disease, with up to 50% mortality in ARDS patients (Lynch and Kajon,2016). Epidemic AdV outbreaks occur in closed or crowded communities, particularly among children (Lion,2019). In highly susceptible, immunosuppressed populations, such as in the transplant setting, AdV infections can also be highly lethal, depending on serotype, AdV can also cause gastroenteritis with prolonged fecal shedding, or keratoconjunctivitis that can lead to blindness (Ismail *et al.*,2019)

Viral infections first require viral entry into the host cell, and entry is mediated by cellular receptors. Significant research efforts have resulted in the discovery of several different primary receptors and co-receptors for human AdVs (hAdVs), In

general, hAdV can be divided into seven different groups, A-G, and almost all but group B AdV use the coxsackievirus and adenovirus receptor (CAR) as a primary receptor. While not yet understood, the convergence of several distinct viruses on a single protein as a primary receptor indicates that CAR manifests beneficial characteristics as a viral receptor. These factors may range from the essential nature of CAR in developmental and adult biology, to receptor localization, to the molecular pathway for internalization of viruses into the host cell (Greber and Flatt,2019).

The coxsackievirus and adenovirus receptor (CAR) is an essential multifunctional cellular protein that is only beginning to be understood. CAR serves as a receptor for many adenoviruses, human group B coxsackieviruses. While named for its function as a viral receptor, CAR is also involved in cell adhesion, immune cell activation, synaptic transmission, and signaling (Lim *et al.*,2008). The human CXADR gene that encodes CAR is found on chromosome 21q21.1 and has eight exons spread over ~ 80 kB of genomic DNA. While several studies have looked for genetic polymorphisms within exonic regions of human CXADR, very few changes have been found and most have been synonymous changes indicating the strength of genetic conservation (Thoelen *et al.*,2002).

Adenovirus tropism is heavily contingent on the generation of particular virus receptors. The attachment of several human-associated adenovirus serotypes, such as adenovirus types 2 alongside 5, is facilitated through the presence of coxsackievirus and adenovirus receptor (CAR), which is an exterior cell receptor. The association among the level of expression of its receptors with the sensitivity of specific cell types for the transfer of genes through Ad2 as well as Ad5 is currently observed (Cohen *et al.*, 2001).

2.8. Genetic Polymorphisms susceptibility to SARS-CoV-2

Numerous risk factors related to the coronavirus were identified, encompassing demographics such as age, sex, diabetes, hypertension, obesity, and geographical region (Buscemi *et al.,* 2021). Furthermore, it is important to acknowledge that genetic characteristics suffer from an important impact in becoming susceptible toward SARS-CoV-2 infected. The sensitivity individuals to SARS-CoV-2 infected is linked to a great deal of genetic polymorphisms (variants) in multiple genes, specifically responsible for encoding host receptors that are involved in the entry of the virus process and triggering immune system cells. Mutations in genes possess the ability to be hereditary, passing from one generation of people to the next, and can be noticed in a minimum of 1% of the people within a specific group. This phenomenon may provide an explanation for the variations in vulnerability to certain diseases, like COVID-19 (Kaltoum,2021).

Genetic variation happens within and between populations, resulting in polymorphisms that may be associated with a genetic characteristic or a phenotype in the presence of an environmental stimulus (Hirschhorn and Daly, 2005).

Single-nucleotide polymorphisms (SNPs) are the most prevalent type of polymorphism identified in the human genome, accounting for 90% of all genetic changes between individuals. SNPs are the most prevalent type of genetic variation and are widely utilized to analyze genetic differences between people and communities. These SNPs may have a role in changes to the genomic sequence (van Dijk *et al.*, 2014). These genetic polymorphisms may have an impact on the outcome of COVID-19 in high or intermediate producers of this cytokine (Silva *et al.*, 2022).

The relationship between immune gene polymorphisms and the outcome of viral infections has long been a source of interest. Because of the role of these genes in

viral clearance and immunopathogenesis, variations in these regions are expected to influence the outcome of a disease like COVID-19 (Hashemi *et al.,* 2021).

The susceptibility or resistance to several viral infections is known to be significantly influenced by host genetic polymorphisms, Because of the role of host genes in the entry and replication of SARS-CoV-2 in cells, as well as the development of an immune response, it appears that a combination of many genes may be involved in COVID-19 pathogenesis (Debnath *et al.*, 2020). As a result, various research on the relationship between genetic polymorphisms and COVID-19 have been performed to date (Elhabyan *et al.*, 2020).

Major changes in cytokine genes have the potential for influencing the generation of cytokines, whereas even slight alterations in such genes may accumulate over time in humanity that result in a substantial plausible biological effect (Cooper, 2003, Bidwell *et al*., 1999). Ahir *et al.,* (2015) have identified numerous its (SNPs) throughout the genes that encode cytokines as well as the receptors for them which demonstrate a strong correlation between the emergence in addition outcome of multiple illnesses.

Variations in COVID19 prevalence and mortality rates among countries may be explained by polymorphisms in various interleukins genes (Karcioglu Batur and Hekim,2021).

Among cells of interest that expressed chemokine receptors are monocytes, dendritic of cells in addition to lymphocytes and basophils. Notably to this, it should be noted which the CCR2 receptor can be detected on non-hematopoietic cell types like endothelially, fibroblasts and mesenchymal stem cells (Salcedo *et al.*, 2000). Monocytes are cells exhibit a constant production of CCR2. During an induction of monocytes, there has been an observed rise in the amount of expression of CCR2,

which is analogous to the greater expression detected onto lymphocytes response for activation using IL-2 (Luster,1998), reported a study of the association of CCR2 SNP V64I gene polymorphism in patients with severity COVID-19 infections to reveal more immune routes leading to towards the seriousness as well as mortality of a disease (Dogan *et al.,* 2022).

2.8.1 *Interleukin 6 receptor* rs4845374 and rs2228145 SNP

The potential influence of a persons genetic variation on disparities in the immune system's reaction among people infected via the novel coronavirus illness is able to be investigated through looking at the potential linkage between polymorphisms within particular genes and rates of infection and death from SARS-CoV-2 in various international populations (Karcioglu Batur and Hekim,2021).

The researcher team in Italian in investigated the distribution of genetic variations *IL-6R* genes (rs2228144, rs2229237, rs2228145, rs28730735, rs143810642), which may be employed as prognostic and pharmacogenetic biomarkers for COVID-19. These variants have been predicted to affect the expression and binding ability of IL6 and IL-6R . additionally, showed that IL6 and IL-6R appeared to be implicated in several pathogenetic mechanisms associated with COVID19 severity and mortality. Thus, the availability of IL6-IL-6R-related biomarkers for COVID19 disease may be helpful to counteract harmful complications and prevent multi-organ failure. Finally, researchers concluded, how IL6 and IL-6R pleiotropic activity could be exploited to meet different clinical needs and realize precision medicine protocols for cases public health emergencies (Strafella *et al.,* 2020).

The predisposition and disease progression of COVID-19 are influenced via recipient genetic variations, especially that relate to immune system reactions. Some polymorphisms of cytokines and chemokines receptors have been discovered

determined for being linked to the development of COVID-19, based on the impact of the happening variability. For instance, polymorphisms made up of single nucleotide within the TNF locus have been related associated greater severity and strong inflammatory reactions. While presence of genetic polymorphisms in four types of Tolls like receptors (3,4,7 and 9) genes may be associated with an increase of serious breathing problems among people suffer from COVID-19 (Vakil *et al.*, 2022). Previous studies also reported that rs2228145 SNPs of interleukin 6 receptors were linked to manifestations severe COVID-19 infections (Smieszek *et al.*, 2021).

A highly prevalent missense mutation within the *IL-6R* locus has been identified at the rs2228145 loci. Notably, it was projected to have consequences for protein activity because of the arrangement of amino acids. The modification takes place within the extracellular portion of the receptor, that plays a crucial role in the interaction between IL-6R in addition exogenous ligands. Hence, it is plausible that the genetic variation could modify the structural arrangement of the protein domain, thereby potentially impeding the determination of IL-6, which Indeed, there exists some association in between the rs2228145 genetic variant as well as elevated amounts of soluble IL-6R in circulation. According to Garbers *et al.* (2018) and Cavieres *et al.* (2019), The IL-6 receptor locus (IL-6R Asp358Ala; rs2228145 A>C) exhibits a prevalent polymorphism which has been connected to elevated the amount of serum sIL-6R. This variant in the gene is thought to be linked with numerous diseases associated with inflammatory disease (Garbers *et al.*, 2014).

The analysis of population variations related to polymorphism of *IL-6R* located at the rs2228145 locus indicates that the majority of governments demonstrate the AC genotype, with the exception of India and the country of Sweden, where the AA genotype is predominantly seen. As of September 2020, a comparison of COVID-19 infection rates and fatalities by country, revealed that Spain and Brazil exhibited the

most considerable values in terms of both COVID-19 infections as well as mortality levels (Karcioglu Batur and Hekim ,2021).

The rs2228145 variant is a missense mutation that has been identified as an important contributor influencing the levels of IL-6R in several bodily fluids (Garbers *et al.,* 2018; Strafella *et al.,* 2020). The previously mentioned mutation in IL-6R is essential for its ability to bind to external ligands. There was a theory that suggested an amino acid return, namely the substitution of aspartic acid for alanine at position 358 (p.Asp358Ala), would have an impact on protein function (Strafella *et al.,* 2020).

2.8.2 *CC chemokine receptor 2* (rs1799864 SNP)

Chemokines have played a pivotal role in facilitating a immunological defense against viral infections. The interaction between the chemokines in addition to their corresponding receptors is of crucial significance in beginning and regulating and recruitment as well promotion of the immune system's cells inside the infected alveoli (Domingo-Gonzalez *et al.*,2016). The *CCR2* plays an indispensable part in the recruitment of both immunological as well non-immunological cells during pathological circumstances. It functions as a receptor for monocyte chemoattractant protein-1 (MCP-1) (Kirsten *et al.,* 2005).

The genetic intermediaries CCR2 has been identified to be genetically associated with serious illnesses triggered by Novel coronavirus through an impartial investigation of the molecular strategies underlying this their phenotype. the researcher focused on finding potential causal variants and determined that serious COVID-19 is correlated with CCR2 mutations that are predictive of elevated levels of CCR2 within pulmonary tissue (Zhou *et al.,* 2020b). it is CCR2 receptor responsible for binding to CCL2, often referred to as MCP-1, is also known as CCR2.

The attraction of monocytes and macrophages towards locations of COVID-19 infection is facilitated through the CCL2/CCR2 pathway. Excessive activation within this axis triggers excessive inflammation with subsequent damage to organs. (Zhao *et al.,* 2020).

The *CCR2* locus is located at chromosomal 3p21, particularly within a collection of genes which generate chemokine receptors. The *CCR2* DNA produces dual isoforms, namely *CCR2A* and *CCR2B*. The *CCR2* gene has a single nucleotide polymorphism (SNP) where a guanine (G) is substituted with an adenine (A) at nucleotide 190. This modification results in a protein's amino acid shift from valine (GTC) for isoleucine (ATC) at position 64, resulting to the designation *CCR2*-V64I. The aforementioned peptide replacement, that's of a conservative nature, arises inside the primary transmembrane area for both CCR2A and CCR2B. This substitution results in more stability and prolonged half-life specifically for CCR2A, while having no discernible impact on the stability of the CCR2B isoform (Nakayama *et al.,* 2004). This *CCR2*-V64I polymorphisms is being subject to important investigation, with several studies demonstrating a correlation between the presence of the *CCR2*-64I variant as well as a decreased likelihood of AIDS development among people infected by HIV, in addition to a lower susceptibility to diseases such as multiple sclerosis and also the development of breast cancer (Fatima-Zahra *et al.,* 2020).

Some study mentioned, participants immunological phenotype/genotype information can assist researchers to genetically comprehend the infection with SARS-CoV-2 as well as detect illness progression. Chemokines are being implicated in the outbreaks of SARS and MERS, which can additionally contribute to COVID-19 symptoms. and researcher observed evaluated relationship between multiple chemoattract types of SNP, especially *CCR2*-V64I DNA mutations and COVID-19 severity for identify immunological mechanisms causing sickness severity and also

mortality (Dogan *et al.*, 2022). *CCR2*-V64I mutations can impact risk of COVID-19 infections throughout Asian countries (Chen *et al.*, 2011).

According to some articles, COVID-19 patients had greater quantities of multiple chemokines and cytokines compared to controls, regardless of their sex. The upregulation of CCR2 levels appeared to be more pronounced among individuals who experienced a more serious medical condition throughout the time they were hospitalized (Pomar *et al.*, 2022).

2.9 Diagnosis of COVID-19 infection

Physicians establish the preliminary diagnosis for individuals who are infected with COVID-19 by evaluating the patients clinical symptoms. Clinical signs and symptoms included in analysis were age, fever, headache, dry cough, fatigue, dysgeusia, anosmia, rhinorrhea, conjunctivitis, dyspnea, perioral cyanosis, chest pain, arthralgia, myalgia, general discomfort, diarrhea, and abdominal pain in addition to chest computed tomography (CT) scans (Iranmanesh *et al.*,2021; He *et al.*, 2020a). And laboratory identification of COVID-19 by Molecular Testing (To *et al.*, 2020; Wang *et al.*, 2020b), Serological tests (Gorse *et al.*, 2020; Lauer *et al.*, 2020; Griffin, 2022).

2.10 Adenovirus Coinfection with SARS-CoV-2

In 1953, Human adenoviruses (HAdV) were identified, a cytopathogenic agent was discovered during the long-term cultivation of tonsil and adenoid tissues following procedures on children (Huebner *et al.*, 1954). This established the viruses' names (adenoid degeneration viruses) and provided a basic description of their ecology in relation to asymptomatic persistence in the lymphoid tissue. Adenoviruses were quickly discovered in samples taken from individuals who had conjunctivitis and acute respiratory illnesses (Robbins *et al.*, 1950).

The adenovirus the genome consists of five primary transcriptional units, notably E1A, E1B, E2, E3, as well as E4, which are sequentially present during the viral replication cycle. Genes that encode proteins are found in these transcription units and process of replication of the viral genome entails the participation of several components, and it is has five later transcription units. Proteins that are involved in capsid construction or are part of the viral capsid are encoded by the letters L1 through L5. The L1-L5 later transcription units are all controlled by the same promoter region and share the same transcription start point (Stasiak and Stehle,2020).

Adenoviruses are resistant to environmental stressors and stable to physical or chemical agents as well as pH conditions, allowing them to survive outside of the body for lengthy periods of time. Adenoviruses can be transmission of the virus occurs by the dissemination of respiratory droplets between individuals, as well as through fecal-oral pathways. The cellular receptors for coxsackievirus and Adenoviruses, the first step in the Penetrate of human adenoviruses is adhesion of a virus particle to a target cell by adhesion receptor is the adenovirus receptor, which facilities the internalization of adenoviruses (Bansal *et al.*, 2003). CAR interacts with a particular location just on fiber knob domain. Importantly, constraints need that the Human Adenoviruses fibers be wide and flexible in order to allow receptor binding (Wu E *et al.,* 2003).

It has been established that Human Adenoviruses species serotype A, C, D, E, and F require CAR for adherence, by contrast, Human Adenoviruses species B has the shortest fibers and relies mostly on fiber binding to CD46 or, less commonly, on desmoglein2. Other receptors, such as sialic acid-containing polysaccharides, GD1a glycan, and SR-A6 receptor, have been demonstrated to induce Human Adenoviruses

internalization in a range of different cell lines for various Human Adenoviruses types (Baker *et al.,* 2019).

CAR helps to create tight junctions and adherens junctions between epithelial cells (Walters *et al.,* 2002). The receptor with a high specificity contact, however, is cannot ability stimulate viral entrance inside cells. In contrast, the process of catching the virus is accelerated by a subsequent interaction that occurs between the penton base protein of the virus as well as v3 or v5 integrins (Wickham *et al.,* 1993).

Mention a previous report that revealed viral titers in human blood as well as other tissue samples, in addition to serologic and histopathological evidence for infected (Tollefson *et al.,* 2017). Human adenoviruses are frequently encountered as a causative agent of infectious. Adenovirus-associated epidemic conditions were reported extensively in the beginning part of the 20th century. It is worth noting that adenoviral infections can give rise to a diverse array of illnesses. Adenoviral strains account for around between two and five percent of pneumonia overall and are projected to contribute to 2-35% of pulmonary illnesses caused by viruses specifically in kids. These viruses are frequently obtained through conjunctiva, throat, as well as fecal samples, with can be spread by pulmonary and fecal-oral routes (Cherry *et al.,* 2014).

Coinfection of Novel coronavirus, along with various other viral infections of the respiratory tract, potentially exerts a substantial influence on the current outbreak of COVID-19. The best course treatment that complicated by co-infection with viral infections, which also makes diagnosis more difficult. Additionally, it can alter clinical symptoms and heighten illness severity, both of which can raise mortality. According to research, 3-21% of COVID-19 patients had additional viral respiratory pathogen infections (Wang *et al.,* 2020b). Human adenoviruses are capable of

causing pneumonia acquired in the community, are linked to about 5-7% of these coinfections (Kim *et al.,* 2020a).

Novel coronavirus with Adenovirus coinfection eventually led to acute respiratory of distress syndrome. ICU admission necessary, and clinical enhancement happened with the injection of hydroxychloroquine. in accordance with regional recommendations, there was uncertainty regarding the precise time of the coinfection, and the individual's comorbidities worsened due to an additional risk factor for the development of a severe disease. (Guan *et al.,* 2020).

The precise pathophysiological processes behind the coinfection with SARS-CoV-2 remain unclear. Some possibilities exist Regarding the relatively low prevalence of the virus that causes SARS-CoV-2 in the absence of a predisposing higher risk factor (Xing *et al.,* 2020). In descriptive investigations, a higher proportion of patients with coinfection developed ARDS and septic shock, necessitating ICU admission (Lai *et al.,* 2020).

Human Adenoviruses may remain in susceptible cells in a latency state for a very long time after the lytic infection (Lion, 2014). Adenoids, tonsils, and Peyer's patches are just a few examples of lymphoid tissues where Human Adenoviruses typically persist during latent infection, Once again creating disease symptoms, these latent virus particles have the potential to reactivate, re-infect, and multiply in epithelial cells (Lynch *et al.,* 2011).

Chapter Three

Materials and Methods

3. Patients, Materials and Methods

3.1. Patients:

3.1.1. Study Design

A case-control study based on 200 individuals was conducted from March to July 2022 in Imam Hussein Medical City, this study was performed after a comprehensive review of literature related to project by a panel of public health physicians in same hospital. As shown in the study scheme bellow:

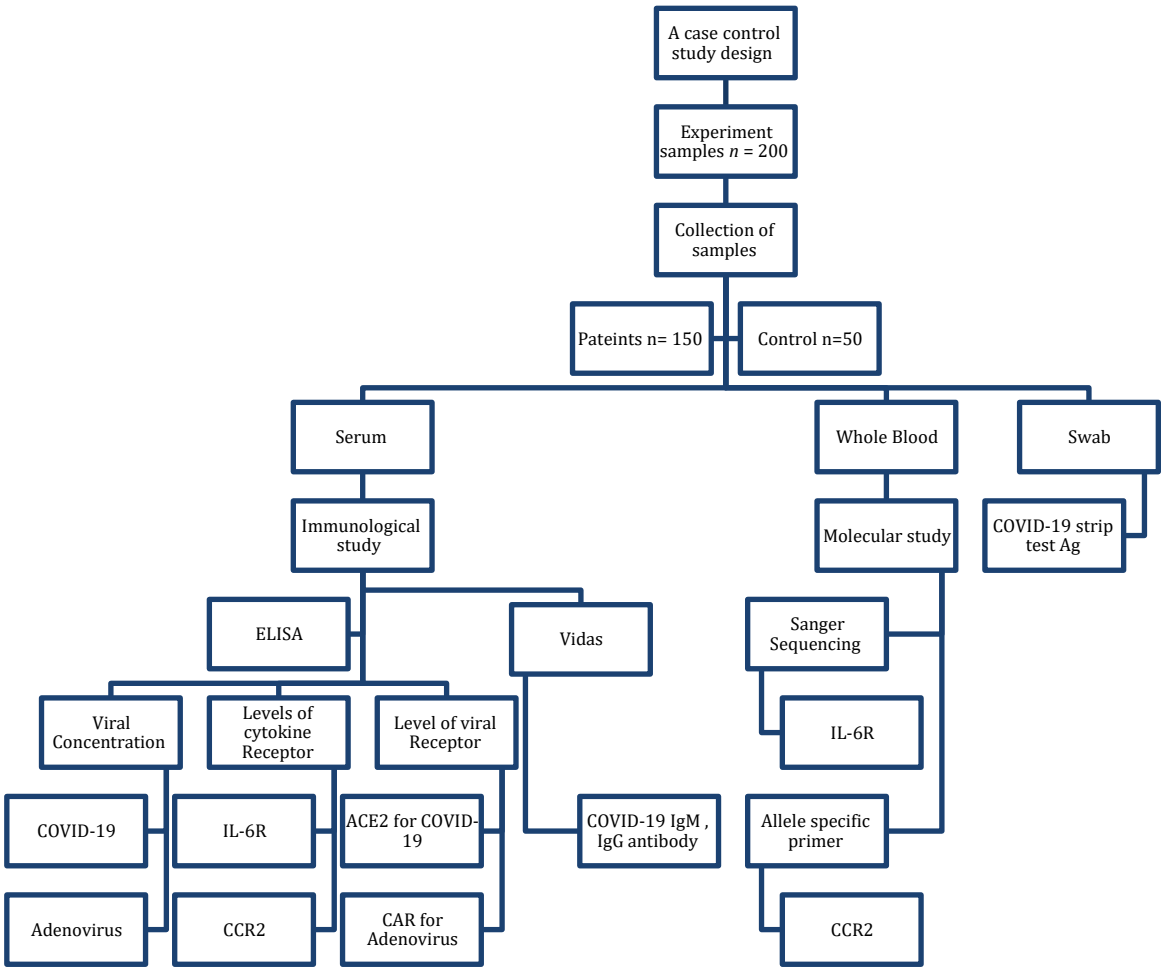


Figure 3.1: Study Design

3.1.2 The Studied Groups

A case-control study based on 200 individuals which included 150 COVID-19 patients of both sex with age range between 20-89 year, who were chosen at random from hospital after diagnosed infected patients by specialist physician with confirmatory by central public health laboratory. In addition to the control group, which contained fifty healthy adults who were not suffering from clinically not infected by other microbes or chronic diseases, this patients group was divided in 3 subgroups depended on progresses of COVID-19 symptoms (mild, moderate and severe) fifty patients for each subgroup , classified in to moderate and sever-critical according to WHO criteria (CT scans involvement :mild <50%, moderate < 50%, sever-critical >50% ,SPO2 saturation: mild≥90% , moderate ≥90% , severe cases <90%) (WHO, Clinical Management of COVID-19,2020).

3.1.3 Ethical Approval

Before collecting specimens, each patient provided verbal consent. The ethics committee, Ministry of Health, Kerbala Health Department, Imam Hussein Medical City, and medical research bioethical committee, College of medicine, University of Kerbala, authorized this study as show in Appendix (I).

3.1.4 Questionnaire

The following information were gathered from the patients and case sheets: name, age, sex, onset of disease, other diseases, vaccination, and medication as show in Appendix (II).

3.1.5 Inclusion Criteria

All adult and both sexes were accepted in this study. The inclusion criteria for the patients group included any patient who had recently diagnosed with sever acute respiratory syndrome due to COVID-19 and admitted to the ICU or HDU by a

specialist physician according to positive RT-PCR, % O2 saturation (less than 90% in severe and more than 93% in mild) , CT scan, clinical and laboratory tests by ferritin , D-Dimer and CRP. The inclusion criteria for the control group included those persons who had negative history of COVID-19 in the past six months and confirmed by COVID-19 rapid test and COVID-19 IgG and IgM levels.

3.1.6 Exclusion Criteria

The study excluded individuals were infected by other diseases, individuals had infected with other respiratory infections in addition to chronic or immune diseases like diabetes, Chronic obstructive pulmonary disease, pregnant women, and people were on long-term oral corticosteroid, anti-IL-6, or anti-TNF therapy. Patients with cancer and kidney disease, smokers with systemic immune disease, and thyroid gland diseases were also excluded.

3.2 Materials

3.2.1 Chemicals and biological materials used in current study are listed in

Chemicals and biological materials used in studies are listed in table (3-1)

Table (3-1): Chemicals and biological materials and their suppliers

No.	Chemical Components	Origin company
1	Agarose powder	MBI Fermentas
2	Distilled water	Drugs and medical appliances/Iraq
3	DNA ladder (100 base pair)	SolGent/ Korea
4	Ethanol absolute	Biosolve/ USA
5	Ethidium bromide dye	Promega/ USA
6	Nuclease free water	Biolabs/ England

7	PCR Master Mix (Green)	Promega/ USA
9	Primer pairs	ALPHA DNA /Canada
10	Proteinase K	Biolabs / England
11	TBE buffer 10X	Promega / USA

3.2.2: Instruments and Equipments

Tables (3-2) and (3-3) contain a list of the tools and instruments used in this study.

Table (3-2): Instruments and their Suppliers in current study.

No.	Instruments	Suppliers
1	Beakers	Marienfeld/Germany
2	Cool box	Eskemo /India
3	Conical flasks	Iwaki glass/Japan
4	Cotton swabs	Arth Al-Rafidain/China
5	Disposable plastic cup (50 ml)	Changazhou medical appliances /China
6	Disposable syringes	Changazhou medical appliances /China
7	Disposable tips	CAPP/Denmark
8	Disposable tips with filter	Bioneer/Korea
9	EDTA Tubes	AFCO-DISPO /Jordan
10	Eppendrof rack	China
11	Eppendrof tube (1.5)ml	Heittch/Germany

12	Gel Tubes	Lassco /India
13	PCR tubes	Gilson /France
14	Sterile Mask	Bioneer/Korea
15	Test tubes	Arth Al-Rafidain/China

Table (3-3): Equipments with their Suppliers in current study.

NO.	Equipment's	Suppliers
1	ABI3730XL, automated DNA sequences	Macrogen Corporation – Korea
2	Cooling Centrifuge	Hettich / Germany
3	Deep Freeze	GFL / Germany
4	Elisa reader and washer	Human- France
5	Incubator	Memmert – Germany
6	Microwave oven	GOSONIC,China
7	Ordinary centrifuge	Hettich / Germany
8	OWL Electrophoresis System	Thermo, USA
9	PCR/ Thermo cycler devise	Bio-base, china
10	Sensitive Balance	Denevr INStrument/ Germany
11	UV- Transilluminator	Med future, China
12	Vortex	Bioneer/ Korea
13	Water bath	GFL / Germany

3.2.3 Kits

Table (3-4) showed the kits with their suppliers that used in the current study.

Table (3-4): Kits with their suppliers that used in this study.

No.	Chemical Components	Origin company
1	Coronavirus 2019 IgM Vidas	BIOMÉRIEUX (USA)
2	Coronavirus 2019 IgG Vidas	BIOMÉRIEUX (USA)
3	DNA Extraction kit	Favorgen – Korea
4	Human ACE2 ELISA kit	BT lab (china)
5	Human Adenovirus Antigen ELISA kit (Quantification)	Sunlong (china)
6	Human CAR ELISA kit	BT lab (china)
7	Human CCR2 ELISA kit	BT lab (china)
8	Human COVID-19 Spike Protein ELISA kit (Quantification)	Sunlong (china)
10	Human IL-6R ELISA kit	BT lab (china)
11	Panbio™ COVID-19 Ag Rapid Test	Abbott, USA
12	PCR Clean-Up Mini kit	Favorgen – Korea

3.2.3.1 COVID-19 Ag rapid test instruments

Coronavirus 2019 Ag rapid test instruments that used in present study found in table (3-5)

Table (3-5): Contents of COVID-19 Ag Rapid test instruments

Item	Specifications	Storage
Test devices with desiccant in individual foil pouch	25 strips	2-8°C/-20°C
Buffer	1 x 9 ml/bottle	2-8°C
Extraction tubes	1 x 25	2-8°C
Extraction tube caps	1 x 25	2-8°C
Positive control swab	1 piece	2-8°C
Negative control swab	1 piece	2-8°C
Sterilized nasal swabs for sample collection	1 x 25	2-8°C
Tube rack	1 piece	
Quick Reference Guide	1 copy	
Instructions for use	1 copy	

3.2.3.2 Human COVID-19 Spike Protein ELISA Kit

Human COVID-19 Spike Protein ELISA Kit listed in table (3-6)

Table (3-6) : Contents of Human COVID-19 Spike Protein ELISA Kit

Item	Specifications (96T)	Storage
ELISA Microplate	8×12	2-8°C/-20°C
Standardized Lyophilized	2vial	2-8°C/-20°C
Buffer for Sample/Standard Dilution	10ml	2-8°C
Concentrated Biotin-Labeled Antibody	120ul	2-8°C (Avoid Direct Light)
Dilution of Assay	5ml	2-8°C
Antibody Dilution Buffer	10ml	2-8°C
Stratavidin-HRP Conjugate (SABC)	120ul	2-8°C (Avoid Direct Light)
Buffer for SABC dilution	10ml	2-8°C
Substrate made of TMB (A)	5ml	2-8°C (Avoid Direct Light)
Substrate made of TMB (B)	5ml	2-8°C (Avoid Direct Light)
Stop Solution	10ml	2-8°C
Cleaning Buffer (25X)	30 ml	2-8°C
Sealer for Plates	Five pieces	

Details of the Product	1copy	

3.2.3.3 SARS-CoV-2 IgM VIDAS

SARS-CoV-2 IgM VIDAS listed in table (3-7)

Table (3-7) : Contents of SARS-COV-2 IgM VIDAS

Items	Description	Storage temperature
60 Strips[(a)] (9COM)	STR	Read usable
60 Solid Phase Receptacles (9COM) 2 x 30	SPR	Read usable The inside of the SPR apparatus was covered with recombinant Coronavirus 2019 antigen (the Spike Coronavirus 2019 protein's attachment receptor domain (RBD)
Standard[(b)] (9COM) 1 x 0.5 mL (liquid)	S1	Read usable The buffer solution consists of humanized transgenic anti-SARS-CoV-2 antibodies IgM + stabilizer of animal origin + preservatives. MLE data indicate the acceptable range in "Relative Fluorescence

		Value" ("Standard (S1) RFV Range").
Positive control[b] (9COM) 1 x 0.5 mL (liquid)	C1	Read usable Buffer containing humanized recombinant anti-SARS-CoV-2 IgM antibody + stabilizer derived .from animals with preservatives The permissible range is shown as an index using MLE data ("Control C1 (+) Test Value Range").
Control Negative [b] 1 x 0.5 mL (liquid)	C 2	Read usable stabilizer and Buffer of origin animal + stabilizers. MLE statistics show the acceptable range as an index ("Control C2 Test Value Range").

3.2.3.4 SARS-COV-2 IgG VIDAS

SARS-CoV-2 IgG VIDAS listed in table (3-8)

Table (3-8) : Contents of SARS-COV-2 IgG VIDAS

Items	Description	Storage temperature
60 Strips [a] (9COG)	STR	Ready-to-use.

60 Solid Phase Receptacles (9COG) 2 x 30	SPR	Ready-to-use. Interior of SPR device coated with recombinant SARS-CoV-2 antigen (receptor binding domain (RBD) of the Spike SARS-CoV-2 protein).
Standard[(b)] (9COG) 1 x 0.5 mL (liquid)	S1	Ready-to-use. Buffer containing humanized recombinant anti-SARS-CoV-2 IgG antibody + stabilizer of animal origin + preservatives. MLE data indicate the acceptable range in "Relative Fluorescence Value" ("Standard (S1) RFV Range").
Positive control[(b)] (9COG) 1 x 0.5 mL (liquid)	C1	Ready-to-use. Buffer containing humanized recombinant anti-SARS-CoV-2 IgG antibody + stabilizer of animal origin + preservatives. MLE data indicate the acceptable range as an index ("Control C1 (+) Test Value Range").
Negative control[(b)] 1 x 0.5 mL (liquid)	C2	Ready-to-use. Buffer + stabilizer of animal origin + preservatives.

		MLE data indicate the acceptable range as an index ("Control C2 Test Value Range").

3.2.3.5 Human Angiotensin Converting Enzyme 2 receptors

Human Angiotensin Converting Enzyme 2 listed in table (3-9)

Table (3-9) : Contents of Human Angiotensin Converting Enzyme 2 ELISA kit

Items	Description	Storage
Pre-coated ELISA plate	8 wells× 12 strips	(4-20)℃
Standard Solution (24 ng/ml)	0.5 ml × 1	4C
Standard diluent	3 ml × 1	(4-20)℃
Biotinylated Human ACE2 antibody	1 ml × 1	4C
Streptavidin-HRP	6 ml × 1	(4-20)℃
Substrate solution A	6 ml × 1	4C
Substrate solution B	6 ml × 1	4C (dark)
Wash buffer Concentrate (25x)	20 ml × 1	(4-20)℃
Stop solution	6 ml × 1	(4-20)℃
Plate sealer	2 pieces	
User instruction	1 copy	

3.2.3.6 Human Adenovirus Antigen (HAdV-Ag)

Human Adenovirus Antigen listed in table (3-10)

Table (3-10) : Contents of Human Adenovirus Antigen (HAdV-Ag) ELISA Kit

Items	Description	Storage
User manual	1	R.T.
Closure plate membrane	2	R.T.
Sealed bags	1	R.T.
Microelisa stripplate	1	2-8℃
Standard: 270pg/ml	0.5ml×1 bottle	2-8℃
Standard diluent	1.5ml×1 bottle	2-8℃
HRP-Conjugate reagent	6ml×1 bottle	2-8℃
Sample diluent	6ml×1 bottle	2-8℃
Chromogen Solution A	6ml×1 bottle	2-8℃
Chromogen Solution B	6ml×1 bottle	2-8℃
Stop Solution	6ml×1 bottle	2-8℃
wash solution	20ml (30X)×1bottle	2-8℃

3.2.3.7 Human Coxsackievirus and Adenovirus Receptors

Human Coxsackievirus and Adenovirus Receptors listed in table (3-11)

Table (3-11): Contents of Human Coxsackievirus and Adenovirus Receptors, ELISA Kit

Items	Description	Storage
Pre-coated ELISA plate	8 wells× 12 strips	(4-20)℃
Standard Solution (64 ng/ml)	0.5 ml × 1	4C
Standard diluent	3 ml × 1	(4-20)℃
Biotinylated Human CAR antibody	1 ml × 1	4C
Streptavidin-HRP	6 ml × 1	(4-20)℃
Substrate solution A	6 ml × 1	4C
Substrate solution B	6 ml × 1	4C (dark)
Wash buffer Concentrate (25x)	20 ml × 1	(4-20)℃
Stop solution	6 ml × 1	(4-20)℃
Plate sealer	2 pieces	
User instruction	1 copy	

3.2.3.8 Human soluble interleukin – 6 Receptors ELISA kit

Human soluble interleukin – 6 Receptors ELISA kit listed in table (3-12)

Table (3-12) : Contents of Human soluble interleukin – 6 Receptors, ELISA Kit

Items	Description	Storage
Pre-coated ELISA plate	8 wells× 12 strips	(4-20)℃

Standard Solution (48 ng/ml)	0.5 ml × 1	4C
Standard diluent	3 ml × 1	(4-20)℃
Biotinylated Human sIL-6R antibody	1 ml × 1	4C
Streptavidin-HRP	6 ml × 1	(4-20)℃
Substrate solution A	6 ml × 1	4C
Substrate solution B	6 ml × 1	4C (dark)
Wash buffer Concentrate (25x)	20 ml × 1	(4-20)℃
Stop solution	6 ml × 1	(4-20)℃
Plate sealer	2 pieces	
User instruction	1 copy	

3.2.3.9 Human soluble Human Cc-Chemokine Receptor 2

Human soluble Human Cc-Chemokine Receptor 2 listed in table (3-13)

Table (3-13) : Contents of Human Cc-Chemokine Receptor 2 ELISA kit

Items	Description	Storage
Pre-coated ELISA plate	8 wells× 12 strips	(4-20)℃
Standard Solution (4000 ng/L)	0.5 ml × 1	4C
Standard diluent	3 ml × 1	(4-20)℃
Biotinylated Human CCR2 antibody	1 ml × 1	4C
Streptavidin-HRP	6 ml × 1	(4-20)℃
Substrate solution A	6 ml × 1	4C

Substrate solution B	6 ml × 1	4C (dark)
Wash buffer Concentrate (25x)	20 ml × 1	(4-20)℃
Stop solution	6 ml × 1	(4-20)℃
Plate sealer	2 pieces	

3.3 Methods

3.3.1 Blood Sample Collection

Disposable syringes were used to collect venous blood samples from both patients and controls (5mL). Each individual received 5 ml of blood through vein puncture, with 2 ml deposited in EDTA tubes and the remaining 3 ml progressively put into disposable gel-containing tubes the blood and the serum sample were used as shown in scheme (3.1). Blood in EDTA tubes was stored at -70 C until use in the genetic portion of the study, while blood in tubes containing gel was permitted to coagulate at room temperature for about 10 - 15 minutes before being centrifuged at 4000 rpm for approximately 4-5 minutes, after which the serum was collected and kept at -70 C until use in the immunological study.

3.4. Parameters of study

3.4.1.: Viral Diagnosis

3.4.1.1.: Clinicals COVID-19 Symptoms

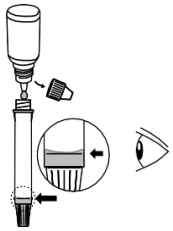
Clinical symptoms associated with COVID-19 were collected from the clinical files of patients. The analyzed clinical manifestations included fever, headache, dry cough, exhaustion, dysgeusia, anosmia, dyspnea, chest pain, arthralgia, myalgia, general pain, diarrhea, and abdominal pain.

3.4.1.2.: Panbio™ COVID-19 Ag Rapid Test

3.4.1.2.1: Principle of the assay

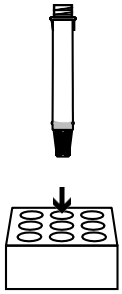
The Panbio™ COVID-19 Ag Rapid Assay measures COVID-19 Ag in a persons nasopharyngeal swab, following manufacturer's instructions (see appendix III).

3.4.1.2.2: Assay Procedure of panbio™ COVID-19 Rapid Ag



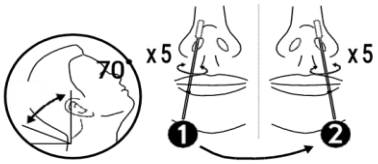
1- Held the buffer bottle vertically and fill the extraction tube with buffer fluid

2- until it flows up to the Fill-line of the extraction tube (300µl).



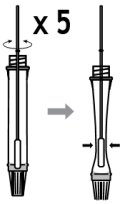
3- Placed the extraction tube in the tube rack.

4- Returned the patient head to a 70-degree angle. Insert the swab less than an inch (about 2 cm) into the nose while slowly spinning it. Swipe the swab against the nasal wall five times. Using the same swab repeat the collection procedure with the second nostril. Slowly remove swab from the nostril.

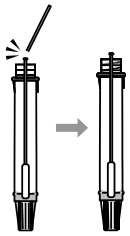


5- Inserted the swab specimen in the extraction tube.

Swirl the swab tip in the buffer fluid inside the extraction tube, pushed into the wall of the extraction tube at least five times and then squeeze out the swab by squeezing the extraction tube with your fingers**.**

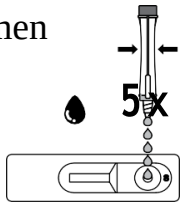


6- Broke the swab at the breakpoint and close the cap of extraction tube.

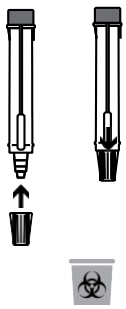


7- Open the dropped nozzle cap at the bottom of the extraction tube.

8- Dispensed 5 dropped of extracted specimens vertically into the specimen well (S) on the device.

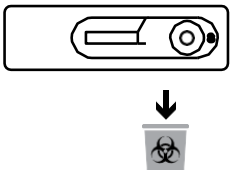


9- Close the nozzle and disposed of the extraction tube containing the used swab according to your local regulations and biohazard waste disposal protocol.



10- Started timer. Read result at 15 minutes. Do not read results after 20 minutes.

11. Disposed of the used device according to your local regulations and biohazard waste disposal protocol.



TEST INTERPRETATION Panbio COVID-19 Ag Rapid Test Device

Positive Negative Invalid

3.4.1.3.: Human COVID-19 Spike Protein ELISA Kit quantitative (pg/ml)

3.4.1.3.1: Principle of the assay

The Human COVID-19 Spike Protein ELISA Kit can be accurately and quantitatively detected in blood, plasma, cell culture supernatants, ascites, tissue homogenates, or other biological fluids using this Sandwich kit. The testing process was carried out in accordance with the manufacturer's directions, which are shown in Appendix IV.

3.4.1.3.2: Assay Procedure

It is necessary to thoroughly and uniformly combine specimens as well as agents before diluting them. Drawing a standard curve is advised for every test.

1. Before beginning the assay technique, prepared all reagents in accordance with the manufacturer's instructions.

2. The standard test, sample, and control (blank) wells were placed in the appropriate locations on the pre-coated plate, and then noted the positions of them. It was advised to measure every sample and standard twice.

3. Two times wash plate and then added 50μL of Assay Diluent to each well.

4. Prepared Standards: 50ul each of the following tubes: sample dilution buffer (blank), zero tube, first tube, second tube, third tube, fourth tube, fifth tube, and sixth tube were added.

5. Incubate: For ninety minutes, at 37°C, covering as well as covered the plate.

6. Washing: washed the plate involves took off the lid, threw away the contents, and washed it twice with Wash Buffer**.** At no point can the wells be allowed to dry out entirely.

7. Biotin-labeled Antibody: 100 ul of the Biotin-labeled antibodies solution that works (standard, test sample, and blank wells) was poured into the wells above. Avoiding contacting the side wall, add the solution to the bottom of every well, covering the plate, and incubate for sixty minutes at 37 C.

8. Wash: Uncover the plate, was washed by use Wash Buffer three times, allowing it to remain in the wells for one to two minutes every time.

9. HRP-Streptavidin Conjugate (SABC): Placed 100ul of SABC Test Solution to each of the wells, putted a plate cover over it, and I was letting it sit at 37 degrees Celsius for half an hour.

10. Wash: Plate was uncovered, then used Wash Buffer five times, allowed it to remain in the wells for one to two minutes every time.

11. TMB Substrate Working Solution: The TMB Substrate Working Mixture should be added in a volume of 90ul to every well. The plate should then be covered as well as incubated at a temperature of 37°C in a darkened room for a period of ten to twenty minutes.

12. Stop: The addition of 50 microliters of Stop Solution should be made to each separate well. The change in color will occur rapidly, resulting in a yellow.

13. OD Measurement: The optical density (O.D.) absorbency at a wavelength of 450nm should be measured using a Microplate Reader promptly after the addition of the stop solution, as depicted in Figure in Appendix IV.

3.4.1.4.: COVID-19 IgM, IgG Antibody Concentration (VIDAS)

3.4.1.4.1: Principle of the assay

The purpose of the SARS-CoV-2 IgM, IgG Antibody quantification test is to measure the levels of COVID-19 IgM alongside IgG Antibodies in persons plasma and serum. The testing technique was carried out in accordance with the instructions provided by the manufacturer, as outlined in Appendix V and VI.

3.4.1.4.2: Assay Procedure

1. The kit removed from storage at +2°C+8°C and take out the required reagents. The SPR package should be resealed with caution and the kit should be stored at a temperature range of +2°C to +8°C. The reagents may be utilized promptly.

2. Every specimen, control, and standard were evaluated with one strip and SPR instrument. After removing the SPR devices, gently reseal the bag.

3. The instrument's 9COM or 9COG code serves as testing identification. S1 designates the standard, which needs to be tested twice. C1 and C2 stand for the controls, which need to be checked separately.

4. If necessary, clarified samples by centrifugation.

5.The standard and controls mixed by using a vortex-type mixer.

6. For optimal results, referred to all the paragraphs in the samples section.

7. Before pipetting, ensured that the samples, standard and controls are free of bubbles.

8. The test contained 100 µL for the standard, controls, and test specimen components.

9. The strips as well as SPR devices placed into the device.

10. Started the scan as directed in the user manual. All scanning steps are performed automatically by the device.

11. In around 27 minutes, the scan will be finished. took the SPR devices and strips out of the gadget once the scan is finished.

12. Closed vials with return them to the required temperature after pipetting.

13. Properly discarded utilized SPR devices along with strips into a suitable receptacle.

3.4.1.5: Human Adenovirus Antigen ELISA Kit(quantitative) (pg/ml)

3.4.1.5.1: Principle of the assay

The quantitative Human Adenovirus Antigen by Enzyme-Linked Immunosorbent Assay (ELISA) Kit. The purpose of our quantitative Human Adenovirus Antigen is to measure the amounts of Adenovirus -Ag in various biological fluids. The testing technique was conducted in accordance with the guidelines provided by the manufacturer, as outlined in Appendix VII.

3.4.1.5.2: Assay Procedure

1. Before beginning the assay technique, prepared all reagents in accordance with the manufacturer's instructions.

2. In the Microelisa stripplate, it is recommended to designate one well as a blank by leaving it empty. In the wells designated for sampling, a volume of 40µl of Specimen Dilution Reagent is combined with 10µl of the sample, resulting in a dilution factor of 5. The process of placing of samples onto the bottom of the well should be done in a manner that avoids any contact to the well the wall. Thoroughly incorporate by using a gentle shaken movement.

3. The incubation process involves sealing the Cover plate membrane then incubated it at a temperature of 37℃ for a period of 30 minutes.

4. Dilution is performed by combining the highly concentrated cleaning buffer with distilled water for 30 time

5. The washing procedure involves the meticulous removal of the cover plate membrane, followed by aspiration and subsequent refilling with the designated wash solution. Dispose of the washing solution following a 30-second incubation period. Perform the washing operation a total of five times.

6. To each well, excluding the blank well, introduce 50 µl of the HRP-Conjugate reagent.

7. The process of incubation, as outlined in Step 3.

8. The process of washing, as outlined in Step 5.

9. Coloring was performed by adding 50 µl of Chromogen Solution A to all wells and 50 µl of Chromogen Solution B to every well. The mixture was shaken lightly and incubated at a temperature of 37 ℃ for a period of 15 minutes. It is advisable to restrict exposure to direct sunlight when engaging in the activity of coloring.

10. Termination of the reaction was achieved by adding 50 µl of a stopping solution to each well. The alteration in color inside the well is expected to go from a color that is blue to a yellow.

11. The absorbance at 450nm was measured using a Microtiter Plate Reader. The optical density (OD) measurement of the blank well without any sample is designated as zero. The assay should be conducted within a time frame of 15 minutes subsequent to the placing of the stop solution, as depicted in Figure within the Appendix VII.

3.4.2: Immunological Parameters

3.4.2.1: Human Angiotensin Converting Enzyme 2 Concentration (ng/ml).

3.4.2.1.1: Principle of the assay

The Human Angiotensin Converting Enzyme 2 can be accurately with quantitatively detected in blood, plasma, cell culture supernatants, ascites, tissue homogenates, or other biological fluids using this Sandwich kit. The testing technique was conducted in accordance with the instructions provided by the manufacturer, as outlined in Appendix VIII.

3.4.2.1.2: Assay Procedure

1. The reagents, standard solutions, as well as samples should be prepared in accordance with the provided instructions. It is advisable to ensure each of the reagents are equilibrated to room temperature prior to their utilization. The experiment is conducted in the environment.

2. The number of strips were determined for the assay and inserted the strips in the frames for use. The unused strips should be stored at 2-8°C.

3. 50ul standard to standard well was added. Note: Don't add antibody to standard well because the standard solution contains biotinylated antibody.

4. Added 40ul sample to sample wells and then add 10ul Human ACE2 antibody to sample wells, then add 50ul streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.

5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.

6. Putted 50ul substrate solution A to each well and then add 50ul substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

7. 50ul Stop Solution was added to each well, the blue color will change into yellow immediately.

8. Determined the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution, as shown in figure in Appendix (VIII).

3.4.2.2: Human Coxsackievirus and Adenovirus Receptor Concentration (ng/ml)

3.4.2.2.1: Principle of the assay

Human Coxsackievirus and Adenovirus Receptor (also known as CAR) can be accurately and quantitatively detected in blood, plasma, cell culture supernatants, ascites, tissue homogenates, or other biological fluids using this Sandwich kit. testing procedure was done according to the manufacturer's instruction as illustrated in appendix IX.

3.4.2.2.2: Assay Procedure

The procedure was similar to Human Angiotensin Converting Enzyme 2 ELISA kit and at 450 nm, examine the optical density (O.D) as illustrated in figure in Appendix (IX).

3.4.2.3: Human Soluble Interleukin 6 Receptor Concentration (ng/ml)

3.4.2.3.1: Principle of the assay

The Human Soluble Interleukin 6 Receptor (also known as sIL-6R) can be accurately and quantitatively detected in serum, plasma, cell culture supernatants, ascites, tissue homogenates, or other biological fluids using this Sandwich kit. testing procedure was done according to the manufacturer's instruction as illustrated in appendix X.

3.4.2.3.2: Assay Procedure

The procedure was similar to Human Angiotensin Converting Enzyme 2 ELISA kit and at 450 nm, examine the optical density (O.D) as illustrated in figure in appendix X.

3.4.2.4: Human Cc-Chemokine Receptor 2 Concentration (ng/ml)

3.4.2.4.1: Principle of the assay

The Human Cc-Chemokine Receptor 2 (also known as CCR2) can be accurately and quantitatively detected in blood, plasma, cell culture supernatants, ascites, tissue homogenates, or other biological fluids using this Sandwich kit. testing procedure was done according to the manufacturer's instruction as illustrated in appendix XI.

3.4.2.4.2: Assay Procedure

The procedure was similar to Human Angiotensin Converting Enzyme 2 ELISA kit and at 450 nm, examine the optical density (O.D) as illustrated in figure in appendix XI.

3.4.3: Genetic Study

3.4.3.1: DNA Extraction

Genomic DNA was extracted from white blood cells (WBCs) from both COVID-19 patients with the healthy control using a Human Blood DNA extraction kit (favorgen/korea), Kit components was explained in appendix XII.

3.4.3.1.1: Extraction protocol for frozen blood

For the extraction of DNA from frozen blood samples, the manufacturer protocol (favorgen / korea) was followed, which can be summarized as follows:

❖ **Preparation of Sample**

1. Putted 200 microliters of blood into a micro centrifuge container with a volume capacity of 1.5 milliliters. In the event that the total volume of the sample is below 200 µl, it is advised to supplement the required quantity of PBS.

2. To the sample, added 30 µl of Proteinase K (10 mg/ml), mixed for a brief period of time, and then incubated for 15 min at 60 °C.

❖ **Cell Lysates**

3. Added 200 µl of FABG Buffer and mixed by vortexing with the sample.

4. To lyse the sample, it should be incubated for fifteen minutes at 70 C in a water bath. Every 3 minutes during incubation, rotated the sample over.

5. Prepared the necessary elution buffer in a water bath at 70 °C.

6. (Optional Step) If RNA-free genomic DNA was desired, combined the material by vortexing with 5 µl of 10 mg/ml RNase A. At room temperature, incubated for 5 minutes.

7. Putted 200 µl of 96–100% ethanol in the sample and vortexed for 10 seconds. If there has been any precipitate developed, pipetted the material to thoroughly mixed it.

8. Placed an FABG column into a collecting tube. Carefully transferred the specimen solution into the FABG Column. For 1 minute, centrifuged at 14,000 rpm or 18,000 x g. removed the FABG Column from the collection Tube and set it in a newer collection Tube.

❖ **Column Washing**

9. Centrifuged the FABG Column with 400 µl of W1 Buffer for 30 seconds at 14,000 rpm or 18,000 x g. Placed the FABG Column backed into the Collecting Tube after discarding the flow-through.

10. Filled the FABG Column with 600 µl of Wash Buffer and centrifuged for 30 seconds at 14,000 rpm or 18,000 x g. Placed the FABG Column back into the Collecting Tube after discarding the flow-through.

- Before using, checked to see if ethanol has been added to the Wash Buffer.

11. Centrifuged the sample for a further three minutes at a speed of 18,000 x g or 14,000 rpm to dry the column.

❖ **A process of elution**

12. Relocated the desiccated FABG Column to a clean 1.5 ml microcentrifuge tube.

13. Filled the membrane center of the FABG Column with A volume of 100 µl of either Preheated Elution Reagent or Tris-EDTA (TE) solution was used.

14. Placed the Column of FAGB in an incubator and heated it to 37 ^{o}C for 10 minutes.

15. To elute the DNA, centrifuged for 1 minute at high speed (18,000 x g or 14,000 rpm).

Elution requires a standard capacity of 100 µl.

16. The piece of DNA should be maintained at a temperature of -20^{o}C.

3.4.3.1.2 Measurement of concentration and purity of extracted DNA

The extracted genomic DNA was measured by using Nanodrop spectrophotometer (THERMO. USA), that check and measure the purity of DNA through reading at 260 and 280 nm as following steps:

1. Select the appropriate application (DNA, nucleic acid) after launching the Nanodrop application.

2. A dry wipe was used to clean the measurement statues several times. To initiate the blanking process, it was necessary to carefully transfer a volume of 2 µl of free nuclease water to the lower of the measuring platforms using a pipette.

3. After cleaning the pedestals and lowering the sample hand, 1 µl of DNA was added for the measurement and the Nanodrop was initialized by clicking OK.

3.4.3.2: Agarose Gel Electrophoresis

3.4.3.2.1: Protocol of Agarose gel electrophoresis (Lee *et al.*,2012)

Protocol of Agarose gel electrophoresis performance according to:

❖ **Preparation of the Gel**

1. Putted on at appropriate weight of agarose in a conical flask so that the concentration of agarose in a gel is suitable for separating the DNA molecules to be separated. It is known that the concentration of agarose ranges between 0.5% -2% in general, and what was used in present study was (1.5%).

2. The vial containing agarose, added running buffer to it with mixing, as the most common types of buffers used in this field are TBE.

3. After that, the components of step 2 are melted using a microwave device until the mixture turns from Turbid to clear. removed the vial from the microwave and mixed it again, and returned it to the microwave. Repeated until complete melting.

4. Carefully added the ethidium bromide (EtBr) dye at a concentration of 0.5 micrograms per milliliter after making sure that the gel temperature is low 45 C .

6. Placed a suitable comb into the gel mold to create the wells after placing the gel tray into the casting device.

7. Poured the molten agarose to gel tray. The agarose should be allowed to solidify at the typical temperature. eliminate the comb with transfer the gel substance into the appropriate gel package.

❖ **Gel Electrophoresis and DNA fragment separation**

1. Added loading dye to the DNA samples to be separated. It is helps to allows the sample to sink into the gel. and also, to track how far your DNA sample has traveled,

2. The power supply to desired voltage should be (5V/cm between electrodes).

3. Added enough running buffer to coat the gel's surface. It is critical that the running buffer be the same as the one used to make the gel.

4. Linked the gel box's leads to the power supply. Switch on the power supply and ensure that both the gel box and the power supply are operating.

5. The lid has been removed. Slowly and carefully load the DNA sample(s) into the gel.

6. Replaced the lid to the gel box. The cathode (black leads) should be closer the wells than the anode (red leads).

7. The power turned on. Run the gel until the dye has migrated to an appropriate distance.

❖ **Observing Separated DNA fragments**

1. The power supply turned off and remove the lid of the gel box after electrophoresis completed.

2. The gel Removed from the gel box. eliminate any extra buffer from the gels surface. To absorb any additional excess buffer, place the gel tray on paper towels.

3. The gel Removed from the gel tray and expose the gel to uv light. The DNA bands should show up as orange fluorescent bands.

4. The gel disposed and running buffer in accordance with institution standards.

❖ **Representative Results**

The DNA standard or ladder should be spaced apart to allow measurement of the sample band sizes.

❖ **Photographic Record**

A Canon digital camera was used to take the photographs, and a UV transilluminator that included in the gel documenting machine was used to see the agarose gel.

3.4.3.2.2: Preparation Tris Borate EDTA (10X TBE) Buffer

To make the TBE, the concentrated TBE buffer was diluted (10X). It was employed in the electrophoresis process and to dissolve agarose powder. 50 milliliters of TBE (10X) stock solution were added to 450 milliliters of dH2O to create 500 milliliters of TBE (1X).

3.4.3.3: Reconstituting and diluting primers (100 pmol/L)

Primers made by Humanizing Genomics Macrogen were frequently delivered in a lyophilized state. A lyophilized primer's units were presented as a mass, measured in picomoles. To generate a stock of primers, the primer should be resuspended in either sterile 1X TE (1 mM Tris, 0.1 mM EDTA, pH 8.0) or clean water that is free from nucleases enzyme. In the interest of producing a master stock that would be utilized to create a working stock with a final concentration of 10 pmol/L was created, the company provided the precise amount of nuclease-free water to be added to each primer. The primers were reconstituted and diluted using the following procedures:

1- the cap was opened with the tube spinet down.

2- The producer of the oligos recommended adding the appropriate amount of nuclease-free water, in order to achieve a concentration of 100 pmoles/l (Master Stock).

3- Next, it appropriately combined via a Re-suspend the primers uniformly using a vortex.

4- The master possessed a volume of 10 µl of stock solution. The sample was transferred to a 0.2 ml the Eppendorf tube containing 90 µl of sterilized free of nuclease water, referred to as the Working Stock.

5- maintained main the stock is currently at a temperature of -20 C.

6- The working inventory was kept at -20 C°.

7- The working material had been vortexed after frozen on ice prior to use in the PCR. Subsequently, it was subjected to storage conditions at a temperature of -20 C.

Table (3-14) : Primers used in the current study

Gene	Type of primer	5 ---------- 3	PCR Product size bp.	Reference
IL-6R	Forward	GAGGGGAAGGTTCCTTTGAG	491	Garbers *et al.*,2014
	Reverse	CATGGCATGCTTTTTGTAGC		
CCR2	Forward wild type	GTGGGCAACATGCTGGTCG	264	Easterbrook *et al.*,1999
	Forward Mutant type	GTGGGCAACATGCTGGTCA		
	Reverse	AGCATGGACAATAGCCAGGT		

3.4.3.4: Polymerase Chain Reaction of *IL-6R* and *CCR2* genes

Polymerase Chain Reaction (PCR) is a rapid, in *vitro* enzymatic method for multiplexing specific DNA sequences into millions of copies of the same target segment, using two oligonucleotide primers that hybridize to opposite strands of the target DNA. This technique includes several cycles and in each cycle denaturation of the DNA template, primer annealing and extension of the annealed primers by DNA polymerase occurs, resulting in an exponential increase of the target sequence. In addition to using the outputs of the first cycle as templates for the second cycle, and so the increase is, so the important role of this technique has emerged in many scientific and medical aspects.

3.4.3.4.1: Preparation of Reaction Mixture To PCR

3.4.3.4.1.1: Primers Selection

PCR primers for detection of *interleukin 6 receptor* gene polymorphism (re4845374 and rs2228145) were depended on reference (Garbers *et al.*,2014), whereas PCR primers for detection of *CCR2B 641* gene polymorphism (rs1799864) were depended on reference (Easterbrook *et al.*,1999). These primers were provided from (Humanizing Genomics Macrogen, Korea). It is worth noting that the primers were diluted by adding 250 microns of free nuclease water.

3.4.3.4.1.2: Polymerase Chain Reaction Mixture

The mixture of polymerase chain reaction was prepared to amplify the *IL-6R* and *CCR2* genes by mixing components master mix with the forward and reverse primers, and the DNA template and Nuclease free water as shown in Table (3-15).

Table (3-15): Polymerase Chain Reaction Mixture

No.	Materials	Volume	Industrial company
1	DNA sample	5 µl	**……**
2	Master Mix	12.5µl	**Promega**
3	Primer Forward or Mutant Forward	1.5µl	**Macrogen**
4	Primer Reverse	1.5 µl	**Macrogen**
5	Nuclease free water	4.5µl	**Promega**
Total	**25 µl**		

3.4.3.4.1.3: Amplification Conditions

Polymerase Chain Reaction-thermocycler was used to amplified the *IL-6R* and *CCR2* genes, the amplification conditions for each gene have been shown in a table (3-16).

Table (3-16): Amplification Conditions for Polymerase Chain Reaction

Gene	Primary denaturation	One cycle condition			Cycles number	Final Extension
		Denaturation	Annealing	Extension		
IL-6R gene	95°C, 2 min	95°C, 30 sec	58.0°C, 30 Sec	72°C, 90.0 Sec	30	72°C, 5 Min
CCR2 gene	95°C, 2 min	95°C, 30 sec	62.0°C, 30 Sec	72°C, 80.0 Sec	30	72°C, 5 Min

3.4.3.4.1.4: PCR Product Electrophoresis

PCR product was subjected to electrophoresis on 1.5% agarose gel. 5 microliters of the ladder DNA (100bp) were applied to the first well of the gel. five micro liters of PCR sample was applied to each well of the gel. The lid of electrophoresis tank was placed and the power was run out for 30 minutes at 60 volts.

3.4.3.4.1.5: Sequencing for *Interleukin 6 receptors*

About 18-20 microliters of PCR product for *Interleukin 6 receptors* SNPs under interest in this study samples were sent to the macrogen company – South Korea to performed the sequence of DNA for detection SNPs under interest by Sanger sequencing the ABI3730XL, an automated DNA sequencing platform. Through 15 days the data of sequencing received by email in three formula; PDF, TEXT and AB1 files which required sequencing reading program by Geneious prime purchased version. software. In addition, the NCBI data tools were used for alignment the gene sequence by BLAST tool of NCBI.

3.4.3.4.1.6: Allele-specific PCR for *CCR2*

Allele-specific PCR as (Imyanitov *et al.*,2002) explain quickly, Allele-Specific Polymerase Chain Reaction (AS-PCR) is also known as an Amplified Refractory Mutation System (ARMS). The method is essentially based on using PCR to detect a single nucleotide polymorphism (SNP) (Newton *et al.,* 1989). pioneered this concept six years after the PCR technology was invented. This approach was created primarily to allow DNA polymerase to amplify the 3-primer end that properly complimented the nucleotide in a variation or wild type sequence (Darawi *et al.,* 2013). AS-PCR is regarded as the most direct approach for SNP detection. Unfortunately, it is still underutilized due to a few insufficient constraints. Allele-specific primers tend to strengthen both appropriate and unsuitable alleles in poor settings, affecting heterozygote proportions (Imyanitov *et al.,* 2002).

3.5 Statistical Analysis

Data were collected, summarized, analyzed and presented through the utilization of the Statistical Package for the Sciences of Society (SPSS) version 20 and GraphPad prime 8. The more quantitative parameters were represented using numerical values along with percentages, whereas, quantitative (numeric) factors were first evaluated for abnormality distribution using the Kruskal-Wallis test, and then accordingly abnormally distributed numeric variables were expressed as mean (an index of central tendency) and standard deviation (an index of dispersion) in addition to range.

The following statistical tests were used:

1. **The Chi-square test** was used to evaluate the association between any two categorical variables provided that less than 20 % of cells have an expected count of less than 5.
2. **Kruskal-Wallis test** was used to evaluate the difference in mean of numeric variables among more than two groups provided that these numeric variables were abnormally distributed.
3. Risk was evaluated using **Odds ratio** and 95 % **Confidence interval**.
4. **Hardy Weinberg equilibrium** was used to compare observed genotype frequency with expected genotype frequency.

The probability of significance was determined to be a P-value of less than 0.05.

Chapter Four
Results and Discussion

4.1.: Demographic Study

The present study had a sample of two hundred participants were divided into four distinct groups for subsequent analysis which included SARS-CoV-2 infection patients as vary groups mild, moderate and severe symptoms in addition to healthy control groups. The present study examined the initial features of the sample individuals, specifically focusing on both sex and age, and evaluated the statistical importance of any observed differences.

4.1.1 Distribution of Patients According to Age

The sample population included 150 patients, they were divided according to age as shown in table (4.1) and figure (4.1), (4.2). According to the infection, the participants were categorized into three different categories (mild, moderate and severe) and their number were 50 patients in each group. The findings of the presented investigation show a statistically highly significant correlation between changes in the mean age of the patient groups ($p<0.0001$), This study determined that the mean as well as the standard deviation of age amongst individuals diagnosed with mild symptoms was 44.04 ±15.96 years, that of moderate patients was 62.16 ±20.02 years, and finally, for severe patients, was 61.14 ±17.52 years. The results showed that the mean age in moderate and severe groups was higher compared to the mild and control groups.

Table (4.1): Distribution of patients and control according to age

Characteristic	**Control *n* = 50**	**Mild n = 50**	**Moderate n = 50**	**Severe n = 50**	***P value***
Age (years)					
Mean ±SD	34.12±10.5	44.04±15.96	62.16±20.02	61.14 ±17.52	*p*<0.0001 S
Range	20 -79	20 -79	20 -89	20 -89	

20-29, *n* (%)	16 (32.0%)	11 (22.0%)	6 (12.0 %)	3 (6.0 %)	
30-39, *n* (%)	17 (34.0 %)	11 (22.0 %)	2 (4.0 %)	3 (6.0 %)	
40-49, *n* (%)	6 (12.0 %)	10 (20.0 %)	3 (6.0 %)	7 (14.0 %)	
50-59, *n* (%)	6 (12.0 %)	6 (12.0 %)	4 (8.0 %)	6 (12.0 %)	
60-69, *n* (%)	2 (4.0 %)	10 (20.0 %)	14 (28.0 %)	9 (18.0 %)	
70-79, *n* (%)	3 (6.0 %)	2 (4.0 %)	10 (20.0 %)	15 (30.0 %)	
80-89, *n* (%)			11 (22.0 %)	7 (14.0 %)	

n: number of cases; **SD**: standard deviation; **S**: significant at ***p*** < 0.05 ; df: (3)

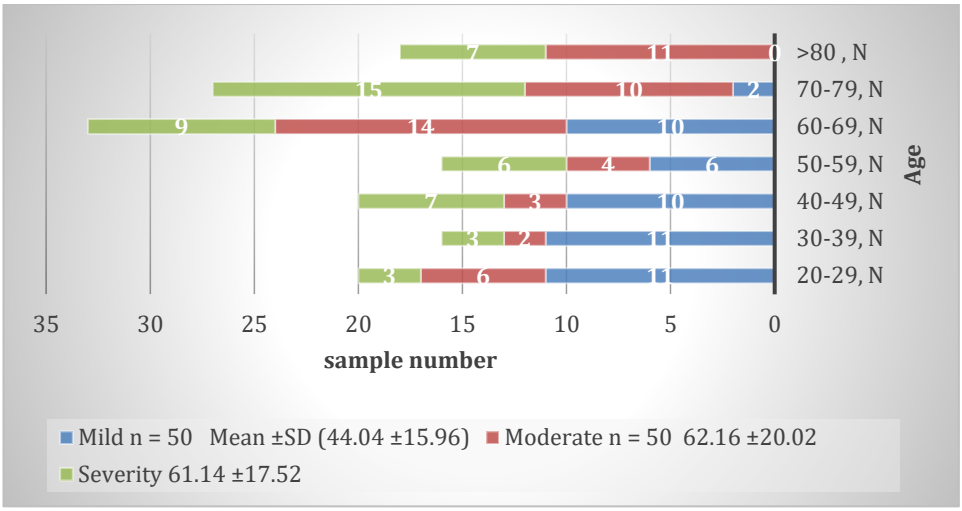


Figure (4.1): Sample numbers of patients and control according to age

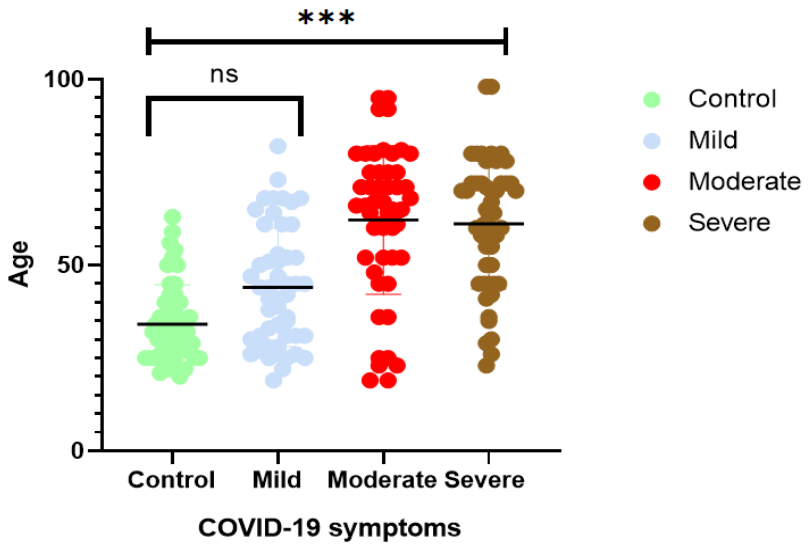


Figure (4.2): Distribution of patients and control according to age, the significance value was indicated as * between the groups. The level of probability was indicated as ***$p \leq 0.001$. Data was presented as mean, t-test, df: (3), n =200.

The current study is consistent with the regional study in the city of Al-Najaf by (Al-Mudhaffer *et al.*,2020), which revealed that 1153 participants examined confirmed SARS-CoV-2 infection, a greater rate of viral infectious had been found in men (743) as opposed to women (410) and all patients have age less than 10 to more than 70 years.

Wu and McGoogan, (2020) reported that a SARS-CoV-2 infection was linked to different levels of severity as well as posed a higher possibility of death, particularly among older individuals with other disease. In severe conditions, the danger of fatality can rise by more than 49%. The incidence of persons exhibiting critical symptoms as a result of a severe acute respiratory syndrome caused by the novel coronavirus disease was 63.93% in men and 36.07% in women and indicated that in-hospital critical state or death was associated with the age of >60 years, the justification for the phenomenon is the idea that the process of ageing contributes to the compromised operation of several physiological systems within the body,

especially the immune response. This weakening of the immune system function is an important factor that contributes to the raised mortality rates observed among elderly individuals contract COVID-19 viral infections (Carvalho *et al*.,2022).

Other study found that patients ages ranged from 43 to 60 years, 63.7% of them were men, and that 41.8% of them, particularly those were elderly, got acute respiratory distress syndrome, furthermore, patient age is a major factor in illness intensity and prognosis, patients aged 65 and up made 80% of all admitted COVID-19 cases, and they had a 23-fold increased chance of mortality compared to younger patients (Wu *et al.*, 2020a).

While the current study had almost no similarity to study conducted locally in Kerbala city by (Al-Ghabban, 2022) found a total of 425 confirmed COVID-19 patients, The more significant infectiousness rate amongst individuals were lower 25 year (66.4%), Al-Ghabban attributed the reason to adult population have high knowledge, negative attitude and weak practice in the precautionary measures about COVID-19.

Finally, some risk factors caused weaken to elderly patients such as exposure to different microbial infections, poor nutrition, other chronic disease, smoking, obesity and weaken of immune system because lost lymphoid tissues during aging, so that COVID-19 patients needed to the medical care specifically elderly and some patients required to CPAP machine during stay in hospitalized (Pan *et al*.,2008).

4.1.2 Distribution of Patients According to Sex

The results in figure (4.3) explained the grouping of patients according to sex. mild group included 36 (72.0 %) men and 14 (28.0 %) women. But in moderate and severe groups included 31 (62.0 %) men and 19 (38.0 %) women, while control group included 23 (46.0 %) men and 27 (54.0 %) women. There was non-significant

difference in the distribution according sex among study groups (p<0.0640) and Chi square (7.26) with degree of freedom (3).

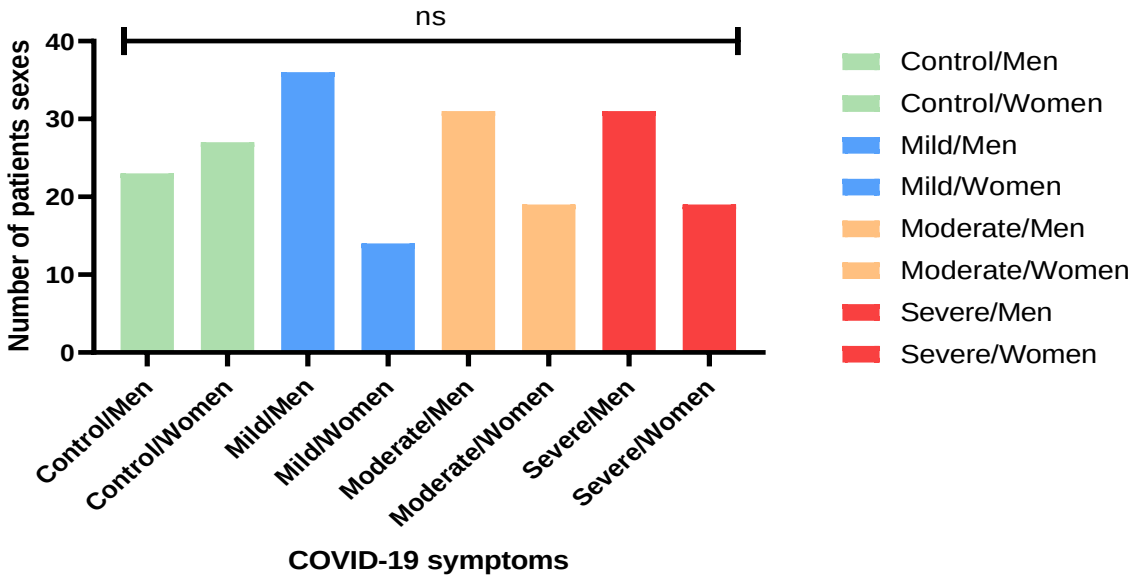


Figure (4.3): Distribution of patients according to Sex, the significance value was indicated as * between the groups. The level of probability was indicated as $p \leq 0.05$. ns: non-significant, Data was presented as mean, t test, df: (3), n =200.

The current study found a higher proportion of men than women, which aligns with the findings of a localized previous survey conducted in Kerbala City (Ahmad *et al.*, 2022). The study revealed that out of the 90 participants tested for the SARS-CoV-2 infection, a greater rate of infectiousness occurred in men (56) in contrast to women (34). Based to a recent global study carried out in Italy, which found a total of 300 confirmed COVID-19 patients, Among the observed cases, it was shown that the rate of infectiousness was significantly greater in men (69.2%) compared to women (30.8%) (Greco *et al.*, 2022).

The difference between infected men and women are likely due to sex specific behaviors, genetics and hormonal factors and sex difference in biological pathways against SARS-CoV-2. In current study the men more infectivity than women, because men are more likely to engage in poor health behaviors and have higher age adjusted

rates of preexisting comorbidities. And regarding women estrogen hormone effect on expression ACE2 receptors and reduces inflammatory complication because gene location of ACE2 receptors at X chromosome (Haitao *et al.*,2020). Hormonal disparities associated with sexuality may contribute to variations in the mechanisms of infection in addition to varied immunological responses in individuals of both sexes has a role in the development of COVID-19, as evidenced by the involvement of the ACE2 receptor, which is modulated through hormones that regulate sex (Badawi *et al.*,2021).

When estrogen hormone is dysfunctional or inadequately expressed, it can cause an escalation in the exposure of ACE2 receptors, increasing the susceptibility to COVID-19 infection (Stilhano *et al.,* 2020). Moreover, a study comparing younger and older women revealed that those with higher levels of progesterone and estrogen are more likely to suffer from milder symptoms of COVID-19, regardless of their age (Lott *et al.,* 2023).

4.2. Determination of the Viral in blood sample of study groups:

4.2.1: Quantitative of Human COVID-19 Spike Protein Antigen (Viral Concentration)

The present results in Figure (4.4) explained that the patient's group included 150 COVID-19 patients with symptoms varying between mild, moderate and severe with mean human COVID-19 spike protein concentrations of 14.32, 67.08, and 77.46 pg/ml, respectively, while 50 individuals were control group with mean spike protein concentration of 0.4094 pg/ml. The current results showed that the COVID-19 Spike protein for patients had increased significantly in severe and moderate spike protein compared to mild and control these results determine the significantly positive relationship ($r = 0.591$, $p<0.001$) between infection developed and spike protein concentration.

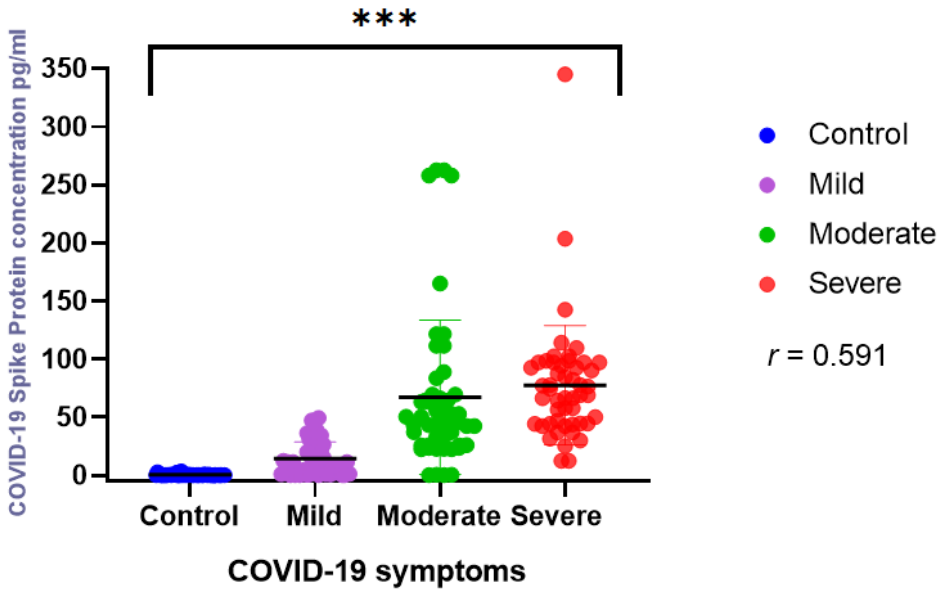


Figure (4.4): The quantitative human COVID-19 Spike (viral load), the significance value was indicated as * between the groups. the level of probability was indicated as ***$p \leq 0.001$. r: correlation, Data was presented as mean, statistical test: Kruskal Wallis test, df: (3) , n =200.

The current results were consistent with Soria *et al.* 2021, which found the severe group exhibited considerably lower average Ct values (Nucleic acid concentration is measured by threshold cycle value; lower Ct values indicate more target nucleic acid in the sample) when compared to both the moderate and mild patient groups. The comparison within the moderate and mild medical classes also showed considerably lower mean Ct values. These findings were statistically significant, with a p=<0.001 in every case.

In a systematic review of 1015 records, the findings yielded equivocal results on the association between COVID-19 severity and the amount of viral load, as an approximately equal number of research provided support for or refuted this theory, nevertheless, the research indicates a clear correlation between elderly age and a raised level of SARS-CoV-2 infection load, which is acknowledged as a significant risk factor for fatality caused by COVID-19, the elevated virus levels observed in

elderly individuals may be a mechanism behind any potential associations between disease severity and viral load, a significant association has been observed between the infectious agent load of SARS-CoV-2 and it disperse capability (Dadras *et al.*,2022).

Finally, current studied significantly raise proportion of severe and moderate patient were shown to possess a greater viral load compared other patients. This could be a reason for more adults and elderly being afflicted seriously by COVID-19 and having greater rates of mortality may be because Immunosenescence, changes in T-cell diversity, inflammation (Mueller *et al.*, 2020), and elderly people are susceptible to severe COVID-19 because they have greater comorbidities (Guo *et al.*,2020b).

4.2.2: COVID-19 Antibodies Level in Study Groups

The level of COVID-19 Antibodies in the serum of patients was conducted to estimate their levels. The results in Figure (4.5) shows that COVID-19 IgG levels had significantly increased in mild, moderate, and severe with a mean (of 14.98, 14.14, and 18.10) respectively, compared to mean control (2.68) instances as evidenced by a correlation that was positive for IgG Antibody ($r = 0.347$, $p<0.001$). In contrast, IgM shows that significance of severe ($p=0.021$) with a mean (0.922) compared to control instances as evidenced through a positive correlation ($r= 0.249$) for IgM Antibody, but was non-significant between mild, moderate that had a mean (0.570, 0.492) and ($p= 0.316, 0.081$) respectively compared with control group (0.12).

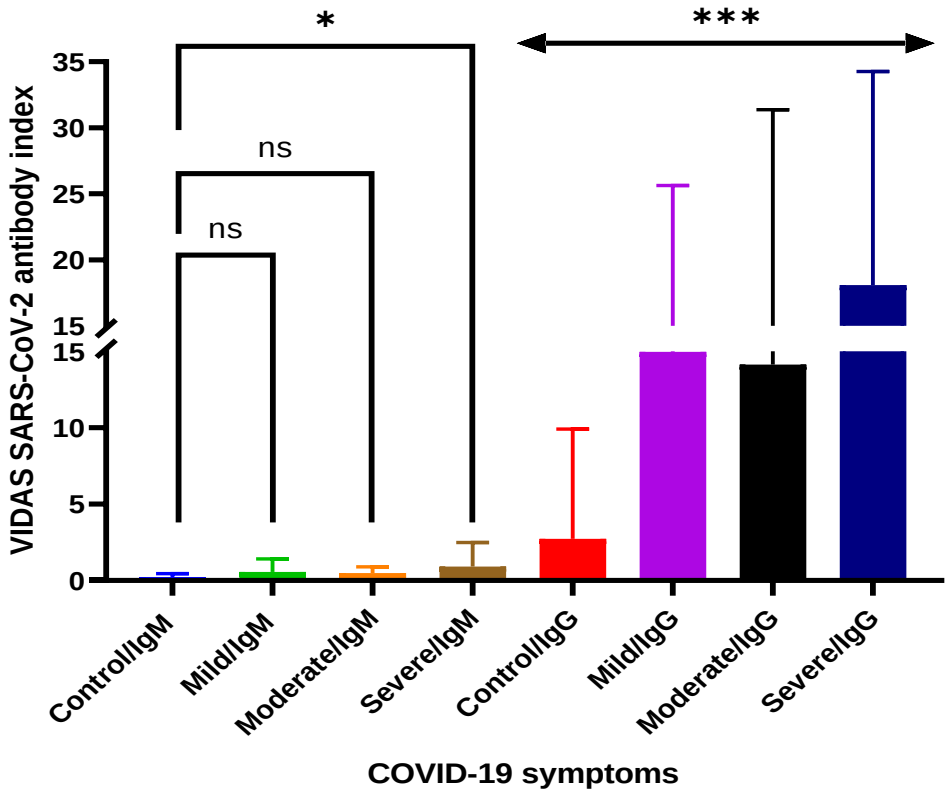


Figure (4.5): The COVID-19 Antibodies level, the significance value was indicated as * between the groups. The level of probability was indicated as *$p \leq 0.05$, ***$p \leq 0.001$, Data was presented as mean, statistical test: Kruskal Wallis test, df: (3) , n =200.

The results of the current study were consistent with the results of the local study in Kerbala City by (Al-Ibraheemi and Al-Saeedi, 2021); despite the limited number of participants in the study, the findings are still significant, the researchers found that a total of 30 patients had raised for IgG in severe symptoms of COVID-19 patients, among them a higher rate of infectivity was in men 22 than women 8. All patients were aged less than 10 to over 70 years, but more cases were between 20 and 40.

The current results of IgG and IgM levels were compatible with study in Al-Najaf city by (Abdalruda *et al.*,2022) the researchers found high level of both Antibodies in severe SARS-CoV-2 infection compare with non-severe patients and control.

Thus, they concluded that the levels of both antibodies classes were a notable rise in the prevalence of this condition among elderly individuals who were infected with the COVID-19 virus.

Based on research conducted international study in Iran by Alibolandi *et al.*, (2022) the researchers found that a high level of IgG Antibody in individuals afflicted from severe of COVID-19 infection, in comparison to other groupings. Following being infected with SARS-CoV-2, immune system cells initiate the production of antibodies known as IgM during the initial phases of infection, which are subsequently followed through the formation of IgG antibodies in the later phases of disease result from SARS-CoV-2. The identification of IgM antibodies signifies recent encounter with SARS-CoV-2, whereas recognition IgG Antibody without measurable IgM Antibody implies previous encounters with viral (Ai *et al.*,2020; Machado *et al.*,2020).

Typically, the IgM antibodies existence may be observed within a span of 3 to 6 days, whilst the presence of IgG Antibodies becomes evident after around 8 days (Lee *et al.*, 2010), Therefore, Antibodies against IgM and IgG are used for identifying an active SARS-CoV-2 infectious, in addition to Lung CT scan with PCR which more accurate (Machado *et al.*, 2020).

This phenomenon may be associated with the manifestation of severe sickness, since those who are exposed to the virus for longer durations might exhibit increased Antibody defenses in opposition to viral infections. Therefore, this group has a more prolonged duration of immunity in comparison with various groups (Alibolandi *et al.*, 2022). The impact of aging and severity of illness upon SARS-CoV-2 Antibody, the two types of Antibodies being referred to are immunoglobulin γ (IgG) as well as immunoglobulin Mu (IgM), is mostly observed, displaying a beneficial relationship.

However, the influence of sex on these Immunoglobulins remains unaffected (Luo *et al.*, 2021).

Finally, concluded that the high IgG and low IgM in patients infected with COVID-19 may be the reason that the study samples were collected within a period of two to three weeks of infection, and this is attributed to Immunosenescence , in addition to the possibility of a delayed immune response in patients of moderate and severe groups.

4.2.3: The Spike Protein Concentration and Mortality of Patients with COVID-19 Infections

Figure (4.6) shows that there was a non-significant increase in mortality and serum spike protein concentration, The mortality rate was 8 (16%) for moderate group and 24(48%) for severe group, where mean spike protein concentration it reached to 77.46 pg/ml in severe and 67.08 pg/ml in moderate compared to mild 14.32 pg/ml and control 0.4094 pg/ml, as indicated by a positive correlation with increased mortality (r=0.866, p=0.134). Nevertheless, the spike protein concentration and mortality increase were substantially higher in patients with severe and moderate conditions.

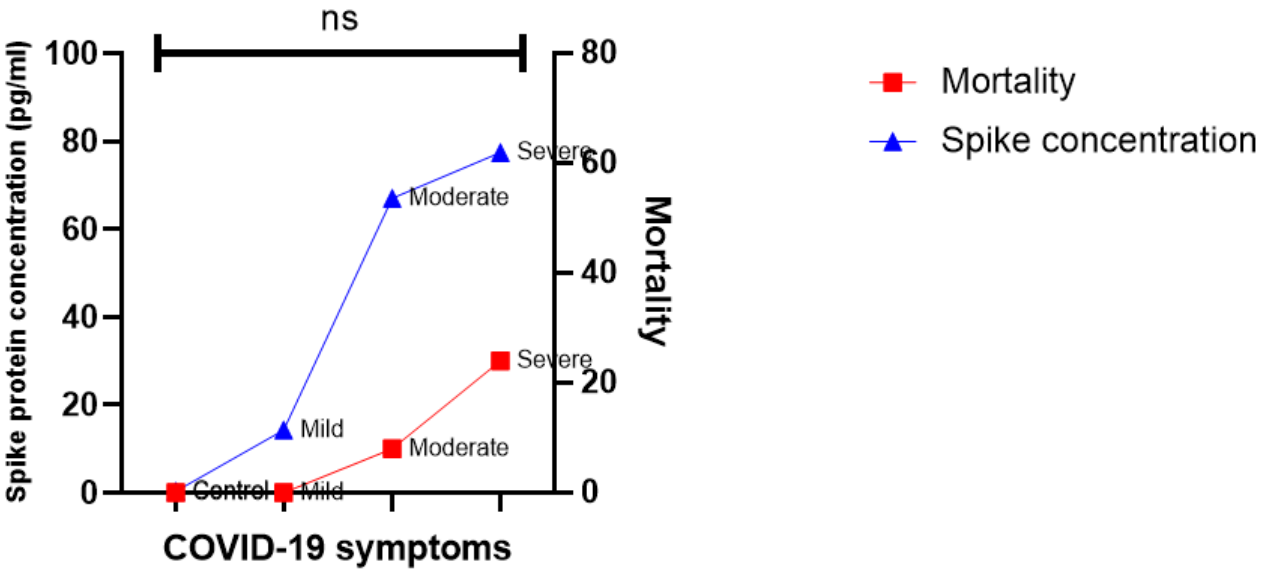


Figure (4.6): The serum spike protein concentration and mortality of patients with COVID-19 infections, the significance value was indicated as * between the groups. The level of probability was indicated as $p \leq 0.05$., Data was presented as mean, statistical test: Kruskal Wallis test, ns: non-significant; df: (3) , n =200.

According to study conducted in New York by (Satlin *et al.,* 2021) which found that individuals demonstrating high of viral load had an increased fatality rate in comparison to individuals presenting decreased viral load. Another study remembers, it has been observed that men exhibit greater quantities of viruses compared to women, while young adults tend to have higher viral loads as opposed to elderly people. This discrepancy may be attributed to the increased in addition extended exposure to airborne particles experienced by younger individuals (Ortiz-Prado *et al.*,2022). and the fact that men who were older had a greater incidence of severe illnesses and fatalities compared to women. Furthermore, older age, smoking patterns, and the presence of multiple disorders, like the medical conditions of hypertension with congestive cardiovascular disease, Numerous risk factors have been identified found as being connected with an elevated severity of illness as well as a rise in mortality in both cancer as well as non-cancer patients. Individuals diagnosed with COVID-19 (Al-Mozaini *et al.*,2021).

Other study mentioned the age, sex, asthma, atrial fibrillation, Chronic obstructive pulmonary disease, renal disease, The medical conditions under consideration include lung illness, diabetes, a condition cardiovascular disease, high blood pressure, as well as the variable of race yielded mortality and viral load have a strong, independent relationship (Pujadas *et al.,* 2020).

4.2.4: The Level of sACE2 Receptors in Patients with COVID-19 Infections

The Figure (4.7) shows the serum soluble ACE2 receptors with COVID-19 symptoms. All patients were significantly decreased between mild, moderate and

severe groups with mean (5.058, 4.796, and 4.71 ng/ml) respectively. Still, these three patients' groups raised when compared to a control group with a mean (1.417 ng/ml), so this parameter indicated a negative correlation with the development of COVID-19 symptoms (r= - 0.34, p<0.001); nevertheless, the decrease in sACE2 receptor in patients groups confirms the importance of this receptor in increasing the severity of the infection caused by the COVID-19. It indicates the virus's ability to infiltrate various cellular structures in the host, and ACE2 reads in a wide range of human cells.

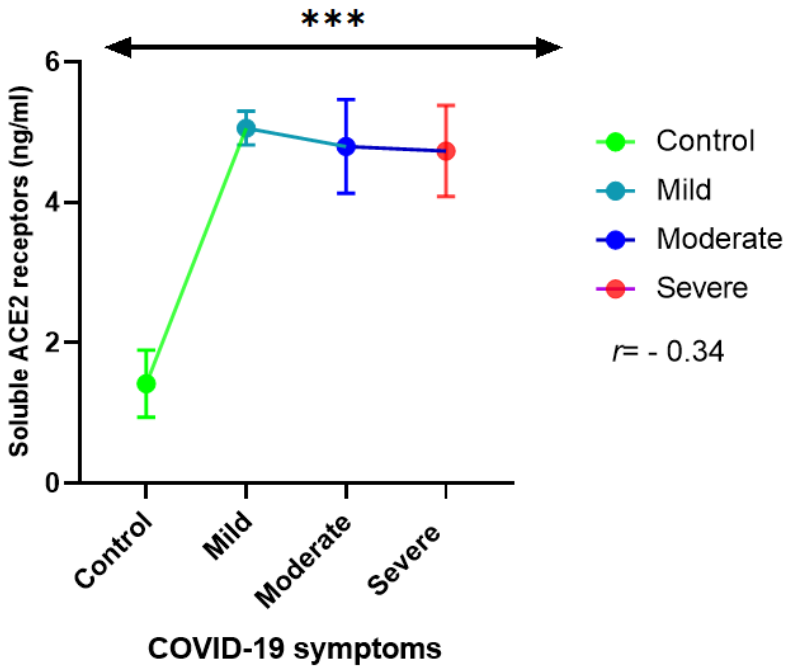


Figure (4.7): Variation in soluble ACE2 receptors level and COVID-19 symptoms, the significance value was indicated as * between the groups. The level of probability was indicated as ***$p \leq 0.001$, r: correlation, Data was presented as mean, statistical test: Kruskal Wallis test, df: (3) , n =200.

Although a higher concentration of ACE2 in the serum might confer some degree of defense against an acute infection, without eliciting an ordinary Antibodies reaction. Some study mentioned, there were reports of conflicting findings regarding

the levels of soluble ACE2 among participants infection by SARS-CoV-2. Its opposition for control healthy with low levels of circulating ACE2 receptors (Patel *et al.*,2021).

The findings of demonstrate soluble ACE2 might potentially function as a biomarker to determine a risk of infection by SARS-CoV-2 (Maza *et al.*,2022). Other studied noted the relationship between increased ACE2 expression in circulation along with inflammation throughout the body, which can lead to the development of liver, heart, and also renal diseases in individuals having COVID-19. Because of this, ACE2 activity has a strong relationship to the seriousness of COVID-19 and complications (Fagyas *et al.*,2022). In the present investigation ACE2 level consistent with local study in Baghdad city by Al-Mashhadani and Al-Thwani (2022) whom collected 84 patients and 27 control and the findings of the study indicate a considerable increase in the concentrations of ACE2 receptor in the hospitalized group when compared with the control group, with a high degree of statistical significance ($P<0.01$). The presence of ACE2 receptors has risen in lung epithelium persons could possibly be an explanation to the heightened seriousness of the respiratory illness caused by the novel coronavirus, has been observed to particularly impact individuals in the older population. In relation to children, study findings indicate that there was a notable increase in the expression of ACE2 in the epithelium of the nose among teens (aged 10 to 17 years) as well as adults (over 18 years) in comparison to a younger age group (under 10 years) (Gheblawi *et al.*,2020). The spike protein of COVID-19 is considered an essential protein to attach ACE2 to respiratory cells. It has unique locations recognized by B and T lymphocytes that encourage the development of antibodies that neutralize the virus (Fung and Liu, 2019 and Walls *et al.*,2020). According to research conducted international study by (Bani Hani *et al.*, 2022) found that a ACE2 level was substantially raised among individuals who infected by SARS-CoV-2 An increased expression of ACE2 is

connected to degree of severity infection of SARS-CoV-2 Furthermore, it possesses the capacity It serves as an indicator of the disease's severity.

ACE2 receptors functions as a bioactive receptor which allows the internalization of SARS-CoV-2 to cells endothelial are cells that present in lungs through the process of endocytosis, this process occurs through the contact between the spike protein, which is a component of the virus as well as the ACE2 receptor, which is embedded in the cell membrane, the protease known as ADAM17 converts ACE2 towards its soluble state from the endothelial cell membrane. In general, the amount of ACE2 in circulation tends to be modest, however, it undergoes an increase in cases associated with different disease (Fagyas *et al.*,2022).

COVID-19 may cause various organ harm due to ACE2, and SARS-CoV-2 has the ability to invade multiple organs, particularly the respiratory system, due to the presence of excessive amounts of angiotensin-converting enzyme 2 (ACE2) production in these tissues. ACE2 is widely expressed in numerous bodily tissues. (Ni *et al.*,2020).

The recognized function of ACE2, which is as the principal receptor upon cellular hosts that are enables the adherence of SARS-CoV-2, the causative pathogen accountable for the worldwide COVID-19 pandemic, the viral replication initiated via the binding spike protein to ACE2 receptors, the occurrence of this particular enzyme is widely observed in multiple organs in humans, such as the heart, arteries, in addition respiratory system. The complex pathogenic mechanisms involving ACE2 in the context infection by SARS-CoV-2 are likely connection with several factors as well as conditions that manifest as a greater severity of symptoms for persons affected by COVID-19 (Smieszek *et al.*, 2021). These factors Constitute of elderly status, the sexes, and the presence of comorbidities, namely heart failure, persistent lung conditions, overweight and obesity, as well as diabetes (Bourgonje *et*

al.,2020). The higher level of ACE2 receptors in the airway epidermal cells, which is dependent on their ages, may potentially contribute towards the heightened incidence of COVID-19 pulmonary disease found in the group of elderly people (Rodrigues and Costa de Oliveira,2021).

4.2.5: Correlation between Spike Protein Concentration with soluble ACE2 Receptors

The current results have shown significantly negative correlation between spike protein concentration in patients and soluble ACE2 receptors ($r = -0.284$, $p<0.001$) , In Figure (4.8) every case with COVID-19 infections were increased significantly in viral load parameter with increased COVID-19 infectivity compared to control group as indicated by positive correlation for this parameter (viral load; $r = 0.591$, $p<0.001$), while decrease in sACE2 receptors with mean of mild , moderate and severe (5.058, 4.796 and 4.71 ng/ml) respectively, and negative correlation (sACE2; $r = -0.341$, $p<0.001$) there for the relationship between these two parameter was an inverse relationship .

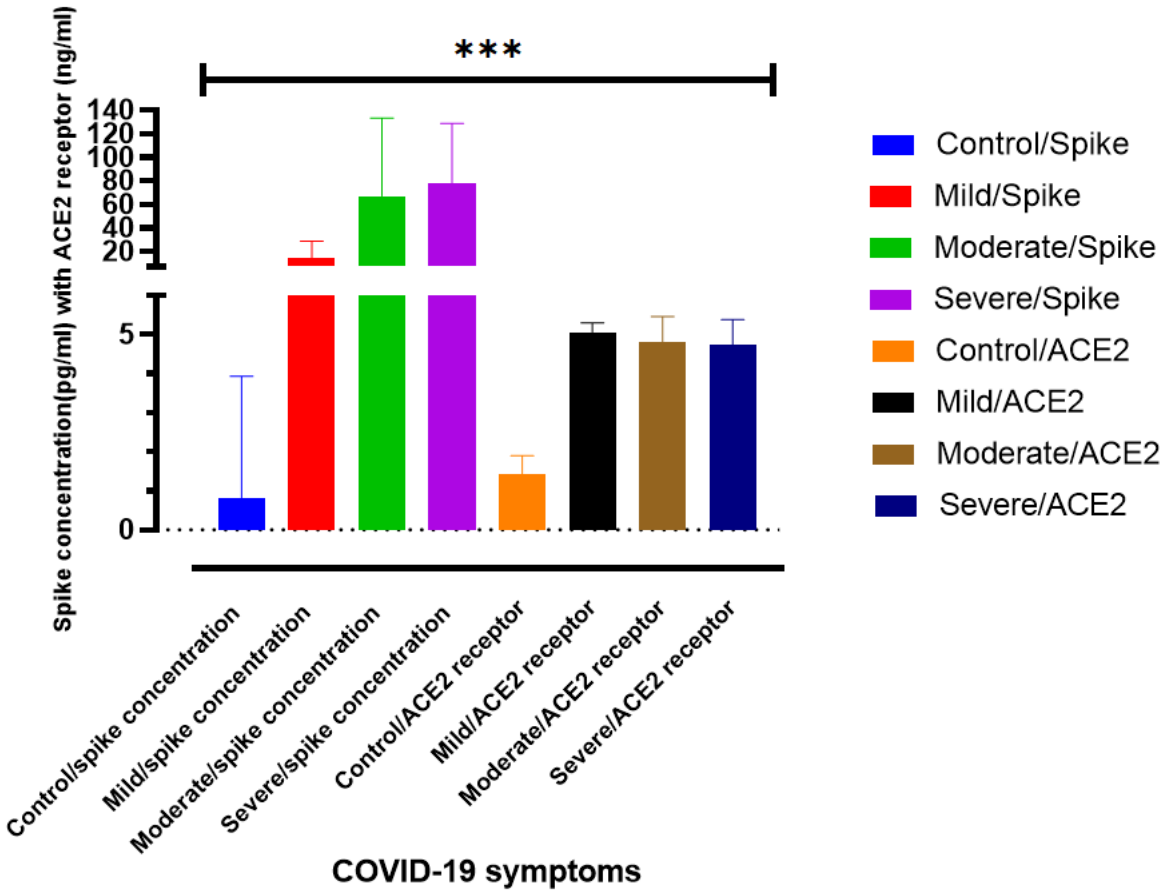


Figure (4.8) : The spike protein with serum soluble Ace2 receptors, the significance value was indicated as * between the groups. The level of probability was indicated as ***$p \leq 0.001$, Data was presented as mean, statistical test: Kruskal Wallis test, df: (3) , n =200.

In reality, those afflicted with higher-grade cases of COVID-19 suffer from elevated viral loads in their breathing passages as well as prolonged viral persistence compared to those who demonstrate easier symptoms (Al mashhadani and AL-Thwani,2022). Elevated plasma ACE2 concentrations were seen in person diagnosed infected by this virus, and a positive linear relationship was found that occur between these levels and viral load alongside pulmonary damage degree (Ni *et al.*,2020). In actuality, people exhibiting serious symptoms of COVID-19 demonstrate elevated levels of viral particles in the airways, encompassing the throat, bronchoalveolar

drainage water, and sputum. Furthermore, they exhibit an extended period of viral presence compared to those with lesser presentations of the illness (Liu *et al.*,2020a).

Indeed, this difference between the first and subsequent weeks of infection appears common to a number of the body defenses. Early on the objective is to avoid or reduce the presence of large amounts of virus that can penetrate into lung cells. So, while at the outset of infection the lung may be protected by having low levels of ACE2 at the cell surface – fewer locks for the virus key to turn (Azizan and Brown,2020).

Subsequently, the first measurement of ACE2 was examined to determine whether it exhibited any correlation with the clinical outcome of disease, based on the data collected from every patient, it was observed that non-survivors exhibited notably elevated activity levels of ACE2 prior to any therapy, in comparison to survivors (Kragstrup *et al.*,2021). After host cells attach with SARS-CoV-2 this linked trigger ACE2 expression as well as the shedding process leads to a notable decrease in enzymatic activity, as it facilitates the cleavage of the extracellular portion of ACE2, resulting in a release of protein that is soluble (Patel *et al.*,2021). The corresponding elevation in soluble angiotensin-converting enzyme 2 (sACE2) levels may function as a surrogate receptor, effectively binding to the spike protein present on circulatory, therefore, an increased expression of ACE2 receptors prior to the initial binding event may result in elevated levels of soluble ACE2 therefore a decrease in the presence of circulation SARS-CoV-2 with referred to as " S protein sites, thereby decreasing the number of cells in the host impacted. Hence, the presence of factors that increase the expression of sACE2 might provide a safeguarding effect, while a decrease in the expression of ACE2 may lead to the development of a more serious condition (Rabelo *et al.*,2015).

4.2.6: Correlation of COVID-19 Spike Protein and ACE2 receptors according to sex

The present results in Figure (4.9) explained that the patient's group included 150 COVID-19 patients with symptoms varying between mild, moderate and severe with mean human COVID-19 spike protein concentrations of (16.10, 71.20, and 69.95 pg/ml) respectively for women patients and (12.52, 62.97 and 84.87 pg/ml) respectively for men patients, while 50 individuals were control group with mean spike protein concentration of 0.27 and 0.54 pg/ml respectively. The current results show that the COVID-19 Spike protein for patients had increased significantly ($p<0.001$) in severe, moderate and mild spike protein compared to control. Additionally, the serum soluble ACE2 receptors were significantly decreased between mild, moderate, and severe groups with mean (5.01, 4.69, and 4.61 ng/ml) respectively for women and (5.09, 4.89 and 4.79 pg/ml) respectively for men. Still, these three patients groups raised when compared to a control group with a mean (1.27 and 1.55 ng/ml) respectively, the results show that the soluble ACE2 receptors for patients had increased significantly ($p= 0.0048$) in severe, moderate and mild compared to control.

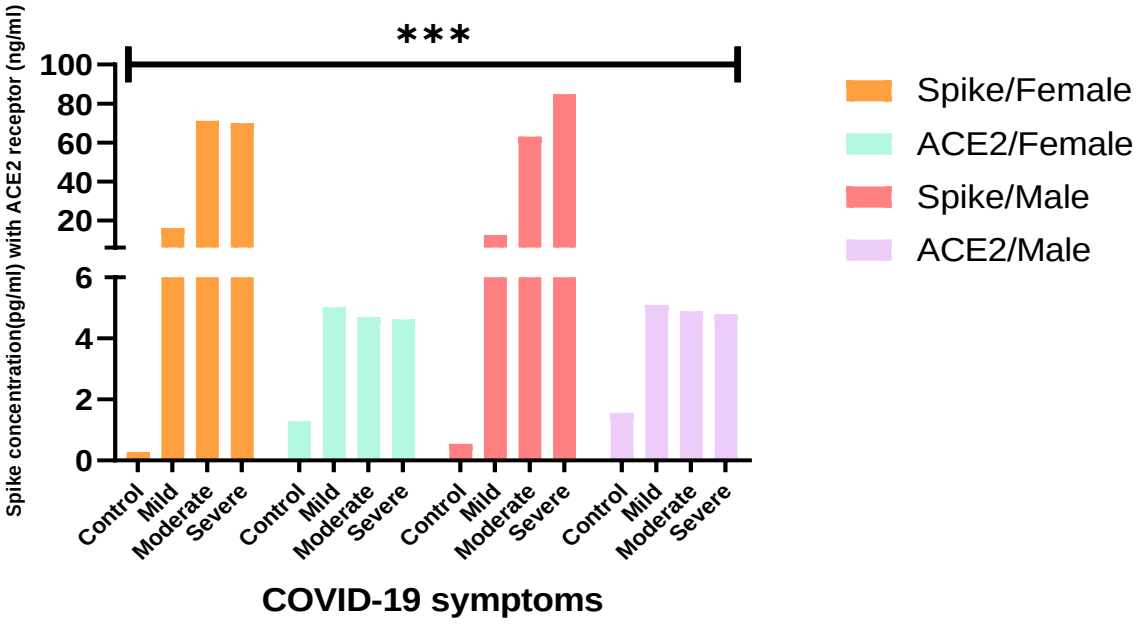


Figure (4.9): Correlation of COVID-19 Spike Protein and ACE2 receptors in both sexes, the significance value was indicated as * between the groups. the level of probability was indicated as ***$p \leq 0.001$, Data was presented as mean, statistical test: Kruskal Wallis test, df: (3) , n =200.

Coronavirus disease 2019 (COVID-19) death rate differs depending on sex: in Chinese confirmed cases, while the infection rate among men and women was similar, the death rate among men was 4.7% compared with 2.8% for women. Moreover, preliminary data from Italian epidemics suggest also a significant sex difference in infection rate, being 52.5% in women and 47.5% in men (Ruggieri and Gagliardi, 2020) .

However, some hypotheses can be put forward on the basis of current knowledge on sex differences in respiratory viral diseases, the sex different lifestyles, such as smoking addiction that is prevalent in men than in women, is considered one of the potential risk factors for developing pneumonia consequent to COVID-19 , in addition, it is known that, in general, innate and immune responses are more intense and stronger in women than in men , this can provide women with a more effective weapon to fight new and infective pathogens, favoring viral clearance (Ghosh and

Klein , 2017 ; Vardavas and Nikitara , 2020). Additionally, further factors could be taken into account in order to explain the sex bias in COVID-19 death rates. In particular, the human angiotensin-converting enzyme 2 (ACE2), an essential enzyme of the renin–angiotensin system (RAS), is the functional receptor for the severe acute respiratory syndrome coronavirus (SARS-CoV) as well as for SARS-CoV-2(Wan *et al.*,2020). It has been shown that ACE2 plays a protective role in chronic pathologies, like cardiovascular diseases, and acute respiratory distress syndrome, Intriguingly, infection with SARS-CoV induces ACE2 downregulation through binding of the viral spike protein to ACE2, thus reducing ACE2 expression in the lung and igniting acute respiratory failure (Hanff *et al*.,2020).

Estrogen, the primary women sex hormone, has been observed to play a protective role in SARS not only by activating immune response but also suppressing directly SARS-CoV-2 replication. To note, estrogen inhibits the activity or expression of different components of the renin–angiotensin system. In particular, estrogen is able to upregulate the expression of ACE2 (Channappanavar *et al*., 2017; Cheng *et al.*,2020).

Finally, hormonal factors could lead to ACE2 over-expression in women. These insights could, at least partially, account for the better outcome and the lower death rate in women SARS-CoV-2 patients with respect to men.

4.2.7: Association of Adenovirus viral load (Coinfection) with COVID-19 infections in study groups

The serum viral load of adenovirus was significantly increased with mean (19.59, 30.71 and 50.04 pg/ml) for mild, moderate and severe respectively, when compared to mean control (14.06) as indicated by positive correlation for Adenovirus viral load as Coinfection ($r=0.457$, $p<0.001$), as shown in figure (4.10).

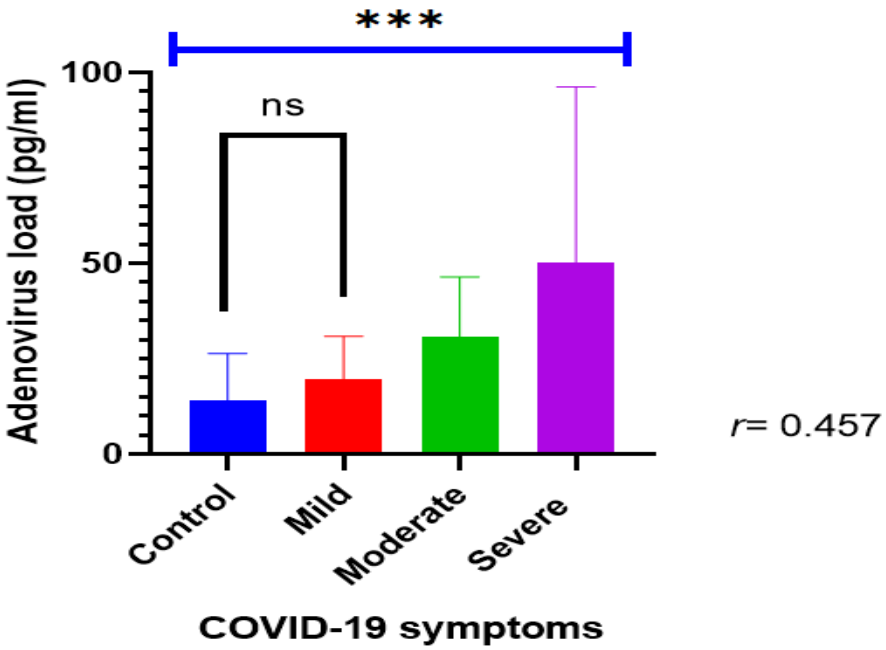


Figure (4.10) : Adenovirus viral load and patients with COVID-19 infections, the significance value was indicated as * between the groups. The level of probability was indicated as ***$p \leq 0.001$, r= correlation, Data was presented as mean, statistical test: Kruskal Wallis test, df: (3) ,n =200.

These results were similar to the findings of (Motta and Gómez, 2020) being mentioned whereas concurrent infections of SARS-CoV-2 with additional respiratory viruses is infrequent, it has been associated with more serious clinical prognosis. It is imperative to consistently consider the potential presence of curable pathogens, regardless of the case of an uncommon coinfection like COVID-19 as well as adenovirus.

The findings of study indicated a potential correlation between elevated viral load in secretions from the lungs with unfavorable clinical forecasting, including mortality (Hijano *et al.*,2023). Another study additionally showed that the presence of the Adenovirus viremia was commonly regarded as an indication of infection spread, with elevated levels of the virus that causes viremia being linked to higher fatality

rates (Zecca *et al.*,2019), Adenovirus viremia as well as mortality appear to be related, however the information were mainly small or single-center study evidence, which raises doubts about how universally applicable those results might be in various centers, Hence, there was a pressing want for comprehensive data derived from global and multicenter research endeavors that assess the correlation with Adenovirus levels and also mortality rates (Mynarek *et al.*,2014).

Case reports involving children and adults with contracted SARS-CoV-2 who also had concurrent illnesses via the influenza virus, humans metapneumovirus, alongside seasonally coronaviruses like CoV-HKU-14 have been published. These studies, while few, raise the possibility that coinfection can have an impact on mortality as well as morbidity (Touzard-Romo *et al.*,2013; Lin *et al.*,2020).

The current study was limited for sample size and the lack of local and international studies similar to this experiment, although there are very few studies that demonstrate the existence of viral particles linked to SARS-CoV-2, in addition, the current study did not determine the serotypes of Adenovirus, but rather estimated the presence of viral load with progress the severity regarding the infection with SARS-CoV-2.

4.2.8: Adenovirus Viral Load and CAR Receptors Concentration

The results in figure (4.11) showed significantly association between serum adenovirus viral load and soluble CAR receptors for all patients groups of COVID-19 infections performed these experiments. In all patients with COVID-19 infections, it was significantly increased in adenovirus viral load with increased COVID-19 pathogenicity compared to control as indicated by positive correlation for this parameter ($r= 0.458$, $p<0.001$), and mean (mild: 19.59; moderate:30.71 and severe :50.04 pg/ml), While the concentration of CAR receptors was decreased with

COVID-19 severity as indicated by negative correlation (r= -0.071, P= 0.046) and mean (control: 5.89 ; mild: 6.04; moderate:5.4 and severe :5.86 ng/ml).

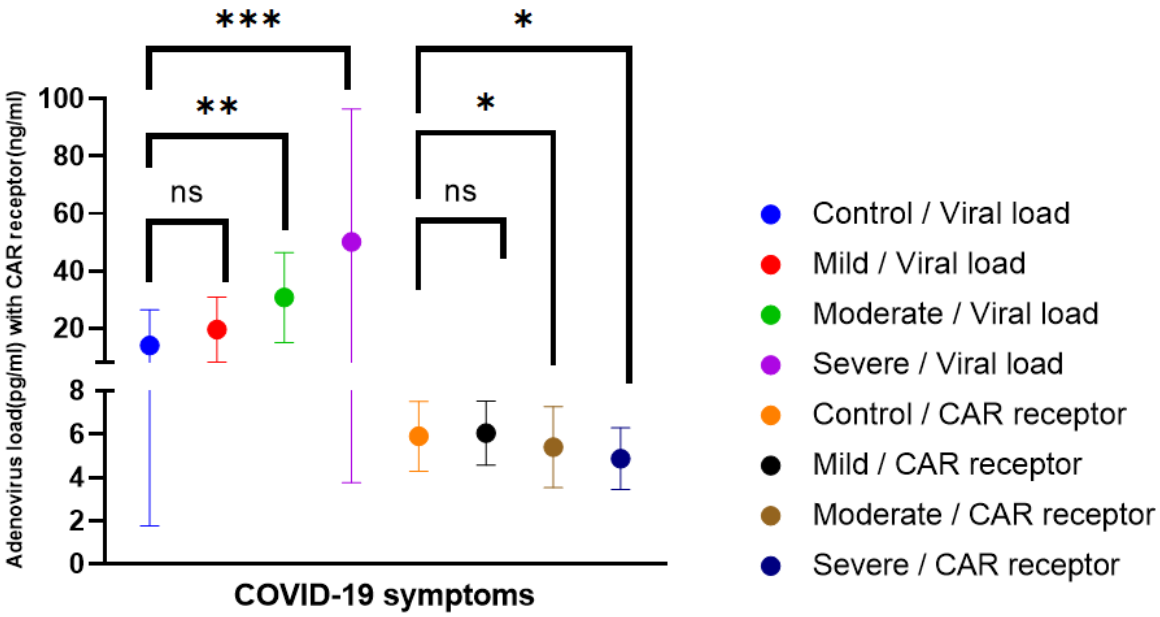


Figure (4.11): The adenovirus viral load with serum soluble CAR receptors, the significance value was indicated as * between the groups. The level of probability was indicated as *$P \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, Data was presented as mean, statistical test: Kruskal Wallis test, df: (3) , n =200.

Human adenoviruses exhibit an analogous affinity for human adenovirus receptor as their way of facilitating their entry into the body, nevertheless, the entire understanding of the impact of the amount of viral load with human adenovirus receptor production on disease severity remained unclear, the results of the current study were opposite to what this researcher had reached, as his study showed a positive relationship between virus load and receptor expression (Sharma *et al.*,2016). The transmembrane protein of human adenovirus receptor is found in cells of epithelium junctions with tight junctions. As its name implies, CAR receptor originally turned out to be the type of C adenovirus and group B coxsackievirus

receptor. Following this, human adenovirus receptor aids intercellular attachment, immune system cell activation, and intracellular communications, inflammation that persists and malignancies often deactivate CAR receptors. Mechanistic understanding of human adenovirus receptor within pathogenesis is inadequate. The results of the current study were opposite to what the researchers had reached, as their study showed a positive relationship between virus load and receptor expression (Nilchian *et al.*,2020).

4.2.9: Evaluation of Interleukin 6 Receptors (IL-6R) in Studied Groups

The serum level of interleukin-6-receptors of studied groups were shown in figure (4.12), the mean of IL-6R included (2.37, 3.87, 6.12 and 6.63 ng/ml) for control, mild, moderate and severe respectively, there was significant difference in distribution of patients and control groups according to symptoms with positive correlation (r= 0.695, p = <0.001).

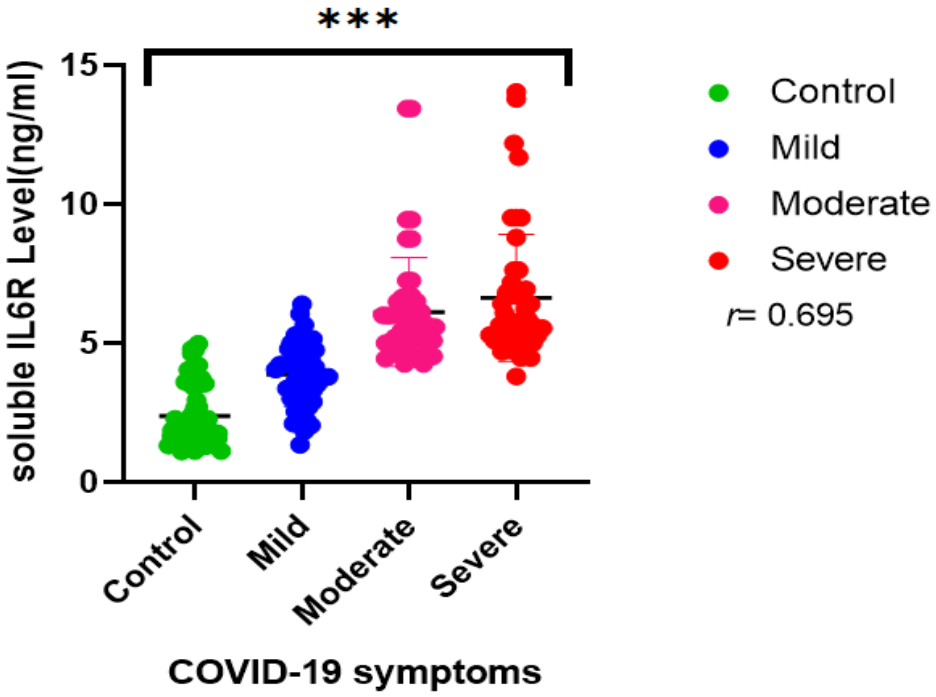


Figure (4.12) : The sIL-6R level in studied groups, the significance value was indicated as * between the groups. The level of probability was indicated as ****$p \leq$ 0.001, Data was presented as mean, statistical test: Kruskal Wallis test, df: (3) , n =200.

Macrophages represent a group of natural immune cells which possess the ability to detect and react to viral stimuli through the production inflammatory. This response serves to eradicate infections and facilitate the process of tissue healing . Nevertheless, an unregulated macrophage reaction may result in harmful consequences on the host's immune system, as evidenced by the activation of macrophages syndrome triggered by viral infections in severe status, such as those attributable to the closely associated virus SARS-CoV-2 (Merad and Martin, 2020).

There are multiple factors that contribute to the manifestation of severe sickness in persons who have contracted infection by SARS-CoV-2 include existing of a compromised interferon response, which may lead to a progression of serious illness. The pathogenesis of COVID-19 involves a significant contribution from an exaggerated inflammatory reaction due to the infectious agent SARS this reaction is thought to be having an essential impact in the disease's intensity and fatality rates. The condition is distinguished by heightened concentrations of cytokines in the circulatory system, significant reduction in macrophage count, and widespread infiltrating of mononuclear cells that occur in several organs including the heart and arteries, lungs, lymph nodes, as well as kidneys apparatus (Hadjadj *et al.*,2020).

The findings of this study were roughly in line with what the researchers, as their study showed a positive relationship between Interleukin 6 receptors circulating level and COVID-19 pathogenicity, when the researcher studied the same genetic mutation in patients (Bovijn *et al.*,2020). Reported previous that he conducted on 46 individuals that exhibit COVID-19 symptoms, 46 The study included individuals diagnosed with asymptomatic COVID-19 with a control group consisting of 46 individuals who were deemed healthy, it is worth noting that the researcher noticed a rise in IL-6R expression in tandem with the development of the illness (Mohammadisoleimani *et al.*,2022). The IL6 and IL-6R-mediated a common characteristic patient viral infected is of storm cytokine, in addition, the protein

concentration of IL6 and IL-6R exhibited arise, while its messenger RNA level remained unaltered, suggesting that its overexpression was occurring within the translational step. The upregulation of IL6 and IL-6R production at the protein level can be observed in individuals affected with COVID-19 (Wang *et al.*,2022a).

Additionally, (Hamilton *et al*.,2023) mentioned sepsis occurrence was directly correlated with IL-6R during the initial days in intensive care unit. wherein the administration of IL-6 receptor inhibition has been demonstrated to enhance survival rates in cases with severe COVID-19.

The study findings indicate that the administration of tocilizumab during the initial two days in intensive care unit admission was found to be related with a decreased risk of fatality among hospital conditions, as compared to patients did not get early treatment by tocilizumab. Tocilizumab, a monoclonal antibody that focuses on the binding protein of interleukin 6, has the potential to mitigate the condition characterized by the production of inflammatory mediators in individuals suffering via COVID-19 (Gupta *et al*.,2021).

4.2.10: Correlation between CCR2 with COVID-19 infection

The serum level of CCR2 of patients and control groups illustrated in figure (4.13), the mean of serum level of CCR2 included (94.27, 157.8, 251.5 and 269.0 ng/ml) for control, mild, moderate and severe respectively, there was significant difference in distribution of patients and control groups according to symptoms with strong positive relationship ($r= 0.564$, $p = <0.001$).

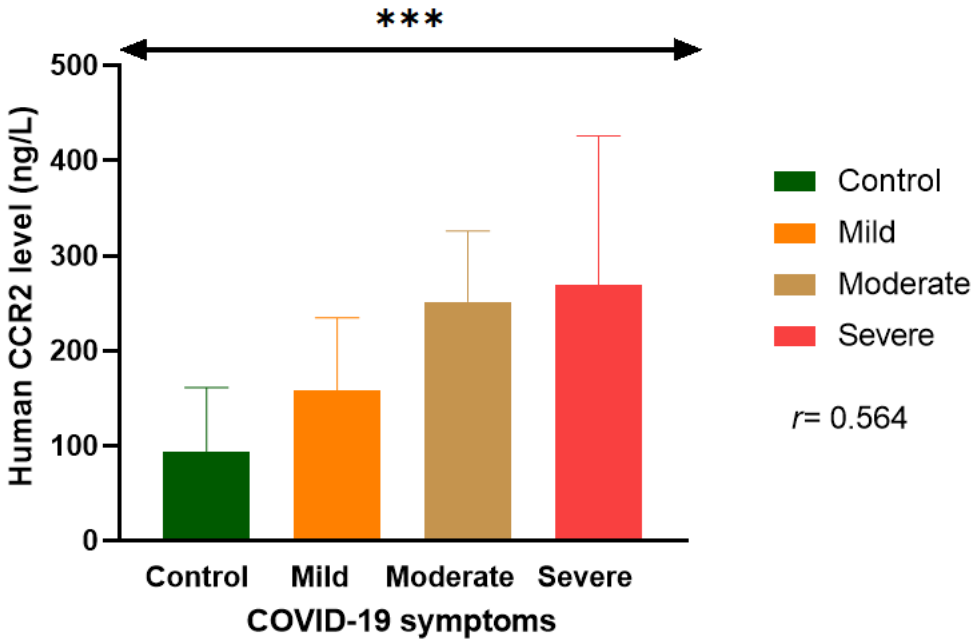


Figure (4.13) : The CCR2 level in studied groups, the significance value was indicated as * between the groups. The level of probability was indicated as ***$p \leq$ 0.001, Data was presented as mean, statistical test: Kruskal Wallis test, df: (3) , n =200.

Chemokines are known to exert a significant influence on the cellular trafficking process that occurs throughout the immune system's response (Dejnirattisai *et al.*,2022). In the context of a bronchial viral infection, the immune response triggers the production of cytokines that are inflammatory along with chemokines. In the case of SARS-CoV-2 infection, there was an attraction of pro-inflammatory cells with the leukocytes to the affected localized tissue. This recruitment was accompanied by an increase in the expression of cytokines as well as chemokines in the cells that have been infected by the strain of virus (Ugel *et al.*,2015).

In this study the CCR2 level consistent with international study in Iran country by (Sharif-Zak *et al.*, 2022) whom collected 470 patients and the findings of the study demonstrated a statistically significant (P<0.0001), increase in CCR2 levels among the participants in the sick group in contrast with healthy control group. and (Stikker

et al.,2022), concluded the CCR2 genetic variations enhance the risk of developing severe COVID-19 via modifying genes regulation of monocyte-macrophage chemotactic receptor production. therefore, increased macrophage and monocyte migratory the ability may be linked to worsening responses to inflammation as well as more serious illness.

Based on the findings collected from multiple studies investigating the mechanisms underlying COVID-19 pathogenesis, a significant dangerous occurrence in cases of serious infectious in SARS-CoV-2 involves infiltration of an inflammation response monocytes in addition macrophages. Furthermore, the imbalanced inflammatory processes resulting from the activity of these types of cells as well their ensuing release of inflammatory cytokines is deemed crucial (Ranjbar *et al.*,2022).

Jafarzadeh *et al*. (2021) showed an increase in MCP-1 levels, which is a ligand for CCR2, in patients with COVID-19, particularly those in critical condition. This elevation of MCP-1 contributes to the activation of inflammatory mechanisms through attaching with CCR2. MCP-1, a chemokine belonging to the group of cytokines storm-related of chemokines, has been identified as an important trigger toward inflammation of the tissues with potential organ dysfunction (Jafarzadeh *et al.*, 2020). Therefore, the modulation of the MCP-1/CCR2 axis has the potential to mitigate undesired responses to inflammation as well as serve as a therapeutic intervention (Bianconi *et al.*, 2018).

The CCR2 receptor is known to have a play a crucial function in the beginning of inflammatory reactions in addition to advancement diseases associated with inflammation. Furthermore, the binding of CCR2 through MCP-1 facilitates the immune system natural reaction via attracting monocytes towards areas of inflammation (Bianconi *et al.*, 2018).

The route between CCL2(MCP-1) and CCR2 serves an important part in the migration of lymphocytes as well as monocytes/macrophages, which can be linked to the occurrence of certain diseases, Included infections attributable to viruses (Fantuzzi *et al.,* 2019). The upregulation the CCR2 within peripheral B cells as well as T cells during the course of infectious illnesses indicates a significant role of CCR2 in the regulation of the immune reaction (Hodge *et al.,* 2012). Furthermore, the suppression of the CCR2 pathway has been shown to decrease the concentrations of storm-related cytokines mediators, such as interleukin-6, circulating tumor necrosis factor α, interferon-γ, as well as macrophage inflammatory processes, thereby impacting the immune system's response, The findings emphasized the significance of CCR2 in relation to the circumstances of coronavirus infections (Dessing *et al.,* 2007). According to Vanderheiden *et al.,* (2021), it has been demonstrated that parenchymal of monocyte-derived cells exhibit a defensive role against SARS-CoV-2. This was evidenced by the fact that animals lacked CCR2 displayed higher concentrations of viral particles in the respiratory tract, increased diffusion of the virus inside the lungs, with heightened cytokines associated with inflammation responses.

4.3. Genetic study

4.3.1 Gene polymorphism of *IL-6R*

The targeted region of partial sequence of *IL-6R* covering the SNPs rs4845374 and rs2228145 as Figure (4.14). In addition to Figure (4.15) was illustrated result of amplification *IL-6R* region with flanking primers, PCR products 491bp for control and COVID-19 patients. The frequency distribution of genotypes according to groups based on Hardy Weinberg equilibrium is shown in tables (4.2), (4.6), There was significant correlation in the frequency distribution of observed and expected count of *IL-6R* for (rs4845374 and rs2228145 SNPs) in all patients compared with control

groups, present study explains significantly for both SNPs (p=0.0097, 0.0001) respectively.

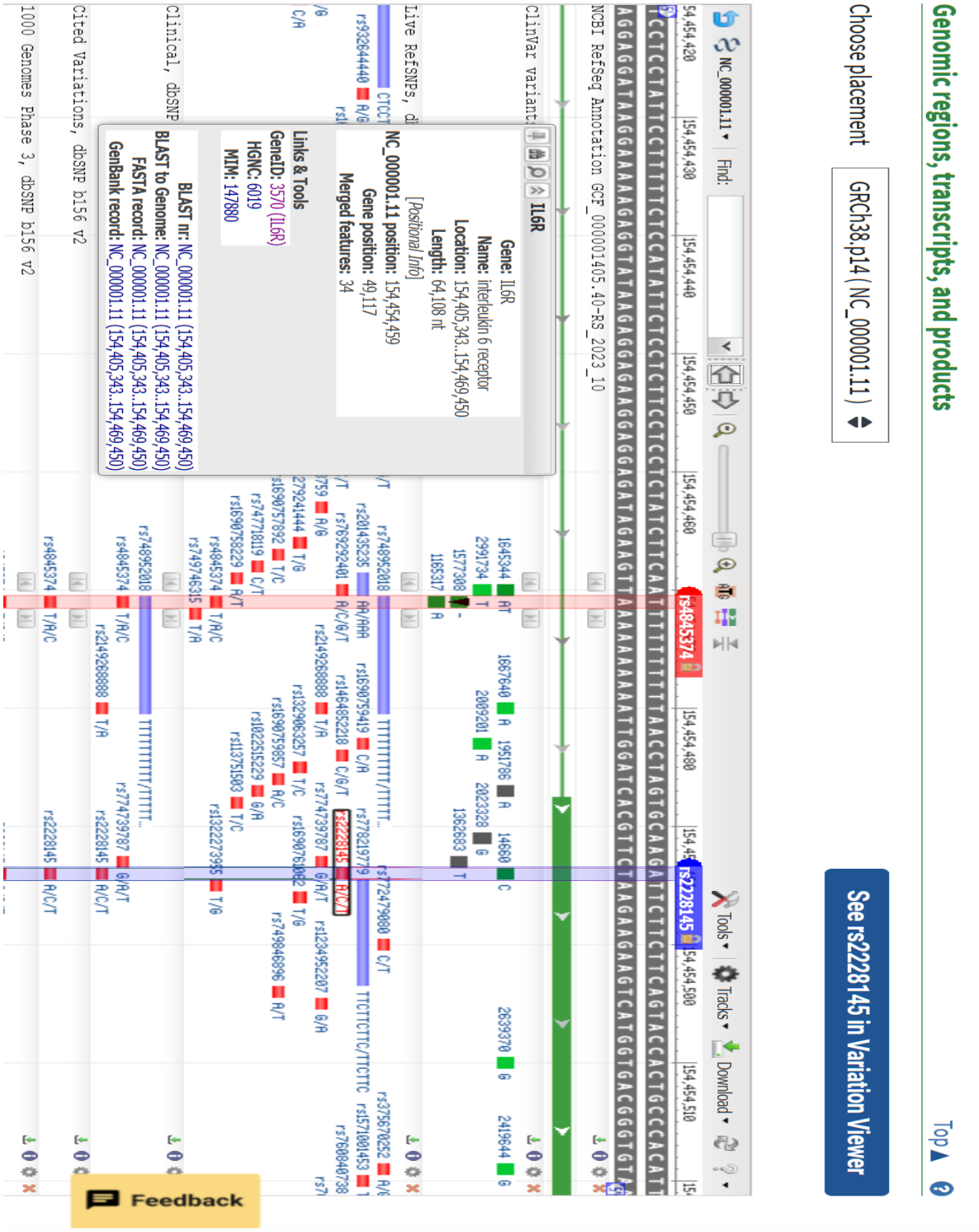


Figure (4.14): The targeted region of partial sequence of *IL-6R* covering the SNPs rs4845374 and rs2228145 (NCBI website).

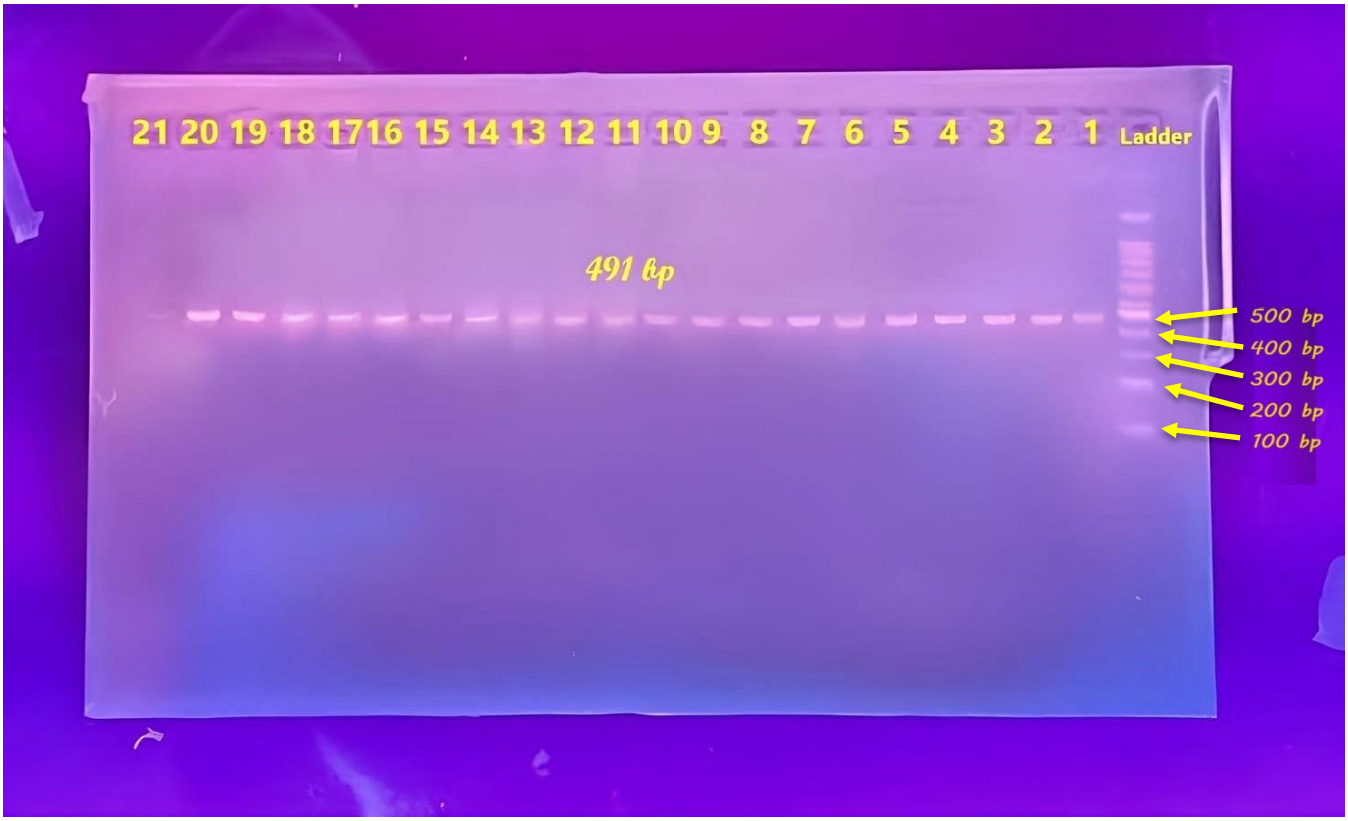


Figure (4.15): Gel electrophoresis of Target DNA region *IL-6R* for study groups, Amplification region with flanking primers, number of patients PCR products 491bp , Ladder = 100bp for each step.

Table (4.2) : Frequency distribution of *IL-6R* genotypes (rs4845374) SNP according to patient groups based on Hardy Weinberg equilibrium

Genotype rs4845374	Control n = 50		Mild n = 50		Moderate n = 50		Severe n = 50	
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
TT	40	30.25	27	30.25	29	30.25	25	30.25
T/A	10	16.75	20	16.75	19	16.75	18	16.75
AA	0	3	3	3	2	3	7	3
$\chi 2$	11.39							
P	0.0097*							

Number of cases (n); (χ^2) = Qi square; significance level (*) at $p \leq 0.05$, df: (3)

The comparison of frequency of *IL-6R* genotypes (rs4845374 T/A/C) as well as the differences in alleles between the COVID-19 patients and control group observed in tables below (4.3), (4.4) and (4.5) with figure (4.16) and (4.17).

Table (4.3): Genotypes and Alleles frequencies of *IL-6R* (rs4845374) SNP in COVID-19 patient group of mild status

Genotype rs4845374	Mild n = 50	Control n = 50	P	OR	95 % CI
TT	27	40	References		
T/A	20	10	0.0183	0.3375	0.1369 to 0.8322
AA	3	0	0.1278	0.0970	0.0048 to 1.9533
Allele	Mild n(allele) = 100	Control n(allele) = 100	P	OR	95 % CI
T	74	90	References		
A	26	10	0.0044	0.3162	0.1433 to 0.6979

(*n*)= cases;(OR)= odds ratio;(CI) =Confidence Interval; Significant: p<0.05, df: (3)

Table (4.4): Genotypes and Alleles frequencies of *IL-6R* (rs4845374) SNP in COVID-19 patient group of moderate status

Genotype rs4845374	Moderate n = 50	Control n = 50	P	OR	95 % CI
TT	29	40	References		
T/A	19	10	0.0364	0.3816	0.1547 to 0.9411
AA	2	0	0.2192	0.1457	0.0067 to 3.1484
Allele	Moderate n(allele) = 100	Control n(allele) = 100	P	OR	95 % CI
T	77	90	References		
A	23	10	0.0157	0.3720	0.1667 to 0.8298

(*n*)= cases;(OR)= odds ratio;(CI) =Confidence Interval; Significant: p<0.05, df: (3)

Table (4.5): Genotypes and Alleles frequencies of *IL-6R* (rs4845374) SNP in COVID-19 patient group of severe status

Genotype rs4845374	Severe n = 50	Control n = 50	P	OR	95 % CI
TT	25	40	References		
T/A	18	10	0.0243	0.3472	0.1383 to 0.8717
AA	7	0	0.0324	0.0420	0.0023 to 0.7669
Allele	Severe n(allele) = 100	Control n(allele) = 100	P	OR	95 % CI
T	68	90	References		
A	32	10	0.0003	0.2361	0.1086 to 0.5134

(*n*)= cases;(OR)= odds ratio;(CI) =Confidence Interval; Significant: p<0.05, df: (3)

Genotype TA mutant type (rs4845374-*IL-6R*) explained in tables (4.3), (4.4) and (4.5) was significantly frequent in mild, moderate and severe groups comparing with control group, (p =0.0183, 0.0364 and 0.0243) respectively; therefore, it was considered a protective factor, it demonstrated an odds ratio of (0.3375, 0.3816 and 0.3472) respectively in three patients groups as demonstrated in figure (4.16) and (4.17). While Genotype AA mutant type was more frequent significantly in severe group in comparison with other groups, (p =0.0324); therefore, with an odds ratio of (0.0420), it was regarded as a protective factor for severe group as showed in figure (4.16) and (4.17). Allele A was more significantly frequent in mild, moderate and severe groups comparing with control group, (p =0.0044, 0.0157 and 0.0003) respectively; therefore, it was considered a protective factor, it demonstrated an odds ratio of (0.3162, 0.3720 and 0.2361) respectively in three groups as demonstrated in figure (4.16) and (4.17).

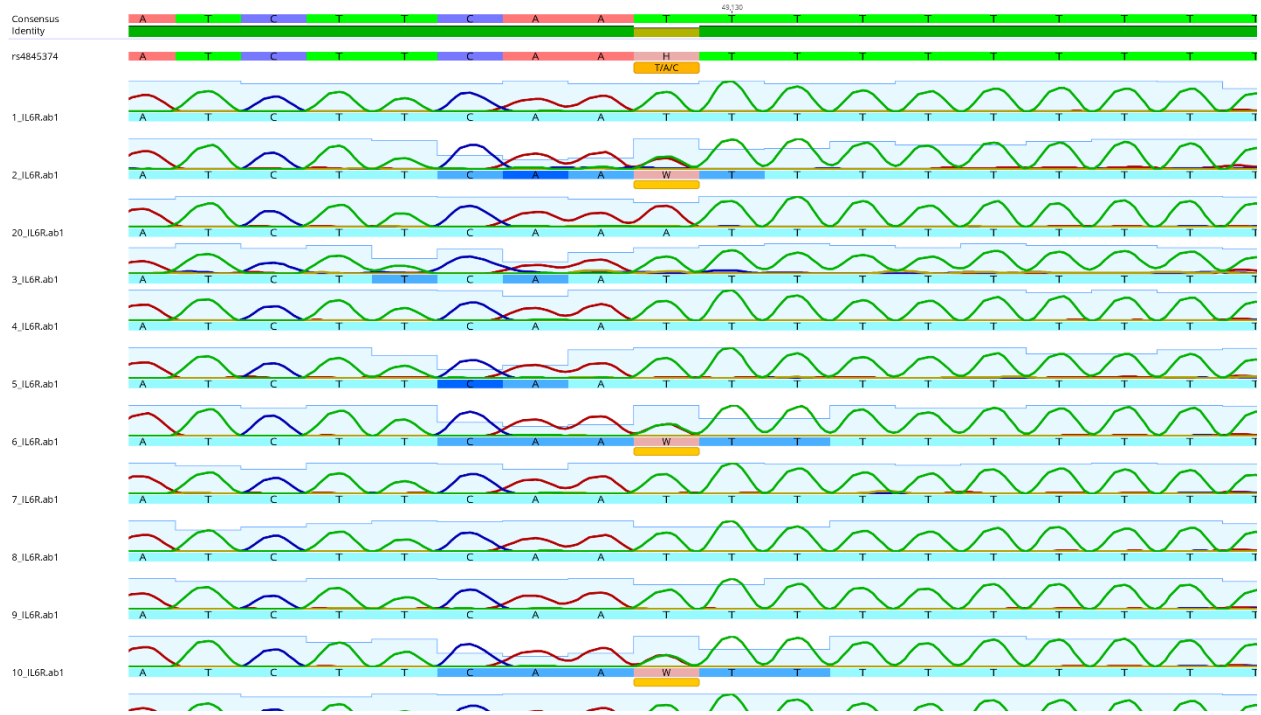


Figure (4.16): Sequencing of *IL-6R* rs4845374 SNP

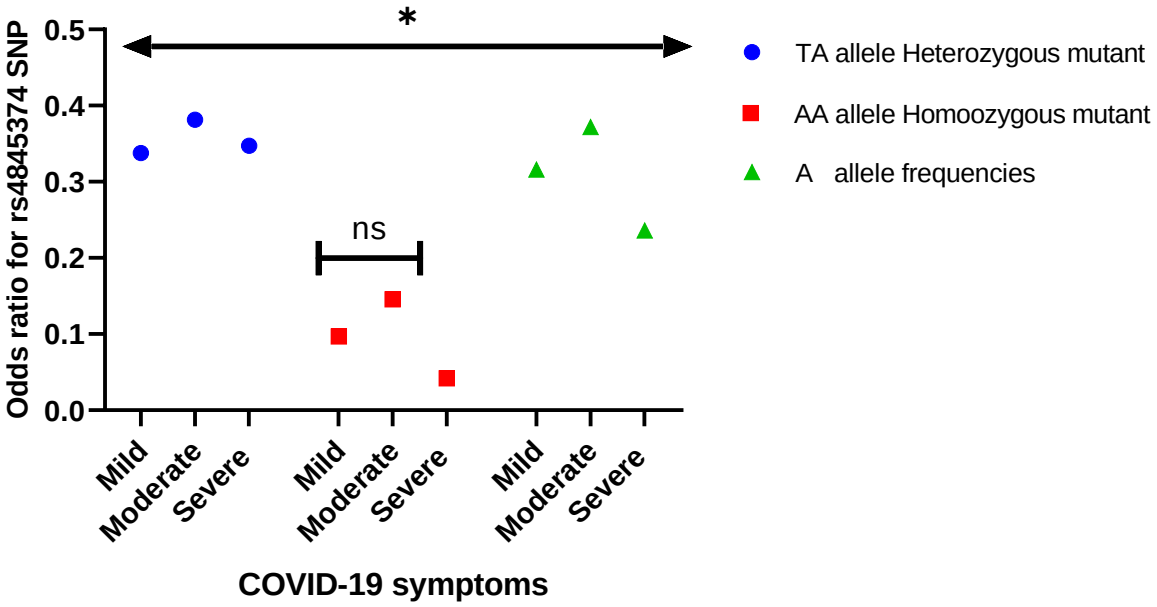


Figure (4.17): Genotypes and Alleles frequencies of *IL-6R* rs4845374 SNP in studied groups, the significance value was indicated as * between the groups. The level of probability was indicated as* $P \leq 0.05$., Data was presented as mean, statistical test: Kruskal-Wallis test, df: (3) , n =200.

Multiple genes regulate the immune response in *vivo*, which determines the organism's response to various microbial infections (Yu *et al.*,2018). Interleukin 6 is secreted by T helper2 cells and regulation of the release of inflammatory mediators during respiratory infection, also, many biological reactions of IL-6 appear after specialized binding between IL-6 and its receptors. Therefore, the locus polymorphisms associated with susceptible person to different response such as the study done by (Yu *et al.*,2018) in which they studied a correlation between *IL-6R* rs12083537 with pediatric asthma susceptibility, and they concluded there was a correlation between polymorphism of *IL-6R* rs12083537 locus and bronchial asthma susceptibility, but this study similar to current results in *IL-6R* rs4845374. This may indicate that correlation between SNP polymorphism and bronchial infection vary greatly depended on both genetic and environmental factors (Lee *et al.*,2014a).

Table (4.6) : Frequency distribution of *IL-6R* genotypes (rs2228145) SNP according to COVID-19 patient groups based on Hardy Weinberg equilibrium

Genotype rs2228145	Control n = 50		Mild n = 50		Moderate n = 50		Severe n = 50	
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
AA	30	12.0	5	12.0	15	12.0	10	12.0
A/C	20	27.25	35	27.25	30	27.25	25	27.25
CC	0	0.75	10	0.75	5	0.75	15	0.75
$\chi 2$	44.545							
P	0.0001*							

(*n*)= cases; (χ^2) = Qi square; (*) = significant at p < 0.05, df: (3)

The comparison of frequency of *IL-6R* genotypes (rs2228145 A/C/T) as well as the differences in alleles between the COVID-19 patients and control group observed in tables below (4.7), (4.8) and (4.9) with figure (4.18) and (4.19).

Table (4.7): Genotypes and Alleles frequencies of *IL-6R* (rs2228145) SNP in COVID-19 patient group of mild status

Genotype rs2228145	Mild n = 50	Control n = 50	P	OR	95 % CI
AA	5	30	References		
A/C	35	20	0.0001	0.0952	0.0319 to 0.2846
CC	10	0	0.0017	0.0086	0.0004 to 0.1689
Allele	Mild n(allele) = 100	Control n(allele) = 100	P	OR	95 % CI
A	45	80	References		
C	55	20	0.0001	0.2045	0.1091 to 0.3836

(*n*)= cases;(OR)= odds ratio;(CI) =Confidence Interval; Significant: p<0.05, df: (3)

Table (4.8): Genotypes and Alleles frequencies of *IL-6R* (rs2228145) SNP in COVID-19 patient group of moderate status

Genotype rs2228145	Moderate n = 50	Control n = 50	P	OR	95 % CI
AA	15	30	References		
A/C	30	20	0.0103	0.3333	0.1440 to 0.7715
CC	5	0	0.0417	0.0462	0.0024 to 0.8906
Allele	Moderate n(allele) = 100	Control n(allele) = 100	P	OR	95 % CI
A	60	80	References		
C	40	20	0.0024	0.3750	0.1992 to 0.7059

(*n*)= cases;(OR)= odds ratio;(CI) =Confidence Interval; Significant: p<0.05, df: (3)

Table (4.9): Genotypes and Alleles frequencies of *IL-6R* (rs2228145) SNP in COVID-19 patient group of severe status

Genotype rs2228145	Severe n = 50	Control n = 50	P	OR	95 % CI
AA	10	30	References		
A/C	25	20	0.0052	0.2667	0.1056 to 0.6733
CC	15	0	0.0024	0.0111	0.0006 to 0.2023
Allele	Severe n(allele) = 100	Control n(allele) = 100	P	OR	95 % CI
A	45	80	References		
C	55	20	0.0001	0.2045	0.1091 to 0.3836

(*n*)= cases;(OR)= odds ratio;(CI) =Confidence Interval; Significant: p<0.05, df: (3)

Genotype AC mutant type (rs2228145-*IL-6R*) explained in tables (4.7), (4.8) and (4.9) was more significantly frequent in mild, moderate and severe groups comparing with control group, (p =0.0001, 0.0103 and 0.0052) respectively; therefore, it was considered a protective factor, it demonstrated an odds ratio of (0.0952, 0.3333 and 0.2667) respectively in three groups as demonstrated in figure (4.18) and (4.19). While Genotype CC mutant type was more frequent significantly in severe group in comparison with other groups, (0.0024); therefore, with an odds ratio of (0.0111), it was regarded as a protective factor for severe group as showed in figure (4.18) and (4.19). Allele C was more significantly frequent in mild, moderate and severe groups comparing with control group, (p =0.0001, 0.0024 and 0.0001) respectively; therefore, it was considered a protective factor, it demonstrated an odds ratio of (0.2045, 0.3750 and 0.2045) respectively in three groups as demonstrated in figure (4.18) and (4.19).

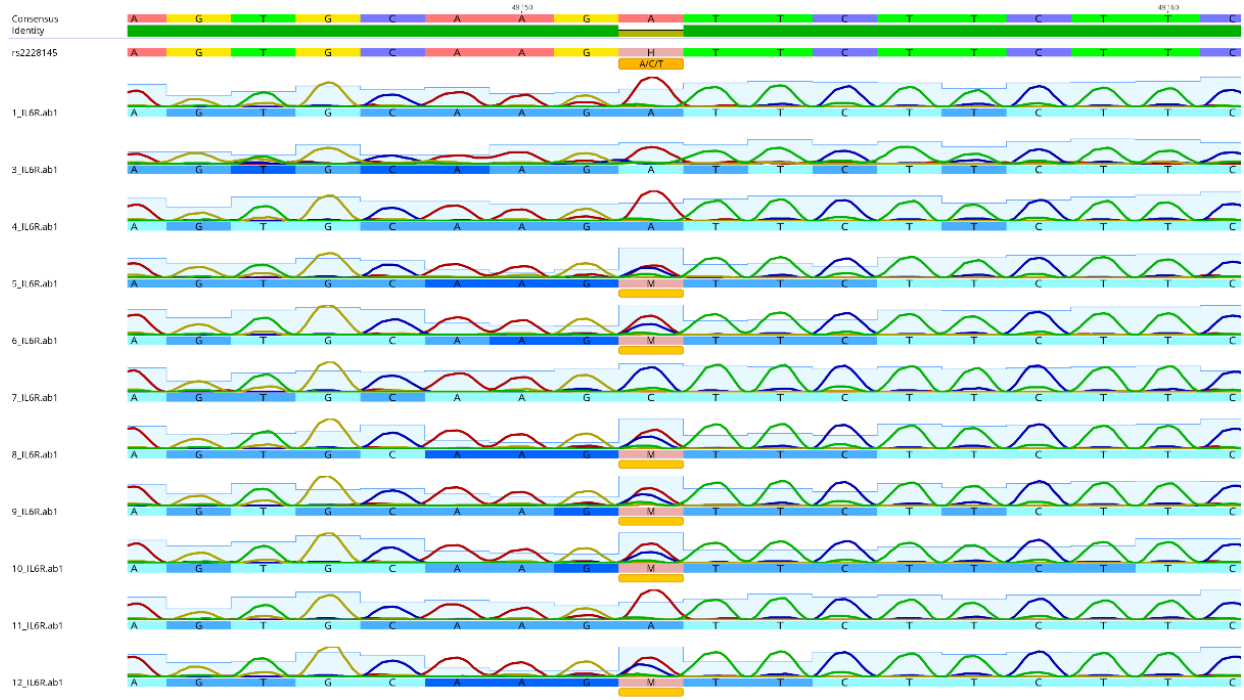


Figure (4.18): Sequencing of *IL-6R* rs2228145 SNP

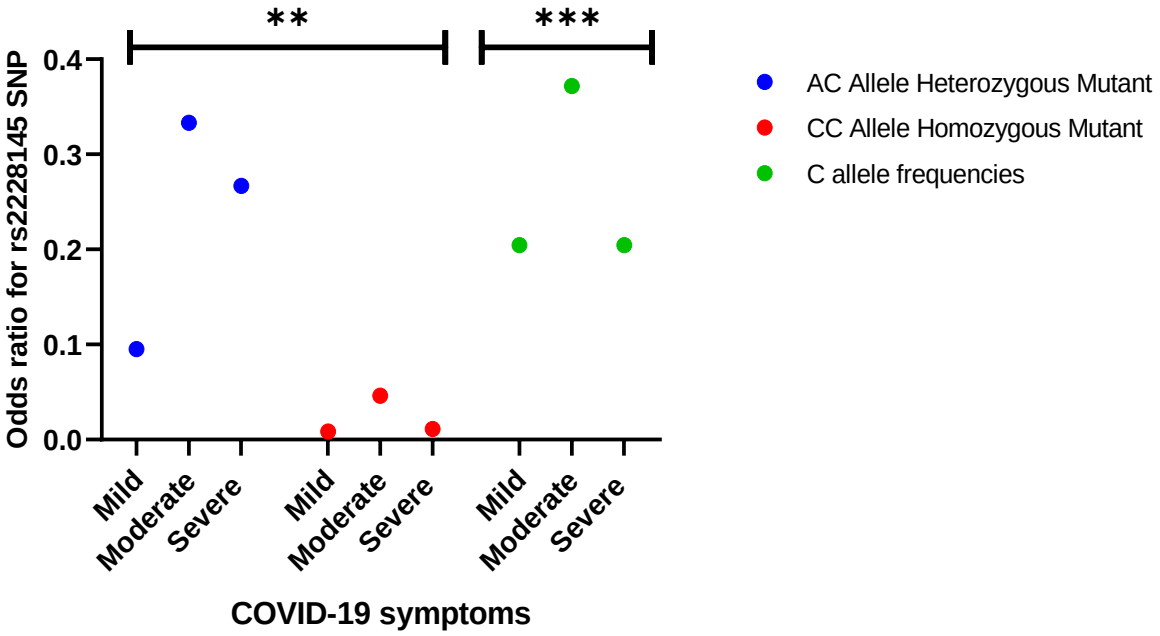


Figure (4.19): Genotypes and Alleles frequencies of *IL-6R* rs2228145 SNP in studied groups, the significance value was indicated as * between the groups. The level of probability was indicated as $**p \leq 0.01$, $***p \leq 0.001$, Data was presented as mean, statistical test: Kruskal-Wallis test, df: (3) , n =200.

The SARS-CoV-2 disease has the potential to impact the cardiovascular system, leading to a variety of consequences, such as acute myocardial damage or renal failure. the myocardium can be suffered damage by either direct viral invasion or indirect processes, which are sustained by systemic inflammation and immune-mediated responses, such as the activation of IL-6/IL-6R signaling pathways (Del Prete *et al.*,2022).

The function of Interleukin-6 is influenced by a specific single nucleotide polymorphism (SNP) known as rs2228145 A/C, which is located in the *IL-6 receptor* (*IL-6R*) gene. Individuals who carry the C allele exhibit a reduced inflammatory response and a lower incidence of ischemic heart disease. The lack of a substantial association between the rs2228145 polymorphism of *IL-6R* and long-term mortality following ST elevation myocardial infarction may account for this observation (Szpakowicz *et al*.,2017), Single nucleotide polymorphisms (A>C rs2228145) of Interleukin 6 receptors it was determined for statistically significant connected with development COVID-19 disease. This current result is in accordance with that obtained by (Batur and Hekim,2020). who found significant effect of this SNP (*interleukin 6 Receptors*) Regarding COVID-19 incidence and mortality rate. In current study almost similarity to obtained by (Rodrigues *et al.*,2023).

The traditional cellular signaling pathway of interleukin-6, the interaction of interleukin-6 to its receptor leads to the creation of a complexities. This complex then interacts with the transmembrane glycoprotein 130, beginning the transduction of intracellular signals. This signaling cascade subsequently generates multiple biological activity, the events including proliferation of cells, differentiating themselves, and stress to cells are considered., and immune system regulation. Notably, IL-6 signaling promotes the proliferation and stimulation of T lymphocyte populations, as well as B lymphocytes specialization. Additionally, it controls the acute phase of the reaction and the secretion of numerous cytokines. Within

circumstances of COVID-19 severe symptoms, a considerable proportion individuals undergo a widespread inflammatory reaction referred to as cytokines releasing syndrome, which is a major contributor to mortality (Zhang *et al.*,2020b). Nevertheless, the conducted assessment did not provide a highly significant association with average frequency of occurrence of rs2228145 polymorphisms within the *IL-6R* genes. This lack of correlation can likely be attributed to the inherent genetic diversity observed in immunological responses throughout various communities (Batur and Hekim,2020).

The genetic variations of *IL-6R* mutations at the rs2228145 locus exhibit biological variety. were investigated, and the findings indicated variations among various populations in Italy, Poland, Mexico, Spain, Russia, Brazil, Japan, Netherland frequently which have AC genotype but AA genotype located in the rs2228145 gene is observed in communities of Indian, Swedish, and South African descent. Notably, the CC genotype exhibited the greatest occurrence exclusively within the UK community, and the researchers concluded the relationship across the frequencies of rs2228145 variation in the *IL-6R* gene, alongside the COVID-19 occurrence and varying fatality rates across different nations (Batur and Hekim,2021).

In addition to the similarity in the results of the researchers (Rashidi *et al.*, 2022) in choosing the same SNP, but their study focused on post COVID patients, where they obtained frequencies for the CC allele of their patients with the progression of disease severity.

The previous investigation observed a lack of significant relationship between the frequency of rs1800796/rs1800795 and rs2228145 variations in the *IL-6* as well as *IL-6R* genes, respectively. This outcome was likely attributable to the inherent variability in genetic factors of immunological characterization among various populations. Hence, the involvement of IL-6 with IL-6R in COVID-19 infection is

not considered to be only attributed to susceptible genes, since there may be additional single nucleotide polymorphisms (SNPs) within these genes that might possibly contribute to the control the expression of these genes. The link between the regulation of the *IL-6* as well as *IL-6R* genes with viral infection has to be determined by more research on associations involving additional SNPs that may change the amount of the expression of genes (Batur and Hekim,2021). The genetic variations in the *IL-6R* locus are being demonstrated to exert influences on a diverse array of clinical along with biochemical characteristics, therefore, the *IL-6R* variants usually raise the possibility of contracting COVID-19 (Hamilton *et al.*,2023).

4.3.2 Gene polymorphism of *CCR2*

The targeted region of partial sequence of *CCR2* covering the SNPs rs1799864 as Figure (4.20). The frequency distribution of genotypes according to groups based on Hardy Weinberg equilibrium is shown in table (4.10), There was significant correlation in the frequency distribution of observed and expected count of *CCR2* for rs1799864 SNP in all patients compared with control groups, present study explains significantly for both SNP (p=0.0001) respectively.

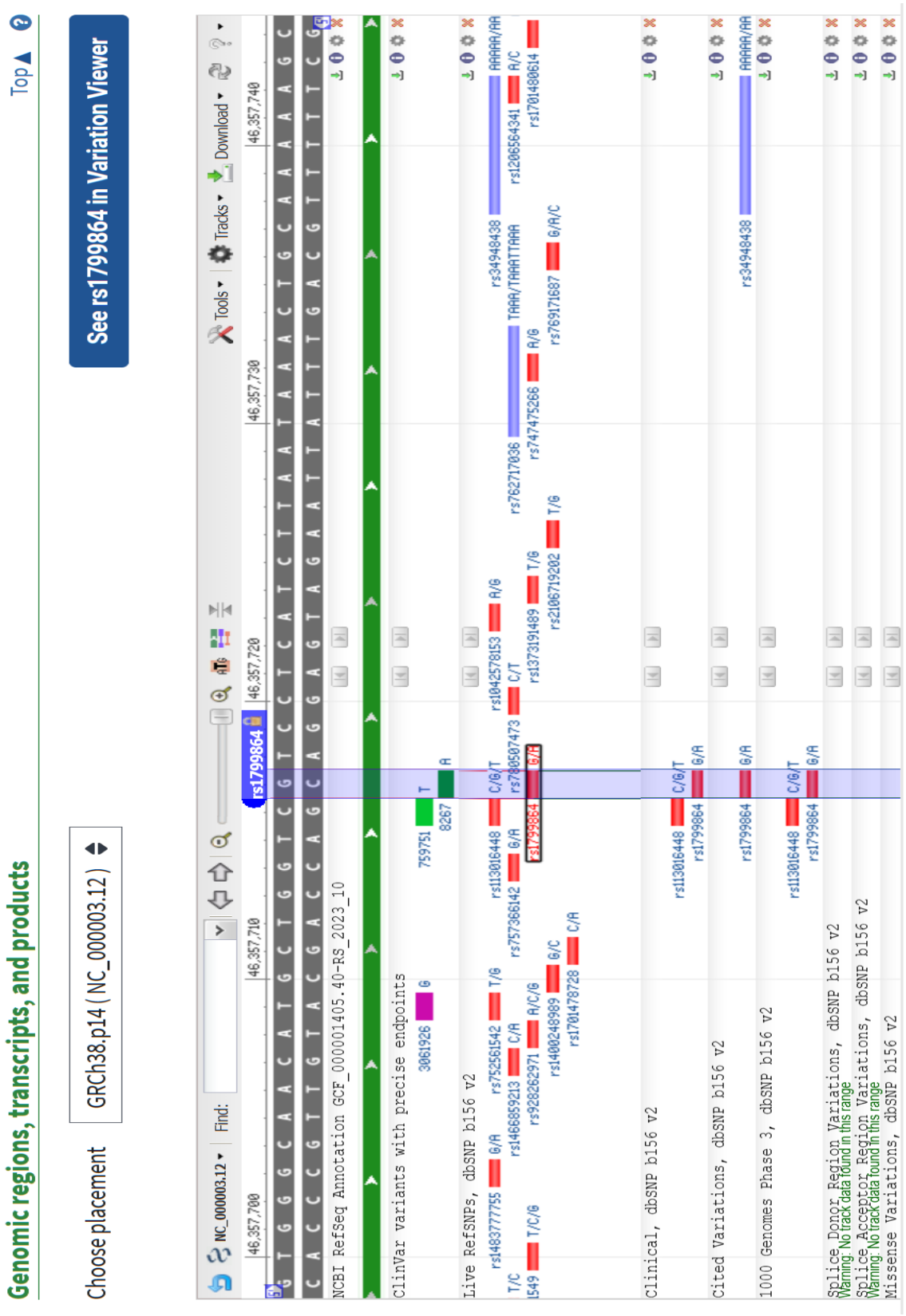


Figure (4.20): The targeted region of partial sequence of *CCR2* covering the SNP rs1799864 (NCBI website).

Table (4.10): Frequency distribution of *CCR2* genotypes (rs1799864) SNP according to COVID-19 patient groups based on Hardy Weinberg equilibrium

Genotype *CCR2* rs1799864	Control n = 50		Mild n = 50		Moderate n = 50		Severe n = 50	
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
GG	16	15	20	15	8	15	4	15
GA	33	27.5	30	27.5	42	27.5	46	27.5
AA	1	7.5	0	7.5	0	7.5	0	7.5
χ^2	62.618							
P	0.0001*							

(*n*)= cases; (χ^2) = Qi square; (*) = significant at p < 0.05, df: (3)

The occurrence of *CCR2* genotyping (rs1799864) as well as alleles was compared between the control group and also COVID-19 patients groups in the tables (4.11), (4.12) and (4.13) in addition to figures (4.21) and (4.22).

Table (4.11): Genotypes and Alleles frequencies of *CCR2* rs1799864 SNP in COVID-19 patient group of mild status

Genotype rs1799864	Mild n = 50	Control n = 50	P	OR	95 % CI
GG	20	16	Reference		
GA	30	33	0.4480	0.7273	0.3195 to 1.6555
AA	0	1	0.4297	0.2683	0.0102 to 7.0285
Allele	Mild n(allele) = 100	Control n(allele) = 100	P	OR	95 % CI
G	70	65	Reference		
A	30	35	0.4507	0.7959	0.4398 to 1.4403

(*n*)= cases;(OR)= odds ratio;(CI) =Confidence Interval; Significant: p<0.05, df: (3)

Table (4.12): Genotypes and Alleles frequencies of *CCR2* rs1799864 SNP in COVID-19 patient group of moderate status

Genotype rs1799864	Moderate n = 50	Control n = 50	P	OR	95 % CI
GG	8	16	Reference		
GA	42	33	0.0573	2.5455	0.9713 to 6.6707
AA	0	1	0.7963	0.6471	0.0237 to 17.647
Allele	Moderate n(allele) = 100	Control n(allele) = 100	P	OR	95 % CI
G	58	65	Reference		
A	42	35	0.3096	1.3448	0.7594 to 2.3815

(*n*)= cases;(OR)= odds ratio;(CI) =Confidence Interval; Significant: p<0.05, df: (3)

Table (4.13): Genotypes and Alleles frequencies of *CCR2* rs1799864 SNP in COVID-19 patient group of severe status

Genotype rs1799864	Severe n = 50	Control n = 50	P	OR	95 % CI
GG	4	16	Reference		
GA	46	33	0.0051	5.4118	1.6597 to 17.646
AA	0	1	0.5301	3.6667	0.0635 to 211.77
Allele	Severe n(allele) = 100	Control n(allele) = 100	P	OR	95 % CI
G	54	65	Reference		
A	46	35	0.1140	1.5820	0.8958 to 2.7940

(*n*)= cases;(OR)= odds ratio;(CI) =Confidence Interval; Significant: p<0.05, df: (3)

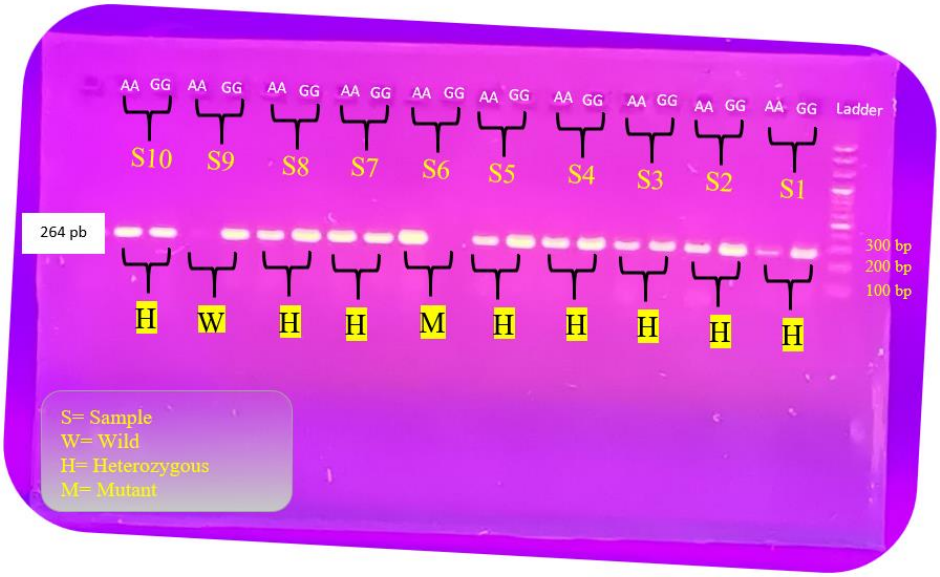


Figure (4.21): - Ethidium bromide-stained agarose gel for *CCR2* polymorphism, the Allele specific primer -PCR products for *CCR2* (rs1799864) polymorphism, (GG wild genotype), (GA Heterozygote mutant genotype) and (AA homozygote mutant genotype).

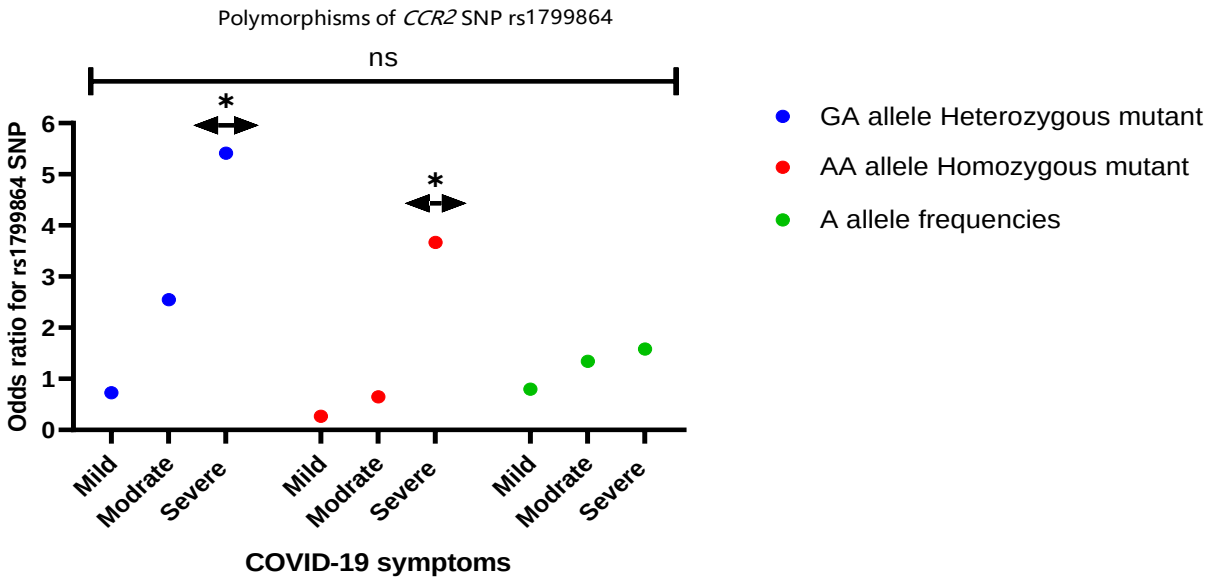


Figure (4.22): Genotypes and Allele frequencies of *CCR2* SNP rs1799864 in studied groups, a significance value was indicated as * between the groups. The level of probability was indicated as * $p < 0.05$., Data was presented as mean, statistical test: Kruskal Wallis test, df: (3) , n =200.

The genotype GA considered Heterozygous mutant type was more frequent in moderate and severe groups in comparison with control group (p =0.0573, 0.0051) respectively; therefore, it was odds ratio of (2.5455, 5.4118) receptively, therefore, it was considered a risk factor for those with moderate and severe conditions. While the opposite occurs in the mild group in comparison with control group. Genotype AA mutant type was absent patients group in comparison with control group. Allele A was more frequent in patients with moderate and severity group (p= 0.3096, 0.1140); therefore, it was with an odds ratio of (1.3448, 1.5820) in comparison with group control, it was regarded simply a risk factor for both patient grouping.

Notably, (Schmiedel *et al.*, 2020) looked at how 679 COVID-19-risk changes affected in process of gene expression to numerous kinds of immune cells system. expression of eleven genes that encode proteins was strongly associated with high COVID-19-risk variations, which also associated with targeted gene promoters or as cis-regulatory areas which interact with target promoters within the cell types, for instance, and found that the *CCR2* activator in monocytes preferentially interacts with a functioning cis-regulatory location, which is probably the mechanism mediating the relationship between variations at the 3p21.31 risk locus and *CCR2* expression in traditional monocytes.

An additional investigator conducted a study aimed at finding variations in a single nucleotide in genes that are related with sensitivity or the serious effects of COVID-19. In one study, a cohort of 319 genetic DNA specimens collected from patients exhibiting different levels of disease severity, together with 78 controlling DNA, were analyzed. The investigation focused on the association between the odds of illness or life-threatening symptoms as well as the presence of seven single nucleotide polymorphisms (SNPs). Notably, statistical analysis revealed a significant correlation between the rs1799864 SNP located within the *CCR2* gene and the likelihood of

developing sickness or experiencing severe symptoms (allele A, OR = 2.21, 95% CI 1.12–4.39, p = 0.015) (Minashkin *et al.*,2022).

Conclusions and Recommendations

Conclusions:

1. The mean age of the mild COVID-19 infection patients is less than the mean age of the moderate and severe cases.
2. Human COVID-19 Spike protein and Adenovirus viral load (as coinfection) increased blood viremia blood appears in the most severe cases (moderate to severe) but less in the mild cases.
3. The levels of COVID-19 Antibody IgM, IgG, IL-6R and CCR2 were elevated in all severity groups.
4. There was a positive correlation between Spike protein, adenovirus, COVID-19 IgM, IgG, IL-6R and CCR2 and the development of COVID-19 symptoms indicating that it's affected by this proinflammatory cytokine and viral loads.
5. A negative correlation between ACE2 (for COVID-19) and CAR receptor (for Adenovirus) may indicate that this viral receptor possibly has a direct binding to the virus.
6. Mutant Heterozygous TA (rs4845374) and AC Genotype (rs2228145) SNPs of *IL-6R* were considered as a protective factor for COVID-19 and Mutant Heterozygous GA (rs1799864) it was considered as a risk factor for *CCR2* .

Recommendations:

1. Use of COVID-19 spike protein, IL-6R and CCR2 serum levels as routine indicator for every COVID-19 case because their levels are well correlated with the severity of the disease.
2. Determine the correlation of IL-6R, CCR2, ACE2 serum levels with mortality of COVID -19 patients.
3. More studies of genotyping COVID -19 about correlate IL-6R, CCR2, ACE2 with COVID -19 complications.
4. Clinical study to evaluate the efficacy of drugs targeting IL-6R, CCR2 receptor.

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Appendices

Appendices

Appendix (I): Ethical Approval of study

University of Kerbala
College of Medicine
Medical Research Bioethical Committee
No: ٤٣
Date: ٢١/٩/٢٠٢١

FINAL APPROVAL LETTER

Dhurgham Hasan Shatti
Department of Biology\ College of Science \ University of Kerbala

Title of Project:
"Immunomolecular detection of some chemokines and interleukin 6 receptors polymorphism in Covid-19 patient suffering from pneumonia"

This is to certify that proposal provided have satisfactorily addressed the research bioethical guidelines..

Please consider the following requirements of approval:

1. Approval will be valid for one year. By the end of this period, if the project has been completed, abandoned, discontinued or not commenced for any reason, you are required to announce to the Committee. And you should inform the committee if the study extends over one year.
2. Please remember the Committee must be notified of any alteration to the project.
3. You must notify the Committee immediately in the event of any adverse effects on participants or of any unforeseen events that might affect continued ethical acceptability of the project.
4. Always consider the confidentiality of participants/ patients' information and/or opinions. And they must never be obligated to participate in the study and can withdraw at any time.
5. At all times you are responsible for the ethical conduct of your research in accordance with the standard bioethical guidelines.
6. The Committee should be notified if you will be applying for or have applied for internal or external funding for the above project.
7. All participants must be clearly informed about the research issue prior to taking blood samples.
8. This document does not compensate administrative or ethical approval might be required from hospitals/ health authorities.

Professor Dr. Ali A. Abutiheen
Chair, Medical Research Bioethical Committee
College of Medicine – University of Kerbala

وزارة الصحة
دائرة صحة كربلاء
مركز التدريب والتنمية البشرية
لجنة البحوث

استمارة رقم ٣/٢٠٢١
رقم القرار ٤٣
تاريخ القرار ٢٠٢٢/٢/٢٠

قرار لجنة البحوث

درست لجنة البحوث في دائرة صحة كربلاء مشروع البحث ذي الرقم (٢٠٢٢/٤٣/كربلاء) المعنون:

الكشف الجزئي المناعي عن تعدد الاشكال في بعض الكيموكينات ومستقبلات انترلوكين ٦ في مريض كوفيد ١٩ المصابين بالتهاب رئوي

والمقدم من الباحثين (**ضرغام حسن شاطي**) الى وحدة ادارة البحوث والمعرفة في مركز التدريب والتنمية البشرية في دائرة صحة كربلاء بتاريخ ٢٠٢٢/٢/٢٠ وقررت:

قبول مشروع البحث اعلاه كونه مستوفيا للمعايير المعتمدة في وزارة الصحة والخاصة بتنفيذ البحوث ولا مانع من تنفيذه في مؤسسات الدائرة.

نعيمة عبد المهداني
مقرر لجنة البحوث

21/02/2022

دائرة صحة كربلاء المقدسة
لجنة البحوث

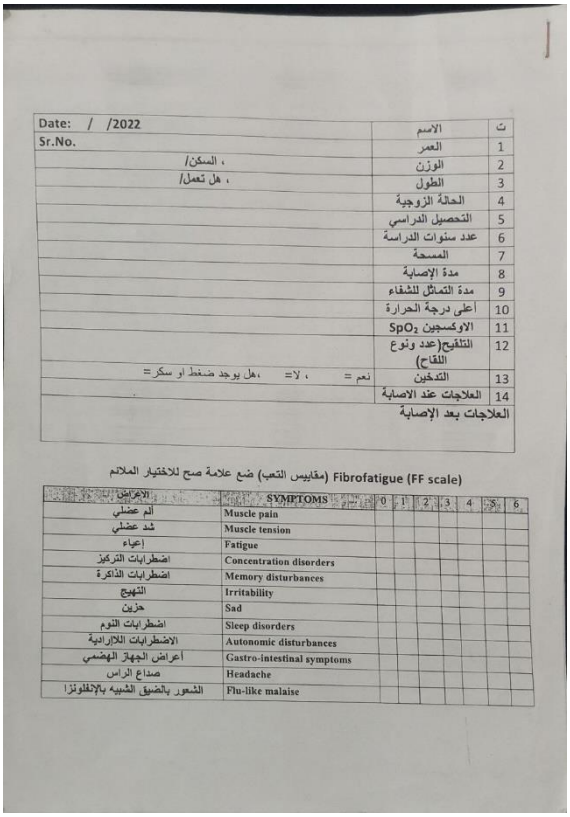
المرفقات:

ملاحظات:

- تم تخويل عضو لجنة البحوث (د.تقوى خضير عبد الكريم) او مقرر اللجنة (د.نعيم عبيد طلال) للتوقيع على هذا القرار استنادا الى النظام الداخلي للجنة البحوث.
- الموافقة تعني ان مشروع البحث قد استوفى المعايير الاخلاقية والعلمية لاجراء البحث والمعتمدة في وزارة الصحة. اما التنفيذ فيعتمد على التزام الباحث بتعليمات المؤسسة الصحية التي سينفذ فيها البحث.

Ethical Approval of study

Appendix (II): List of questioners studies



Date: / /2022

Sr.No.

ت	الاسم
1	العمر
2	الوزن
3	الطول
4	الحالة الزوجية
5	التحصيل الدراسي
6	عدد سنوات الدراسة
7	المسحة
8	مدة الإصابة
9	مدة التماثل للشفاء
10	أعلى درجة الحرارة
11	الاوكسجين SpO_2
12	التلقيح(عدد ونوع اللقاح)
13	التدخين
14	العلاجات عند الاصابة
	العلاجات بعد الإصابة

، السكن/

، هل تعمل/

نعم = ، لا= ،هل يوجد ضغط او سكر =

(مقاييس التعب) ضع علامة صح للاختيار الملائم Fibrofatigue (FF scale)

الأعراض	SYMPTOMS	0	1	2	3	4	5	6
ألم عضلي	Muscle pain							
شد عضلي	Muscle tension							
إعياء	Fatigue							
اضطرابات التركيز	Concentration disorders							
اضطرابات الذاكرة	Memory disturbances							
التهيج	Irritability							
حزين	Sad							
اضطرابات النوم	Sleep disorders							
الاضطرابات اللاإرادية	Autonomic disturbances							
أعراض الجهاز الهضمي	Gastro-intestinal symptoms							
صداع الراس	Headache							
الشعور بالضيق الشبيه بالإنفلونزا	Flu-like malaise							

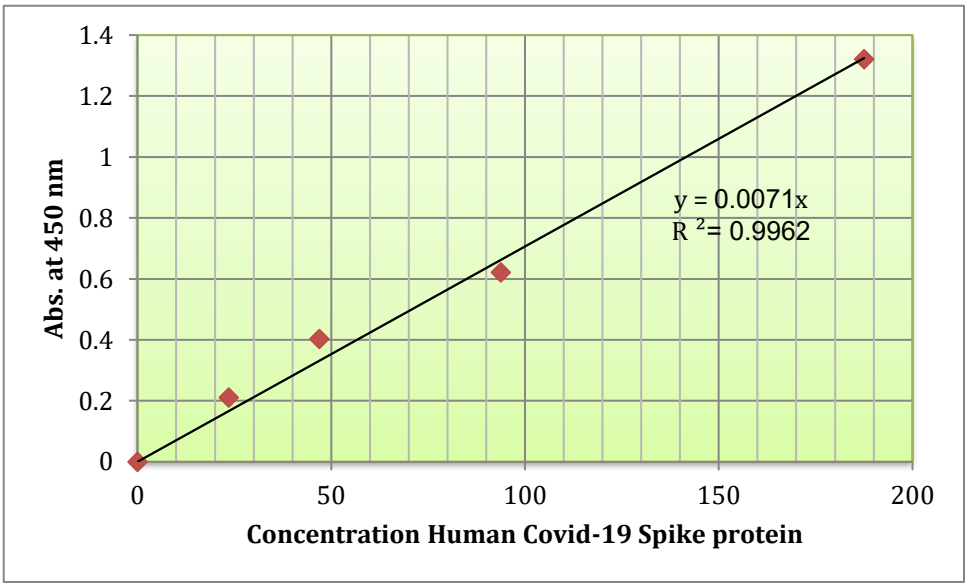
List of Questionnaire studies

Appendix (III): Principle of the Panbio™ COVID-19 Rapid Ag

The Panbio™ COVID-19 Rapid Strip Ag (Abbott Diagnostic GmbH, Jena, Germany) uses a membrane strip that is pre-coated with mouse monoclonal anti-chicken IgY on the control line and immobilized anti-SARS-CoV-2 Antibody on the test line. The membrane moves upward chromatographically as two Human IgG specific to SARS-CoV-2 Ag gold conjugate (binds to the nucleocapsid protein) and chicken IgY gold conjugation are two distinct conjugates that, respectively, react with anti-SARS-CoV-2 Antibody and pre-coated mouse monoclonal anti-chicken IgY. When viewing results, a test line made up of anti-SARS-CoV-2 Antibody and human SARS-CoV-2-specific IgG in gold conjugation will indicate a positive result.

Appendix (IV): Principle and standard curve of the Human COVID-19 Spike Protein ELISA Kit

The sandwich enzyme-linked immune-sorbent assay technology was used in this kit. Pre-coated capture Antibody was used to coat 96-well plates. As detecting antibodies, biotin conjugated antibodies were utilized. Following that, the standards, test samples, and biotin conjugated detection Antibody were added to the wells and rinsed with wash buffer. Unbound conjugates were washed away with wash buffer after HRP-Streptavidin was introduced. HRP enzymatic reaction was seen using TMB substrates. TMB was catalyzed by HRP to create a blue product that became yellow after the addition of an acidic stop solution. The yellow density is related to the amount of sample collected in the plate. In a microplate reader, read the O.D. absorbance at 450nm, and then calculate the target concentration.



Standard curve for determine the Human COVID-19 Spike Protein

Appendix (V): Principle of the SARS-COV-2 IgM VIDAS

The technique includes a two-step sandwich enzyme immunoassay with a final fluorescence detection (ELFA). The single-use Solid Phase Receptacle (SPR)

functions as both the solid phase and the pipetting device. The assay's reagents are ready-to-use and pre-dispensed in sealed single-use reagent strips. The equipment performs all of the assay stages automatically. Many times, the reaction medium is cycled in and out of the SPR device.

Following the sample dilution procedure, recombinant SARS-CoV-2 antigen deposited inside the interior of the SPR device wall captures the SARS-CoV-2 IgM. During the washing process, unbound components are removed. The IgM are selectively recognized in the second phase by anti-human IgM tagged with alkaline phosphatase. During the washing process, unbound components are removed. The substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR device during the final detection process. The conjugate enzyme catalyzes the hydrolysis of this substrate to produce a fluorescent product with a fluorescence wavelength of 450 nm. The results of the assay are automatically calculated by the instrument based on the S1 standard stored in memory at the end of the experiment, and a test value is obtained.

Appendix (VI) : Principle of the SARS-COV-2 IgG VIDAS

The assay principle combines a two-step sandwich enzyme immunoassay approach with a final fluorescence detection (ELFA). The single-use Solid Phase Receptacle (SPR) functions as both the solid phase and the pipetting device. The assay's reagents are ready-to-use and pre-dispensed in sealed single-use reagent strips. The equipment performs all of the assay stages automatically. Many times, the reaction medium is cycled in and out of the SPR device. Following the sample dilution step, recombinant SARS-CoV-2 antigen deposited into the interior of the SPR device wall captures the SARS-CoV-2 IgG. During the washing process, unbound components are removed. The IgG are selectively recognized in the second stage by anti-human IgG tagged with alkaline phosphatase.

During the washing process, unbound components are removed. The IgG are selectively recognized in the second stage by anti-human IgG tagged with alkaline phosphatase. During the washing process, unbound components are removed. The substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR device during the final detection process. This substrate is hydrolyzed by the conjugate enzyme producing a luminous product (4-Methyl-umbelliferone), whose fluorescence is measured at 450 nm. The results of the assay are automatically calculated by the instrument based on the S1 standard stored in memory at the end of the experiment, and a test value is obtained. The outcomes can then be printed.

- **Description of the 9COM and 9COG strip**

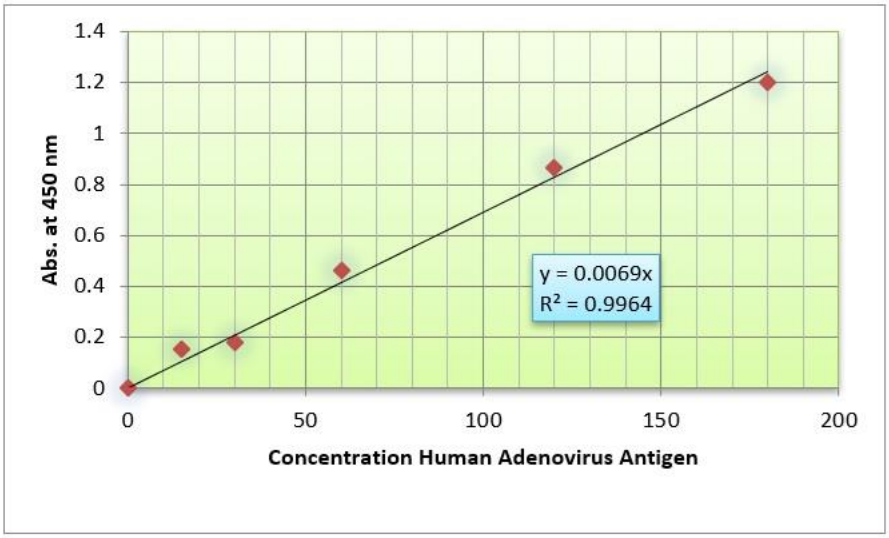
The strip contains diethanolamine and sodium azide. Refer to the hazard statements "H" and precautionary statements "P" indicated above.(a)

Well	Reagents
1	Sample well: dispense 100 µL of standard, control or sample.
2	Sample diluent: buffer + detergent + stabilizer of animal origin + preservative.
3 - 4 – 5	Wash buffer: buffer + detergent + preservative.
6	Conjugate: mouse monoclonal anti-human IgM antibodies conjugated to alkaline phosphatase + stabilizer of animal origin + preservative.
7 – 8	Wash buffer: buffer + detergent + preservative.
9	Empty well

10	Reading cuvette with substrate: 4-Methyl-umbelliferyl phosphate (0.6 mmol/L) + preservative.

Appendix (VII) : Principle of the Human Adenovirus Antigen ELISA

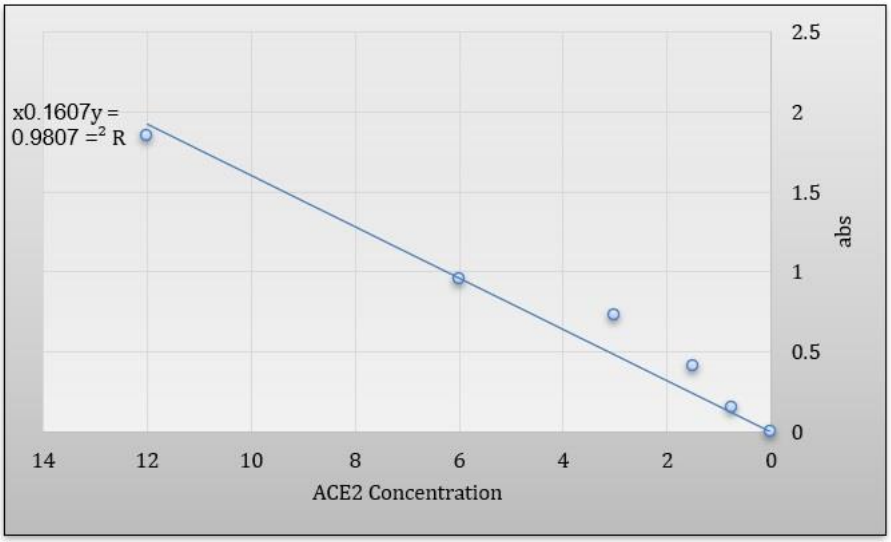
This performance by Human Adenovirus Antigen (HAdV-Ag) ELISA Kit (quantitative) (Sunlong Biotech , China) , Sandwich-ELISA is the technique used in this ELISA kit. This kit's Microelisa stripplate has been pre-coated with an anti-HAdV-Ag Antibody. In the proper Microelisa stripplate wells, the specified Antibody is introduced and combined with standards or samples. Each Microelisa stripplate is then incubated with a specific Antibody for HAdV-Ag that has been HRP-conjugated. While washing, free portions are taken out. The TMB substrate solution is poured into each well. When the stop solution is introduced, only the wells containing HAdV-Ag and HRP-conjugated HAdV-Ag antibodies will initially turn blue before turning yellow. The optical density (OD) is determined spectrophotometrically at a wavelength of 450 nm. The OD value and HAdV-Ag concentration have a linear relationship. The amount of HAdV-Ag in the samples can be calculated by comparing the OD of the samples to the standard curve.



Standard curve for determine the Human Adenovirus Antigen

Appendix (VIII) : Principle of the Human Angiotensin Converting Enzyme 2

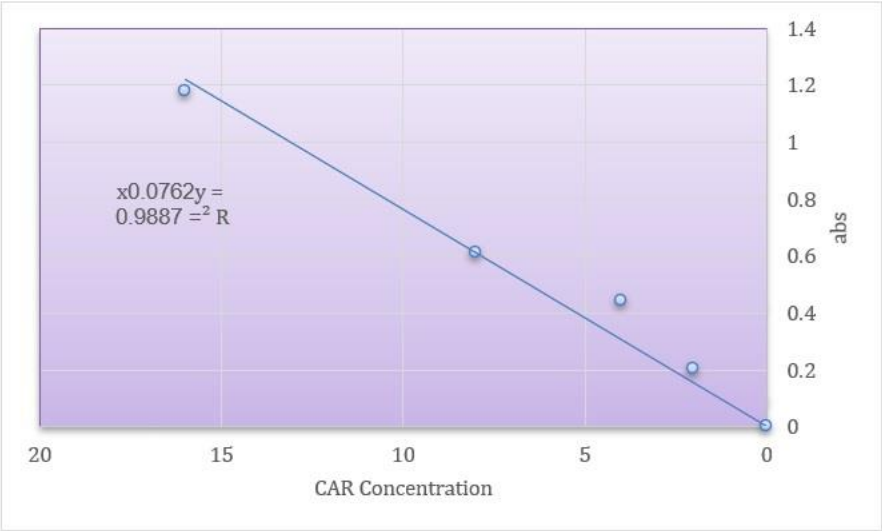
It is an Enzyme-Linked Immunosorbent Assay kit (ELISA) , Human ACE2 Antibody has been pre-coated on the plate. ACE2 is introduced to the sample and binds to antibodies coated on the wells. The biotinylated Human ACE2 Antibody is then added to the sample and binds to ACE2. Streptavidin-HRP is then added, which binds to the biotinylated ACE2 Antibody. During the washing stage after incubation, unbound Streptavidin-HRP is rinsed away. After that, the substrate solution is added, and the color develops in accordance to the amount of Human ACE2. The process is stopped by adding an acidic stop solution, and the absorbance at 450 nm is measured.



Standard curve for determine the human ACE2 Receptors

Appendix (IX) : Principle of the Human Coxsackievirus and Adenovirus Receptor

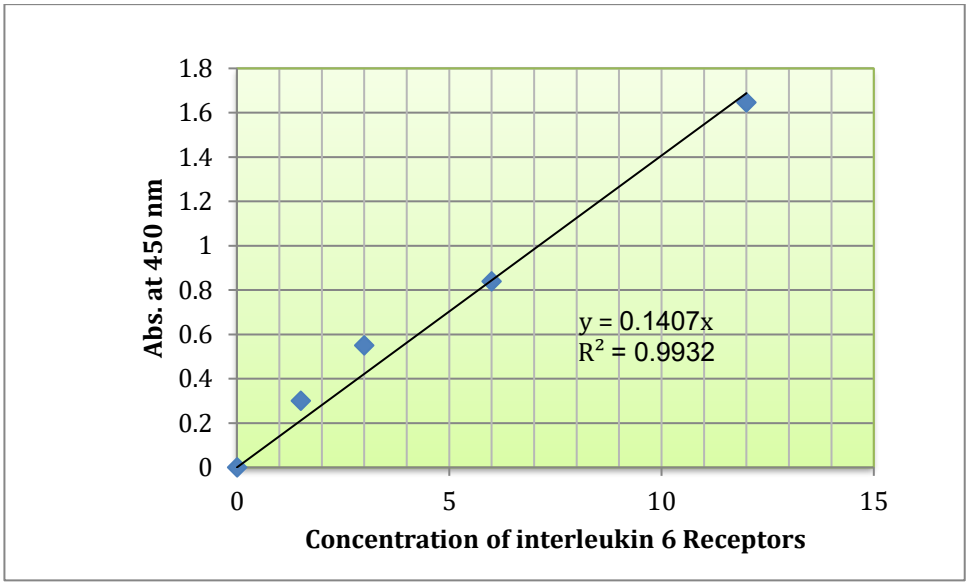
The plate has been pre-coated with Human CAR RECEPTOR Antibody in this Enzyme-Linked Immunosorbent Assay (ELISA) kit. CAR RECEPTOR is introduced to the sample and binds to antibodies coated on the wells. The biotinylated Human CAR RECEPTOR Antibody is then added to the sample and binds to CAR RECEPTOR. Streptavidin-HRP is then added, which binds to the biotinylated CAR RECEPTOR Antibody. During the washing stage after incubation, unbound Streptavidin-HRP is rinsed away. After that, the substrate solution is added, and the color develops in accordance to the amount of Human CAR RECEPTOR. The process is stopped by adding an acidic stop solution, and the absorbance at 450 nm is measured.



Standard curve for determine the Human CAR RECEPTOR

Appendix (X) : Principle of the Human Soluble Interleukin 6 Receptor

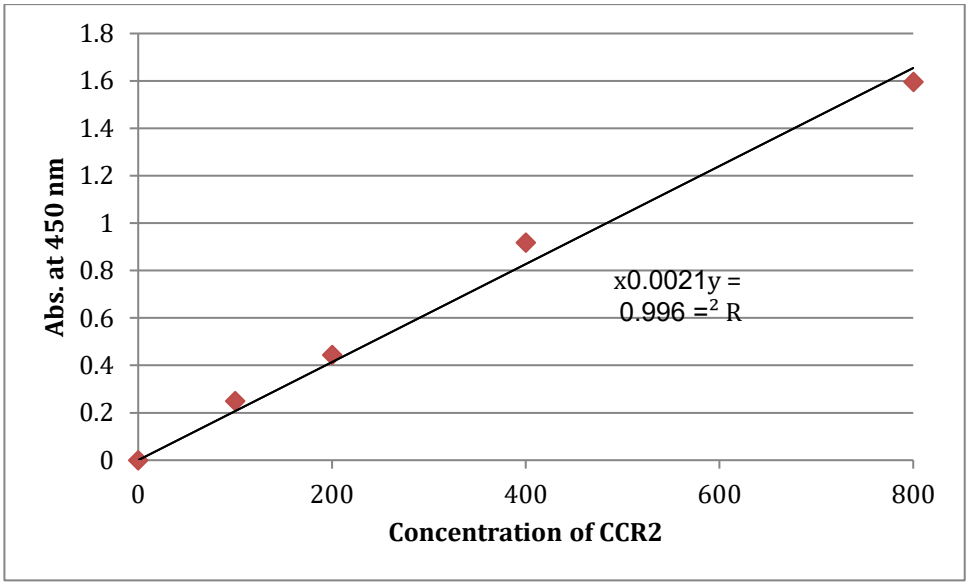
The plate has been pre-coated with Human sIL-6R Antibody in this Enzyme-Linked Immunosorbent Assay (ELISA) kit. The sample's sIL-6R is introduced and binds to antibodies coated on the wells. The biotinylated Human sIL-6R Antibody is then added to the sample and binds to sIL-6R. The biotinylated sIL-6R Antibody is then bound by Streptavidin-HRP. During the washing stage after incubation, unbound Streptavidin-HRP is rinsed away. After that, the substrate solution is added, and the color develops in accordance to the amount of Human sIL-6R. The process is stopped by adding an acidic stop solution, and the absorbance at 450 nm is measured.



Standard curve for determine the interleukin 6 Receptors

Appendix (XI) : Principle of the Human Cc-Chemokine Receptor 2

This is an Enzyme-Linked Immunosorbent Assay (ELISA) kit, with the plate pre-coated with Human CCR2 Antibody. CCR2 is introduced to the sample and binds to antibodies coated on the wells. The biotinylated Human CCR2 Antibody is then added to the sample and binds to CCR2. Streptavidin-HRP is then added, which binds to the biotinylated CCR2 Antibody. During the washing stage after incubation, unbound Streptavidin-HRP is rinsed away. After that, the substrate solution is added, and the color develops in accordance to the amount of Human CCR2. The process is stopped by adding an acidic stop solution, and the absorbance at 450 nm is measured.



Standard curve for determine the CCR2

Calculation for each Enzyme - linked immunosorbent assay:

The approximate ELISA concentration in the patient sample is calculated as follow:

Regarding calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve by software to do this calculation Human Elisa machine or by Excel from following equation:

$$\text{Concentration} = \frac{Absorbance\ at\ 450\ nm}{Slop}$$

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Appendix (XII): Table Substances of Human Blood DNA Extraction Kit

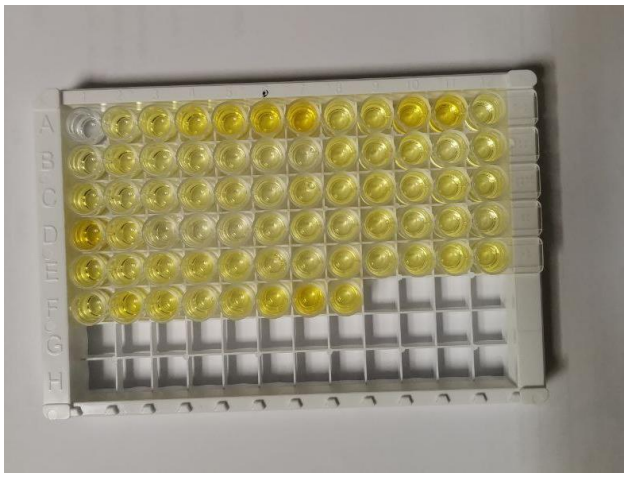
Quantity	Description
(30 ml)	Buffer of FATG
(40 ml)	Buffer of FABG
(45 ml)	Buffer of W1
(25 ml)	Concentrated wash buffer
(30 ml)	Buffer Elution
(135 ml)	Red Blood Cells Lysis Buffer
100 pcs	FABG Mini Column
200 pcs	Collection tube
1 pcs	User Manual

Appendix (XIII): Result of ACE2 and CAR receptors

Appendix (XIV): Result of Human COVID-19 spike protein

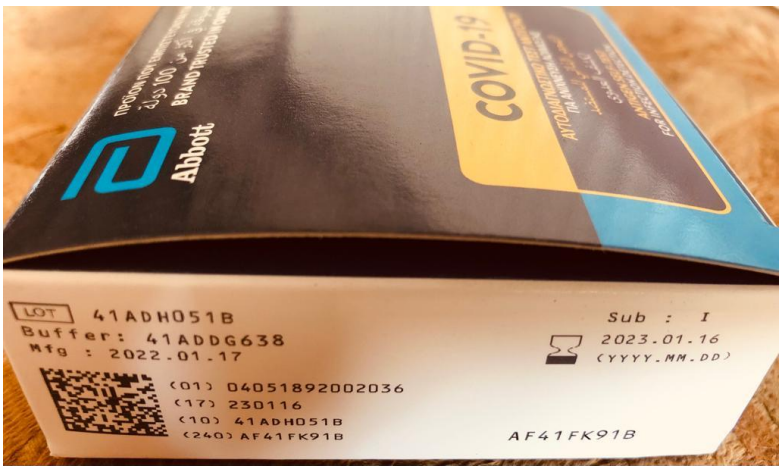
Appendix (XV): Result of CCR2 receptors

Appendix (XVI): Result of Interleukin 6 receptors



Appendix (XVII): Result of Human ELISA machine

Appendix (XVIII): COVID-19 Strip Kit



Appendix (XIX): Vidas machine

Appendix (XX): COVID-19 Antibody IGG kit

الخلاصة

يتميز فيروس كورونا 2019 بحدوث عاصفة من السيتوكينات والاستجابة المناعية المفرطة من خلال ارتفاع إفراز السيتوكينات والكيموكينات مما يؤدي إلى وجود عجز في تنظيم المناعة المرتبطة بالانسجة المدمرة بالمقارنة مع عملية الشفاء وكذلك عملية ترميم الأنسجة التي تضررت والتي تعتبرهذه من أبرز الطرق المستخدمة في علاج الفيروسات و تحديد شدة المرض الناجم عنه.

في هذه الدراسة مائة وخمسون شخصًا تم تشخيص اصابتهم بالعدوى المتعلقة بالسلالة الفيروسية -SARS CoV-2، اذ تم تأكيد اصابتهم بالفايروس من خلال RT-PCR لعينات مسحات فيروسية محددة جنبًا إلى جنب مع المستضد الشريطي، وتلقى المشاركون الرعاية الصحية في مستشفيات مدينة الامام الحسين الطبية من شهر مارس حتى شهر يوليو 2022, بالاضافة الى 50 شخصاً سليم.

ولوحظ أن متوسط العمر (44.04، 62.16، 61.14 سنة) لمجموعات المرضى الثلاث (الخفيف، المعتدلة، الشديدة) وكان المدى العمري بين 20 إلى 79 سنة للمجموعات الاصحاء والخفيفة، مقابل 20 -89 سنة لـ المجموعات المعتدلة والشديدة. وشملت أعداد المشاركين في هذه الدراسة 23 رجلاً و27 امرأة للمجموعة الاصحاء ، مع 36 رجلاً و14 امرأة للمجموعة الخفيفة ، بينما كان هناك 31 رجلاً و19 امرأة ضمن المجموعات المعتدلة والشديدة. تشير النتائج الأولية لهذه الدراسة إلى أن الرجال المسنين المشاركين في هذه الدراسة هم الأكثر عرضة للخطر.

ووجدت الدراسة أن هناك ارتباطًا بين اغلب اختبارات الدراسة وتتطور الأعراض الناجمة عن كوفيد-19 , حيث تضمنت الدراسة (بروتين سبايك لكوفيد-19 البشري، اذ كانت قيمة الارتباط=0.591، وقيمة الاحصائية p =<0.001 ؛ اما الحمل الفيروسي للفيروس الغدي، فقد كان الارتباط = 0.457، والاحصائية p = <0.001؛ كما كان قيمة الارتباط = 0.249 , والاحصائية p = 0.02 للجسم المضاد IgM لـ كوفيد-19 ؛ في حين أن الجسم المضاد IgG لـ كوفيد-19 كانت قيمة ارتباطه = 0.347، والقيمة الاحصائية بلغت p = <0.001؛ بالإضافة إلى ارتباط مستقبلات إنترلوكين 6 = 0.695، وقيمته الاحصائية p = <0.001 اما CCR2 فقد كان الارتباط = 0.564، والاحصائية p= <0.001). اما الإنزيم المحول للأنجيوتنسين 2 (ACE2) ومستقبل فيروس Coxsackievirus and adenovirus (CAR) كانتا ذو علاقة عكسية مع تتطور الأعراض المرتبطة بكوفيد-19 (ارتباط مستقبل الإنزيم المحول للأنجيوتنسين 2 يساوي = - 0.34، والاحصائية p = <0.001؛ في حين ان مستقبل فايروس Coxsackievirus and

adenovirus كان ارتباطه = - 0.071، واحصائيته 0.046 = p على التوالي), حيث ان جميع الفحوصات السابقة تم قياسها باستخدام تقنية , ELISA و ELFA .

بعد ذلك، خضعت كل مجموعات مرضى كوفيد-19 والأصحاء إلى اثنين من تعدد الاشكال الجيني لـ *IL-6R* وواحد لل *CCR2* (rs4845374، rs2228145 وrs1799864 على التوالي)، واظهرت الأنماط الجينية لل *IL-6R* في جميع مجموعات المرضى مقارنة بالاصحاء ارتباط إيجابي (الارتباط = 0.160، والقيمة الاحصائية 0.0097 = p و الارتباط = 0.125، والقيمة الاحصائية 0.0001 = p) على التوالي مع زيادة أعراض كوفيد-19. في حين بين النمط الجيني لل *CCR2* ارتباط إيجابي ايضاً (و الارتباط = 0.564، والقيمة الاحصائية 0.0001 = p) مع ارتفاع أعراض كوفيد-19. كما ان الانماط الجينية لل *IL-6R* تم تحديدها باستخدام Sanger Sequencing اما النمط الجيني للجين *CCR2* فقد تم تحديده باستخدام طريقة Allele specific primer.

وكان النمط الجيني TA لل (rs4845374) الخاصة بجين *IL-6R* أكثر تكرارًا بشكل ملحوظ في المجموعات الخفيفة والمتوسطة والشديدة عند مقارنة بالمجموعة الاصحاء، (0.0183 = p، 0.0364 و 0.0243) على التوالي؛ ولذلك اعتبر عاملاً وقائياً، حيث أظهر نسبة الأرجحية (0.3375، 0.3816 و 0.3472) على التوالي في ثلاث مجموعات من المرضى. في حين أن النمط الجيني AA كان أكثر شيوعا بشكل ملحوظ في المجموعة الشديدة مقارنة بالمجموعات الأخرى، (0.0324 = p)؛ وبنسبة الأرجحية (0.0420)، لذلك اعتبر عاملاً وقائياً للمجموعة الشديدة. كان الأليل A أكثر تكرارًا بشكل ملحوظ في المجموعات الخفيفة والمتوسطة والشديدة مقارنةً بالمجموعة الاصحاء (0.0044 = p، 0.0157 و0.0003) على التوالي؛ ولذلك اعتبر عاملاً وقائياً، إذ أظهرت نسبة الأرجحية (0.3162، 0.3720 و 0.2361) على التوالي في ثلاث مجموعات.

وتبين النمط الجيني AC لل (rs2228145) الخاصة بجين *IL-6R* أكثر تكرارًا بشكل ملحوظ في المجموعات الخفيفة والمتوسطة والشديدة مقارنة بالمجموعة الاصحاء، (0.0001 = p، 0.0103 و0.0052) على التوالي؛ ولذلك اعتبر عاملاً وقائياً، إذ أظهرت نسبة الأرجحية (0.0952، 0.3333، 0.2667) على التوالي في ثلاث مجموعات. في حين كان النمط الجيني CC أكثر شيوعا في المجموعات الخفيفة والمتوسطة والشديدة مقارنة مع مجموعة الاصحاء، (0.0017 = p، 0.0417 و 0.0024) على التوالي؛ ولذلك، وبنسبة الأرجحية (0.0086، 0.0462 و0.0111) على التوالي في ثلاث مجموعات، اعتبر عاملاً وقائياً للمجموعة الشديدة. كان الأليل C أكثر تكرارًا بشكل ملحوظ في المجموعات الخفيفة

والمتوسطة والشديدة مقارنة بالمجموعة الاصحاء (p = 0.0001، 0.0024 و0.0001) على التوالي؛ ولذلك اعتبر عاملاً وقائياً، إذ أظهرت نسبة الأرجحية (0.2045، 0.3750 و0.2045) على التوالي في ثلاث مجموعات.

بالاضافة الى ان النمط الجيني GA لل (rs1799864) الخاصة بجين *CCR2* وجد أكثر شيوعًا في المجموعات المعتدلة والشديدة مقارنة بالمجموعة الاصحاء (p = 0.0573، p =0.0051) على التوالي؛ ولذلك كانت نسبة الأرجحية (2.5455، 5.4118) على التوالي، لذلك اعتبرت عامل خطر للأشخاص الذين يعانون من حالات متوسطة وشديدة. بينما يحدث العكس في المجموعة الخفيفة مقارنة بالمجموعة الاصحاء. كان النمط الجيني AA من النوع غائباً عن مجموعة المرضى مقارنة بالمجموعة الاصحاء. كان الأليل A أكثر شيوعا في المرضى الذين يعانون من المجموعة المعتدلة والشديدة (p = 0.3096 , p = 0.114) وبالتالي، كانت نسبة الأرجحية (1.3448 , 1.582) مقارنة بمجموعة الاصحاء ، والتي اعتبرت ببساطة عامل خطر لكلا المجموعتين من المرضى.

في الختام، جميع اختبارات الفيروسية والمناعية المدروسة (باستثناء مستقبل sACE2 و CAR) والتي تتضمن تركيز بروتين سبايك، والأجسام المضادة IgG لكوفيد-19 ، والحمل الفيروسي للفيروس الغدي، وIL-6R وCCR2 ارتفعت قيمتها لدى الأفراد الذين يعانون من كوفيد-19. كما استنتجنا ان تعدد الاشكال الجيني لجين *IL-6R* فقد اعتبر عاملا وقائياً , في حين ان الجين *و CCR2* كان عاملا خطراً ; ومع ذلك، فمن الضروري أن نأخذ في الاعتبار أنه ليس كل التغييرات في العمليات البيولوجية يمكن أن تشير حصرا إلى ارتفاع هذه المستويات. وبالتالي، فإن البحث الذي أجريناه يقترح مزيدًا من البحث في السيتوكينات الإضافية التي تحفز الالتهاب جنبًا إلى جنب مع مستقبلاتها.

جـــامعة كـــربلاء

كليـــــة العلـــــوم

قسـم علوم الحياة

تعدد الاشكال الجيني ومستويات بعض المعايير المناعية في الالتهاب الرئوي لمرضى كوفيد-19 وارتباطه بالفيروس الغدي كاصابة مشتركة

اطروحـــــة مقدمة الـــى

مجلس كلية العلوم ــ جـامعة كربـلاء

وهي جـزء من متطلبـات نيـل درجـة الدكتـوراة فلسفـة في علوم الحياة

من قبل الطالب

ضرغام حسن شاطي الفتلاوي

بكلوريوس علوم الحياة /جامعة كربلاء, 2007

ماجستير علوم الحياة /جامعة كربلاء, 2012

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علي منصور جاسم العامري

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