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Determination of Some Immunological Parameters associated with miRNA 31, 192 among Patients with Covid-19 infection

A thesis
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بِسْمِ اللهِ الرَّحْمنِ الرَّحِيمِ

((قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَاۖ إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ ﴿٣٢﴾))

صدق اللَّهُ العلي العظيم

البقرة، الاية (32)

Dedication

To the sake of Allah, my Creator and my Master.

To my great teacher and messenger, Mohammed (May Allah bless and grant him), who taught us the purpose of life. To my homeland Iraq.

To the pure spirit who gave me her blood, soul and love …….my lovely Mother

To my ideal in life, who I carry his name proudly…… Dear Father

To my dear husband, who supported and trusted me and my beloved kid jod

To my brothers and friends with my love and respect

Hadeel

Acknowledgment

In the name of Allah, the most gracious, the most merciful. I start my work by the grace of Allah and I finish my work by the strength of Allah. Thank you to everyone who enabled and helped me complete this study. My deep thanks to the Dean of the College of the Science / University of Babylon. Also, many thanks to the head of the biology department I would like to thank my supervisors ***Prof. Dr.Abd alnabe*** *and* ***Dr. Raheem Tuma*** *for their kind advice and continuous encouragement. My thanks and appreciation to each of the patients who did not spare me in giving me samples of them. I would also like to express my deep and sincere thanks to the people who helped me complete the research and allowed me to train and work in the covid-19*

Summary:

Coronaviruses (CoVs) are RNA virus family that causes sickness in humans and other animals by entering the body through Angiotensin converting enzyme2 receptors (ACE2) that located in organs such as the heart, lungs, kidneys, and digestive tract. The big particle viruses known as coronaviruses are spherical, enveloped, and contain surface protrusion-forming spikes in addition to having a positive-sense single-stranded RNA genome.

During September-2021 to January – 2022 ninety samples of blood were collected, 50 blood samples were collected from hospitalized patients who infected with Covid 19, in AL-Hussein Teaching hospital in Kerbala, and 40 samples were collected from healthy individuals as a control group, their age ranged between 20- 80. The Covid-19 infection of patients was checked by using real- time polymerase chain reaction (RT-PCR) technology.

The result found the male patients have higher percentage (52%) than female (48%) of SARS –Cov2 infected patients. The most group of infected patients in the present study have no other chronic disease at the time of study , the diabetes and hypertension were shown in a group of patients (18%) for each disease , 10% of studied patients have both diseases at the same time (diabetes and hypertension), only (6 %) have pulmonary thrombosis. The smoker patients have lower percentage than non-smoker as 14% and 86% respectively.

Some immunological parameters in sera of 50 patients and 40 healthy controls were studied by using enzyme linked immunosorbent assay (ELISA) and fluorescence immunoassay (FIA).

Increased levels of interleuken-6, C-reactive protein in infected patients in comparison with healthy individuals, and increased levels of interleukin-6, C-reactive protein, and WBCs among infected females in comparison with infected males .

The SARS-Cov 2 infected patients with diabetes showed that increase in IL-6 level in comparison with other conditions, while more CRP concentration was shown in complicated patients in which have pulmonary thrombosis, white blood cells have more count in hypertensive condition associated with SARS COV- 2 infection rather than other studied conditions.

Human leukocyte antigens (HLA-DR and HLA-G) levels were estimated by ELISA, the levels of HLA were higher in control group compared with patients group. As for the gender ratio and HLA types (HLA-DR and HLA-G) of patients , there is no noticeable difference between males and females in the values of HLA-DR, while there is a clear increase in the values of HLA-G in male patients than in females. The results of the age groups of patients and the HLA types (HLA-DR , HLA-G) showed that the age group (50-59y) have higher level of HLA-DR (3.82ng/l) in comparison with other age groups, while the result of HLA-G show that the higher level were spotted at age groups (40 -49 y, at 606.66) in comparison with other age groups. The smoker patients have lower level of both HLA –DR and HLA-G in comparison with non-smoker of patients and control , at mean (2.70 , 456.15)ng/l , respectively.

Evaluations of microRNAs were performed in SARS CoV-2 infected patients and healthy individuals, the result revealed microRNA -192 gene have 84 % positive result in comparison with 16 % in which

have negative result, while the other gene MiRNA - 31 have 36% positive result in comparison with 64% as a negative result, as well as 100 % of housekeeping gene, the cycle threshold value showed the high level of MIRNA -31 compared with microRNA - 192 as well as housekeeping gene in infected patients.

The low cycle threshold value of microRNA -31 cycle threshold value (34.76) refer to high level of such gene in studied patients, because there is inverse relationship between cycle threshold value number and microRNA level. While the cycle threshold value of housekeeping gene revealed that (24.39).

This study revealed the aging plays a major role to making persons more susceptible to Covid-19 infection. IL-6 appears to be the most important driver of immune dysregulation and ARDS in Covid-19. The HLA- Class II (HLA-DR) , more predicted work in female rather than male at the infected population , and might be associated with active immune mechanism rather than Class I (HLA-G). The overall result might be refer to that rapid enhancement in immunological response among female patients infected with SARS COV -2 virus

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List of Abbreviations

Abbreviations	Terms
2019-nCoV	2019 Novel coronavirus
ACE2	Angiotensin converting enzyme 2
APCS	Antigen presenting cells
ARDS	Acute respiratory distress syndrome
CD4	Clusters of differentiation 4
CD8	Clusters of differentiation 8
CH B	Chronic hepatitis B
COI	Cut off index
CoV	Coronavirus
COVID-19	Coronavirus disease 2019
CRP	C-reactive protein
CT	cycle threshold
DIF	Direct immunofluorescence
DIFA	Direct immunofluorescence antibody
DM	Diabetes mellitus
Ds RNA	Double stranded RNA
DVT	Deep venous thrombosis
E	Envelope
EGFR	Epidermal growth factor receptor
ELISA	Enzyme-linked Immunosorbent assay
EtBr	Ethidium Bromide
F primer	Forward primer
FIA	Fluorescence immunoassay
FXR	Farnesoid X receptor
HCoV-HKU1	Human coronavirus HKU1
HCoV-NL63	Human coronavirus NL63
HCoV-OC43	Human coronavirus OC43
HLA	Human leukocyte antigen
HT	Hypertension

IC	Internal control
IL-6	Interleuken-6
Kb	Kilo base pair
LTCF	Long term care facilities
M	Membrane
Mcp1	Monocyte chemo attractant protein 1
MERS	Middle East respiratory syndrome coronavirus
MHC	Major histocompatibility complex
MS	Multiple sclerosis
N	Nucleocapsid
NF-κB	Nuclear factor kappa-B
NKs	Natural killer cells
Nsp	Non-structural protein
ORF	Open reading frames
PC	Positive control
PCR	Polymerase chain reaction
PRR	Pattern recognition receptor
R primer	Reverse primer
RFU	Relative flourescent unit
RG-101	Roentgenium-101
RT-PCR	Real Time- Polymerase chain reaction
S	Spike
SAA	Serum amyloid A
SARS	Severe acute respiratory syndrome coronavirus
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SCD	Stearoyl coenzyme desatnrase
SSRNA	Single stranded RNA
TBE	Tris Borate EDTA Buffer
TF	Tissue factore
TG	Triglyceride
TMPRSS 2	Transmembrane protease, serine 2.
TNF	Tumor necrosis factor
UTR	Un translated region
VAT	Visceral adipose tissue
VEGF	Vascular endothelial growth factor

WHO	World health organization
α- CoV	Alpha coronavirus
β- CoV	Beta coronavirus
γ- CoV	Gamma coronavirus
δ- CoV	Delta coronavirus

Chapter One

Introduction

1.1. Introduction:

The new pneumonia epidemic, which started in early December 2019 near Wuhan City Hubei Province, China, is caused by novel coronavirus (CoV) known as ‘2019-nCoV' or ‘2019 novel coronavirus' or ‘COVID-19' by the World Health organization (WHO) COVID-19 is a pathogenic virus, from the phylogenetic analysis carried out with obtainable full genome sequences, bats occur to be the COV-19 virus reservoir, but the intermediate host(s) has not been detected till now (Hue *et al*., 2020).

The word (coronavirus) comes from the Greek korn, which means "crown" or "wreath" and was first used to describe a virus with spike-like projections (Tyrrell and Fielder, 2020). Coronavirus has a single-stranded positive-sense RNA (+ssRNA) genome, the nucleocapsid protein (N) creates the capsid outside of the nucleus, and the RNA is further packed by an envelope consisting of three structural proteins. Envelope protein (E),membrane protein (M), spike protein (S) (Brian and Baric, 2005).

The freshly sequenced SARS-genomic CoV-2's size as a member of the coronavirus family is approximately (29.9 kb). SARS-CoV-2 has sixteen non-structural proteins (nsp1-16) and four structural proteins (S, E, M, and N) (Li *et al*., 2020c).The human leukocyte antigen (HLA), system or complex is a group of genes on chromosome 6 that produce cell-surface proteins that control the immune system (Choo, 2007).

The major histocompatibility complex (MHC) presents in many animals is often referred to as the HLA system, which is found in humans(Trust .,2021).The classical class I (A , B and C) and class II (DR, DQ and DP) HLA molecules are involved in mediating antigen

presentation of intracellular and extracellular peptides respectively (Wieczorek *et al*., 2017). Non-classical class I Other significant products produced by the HLA region's genes include the HLA molecules (E, F, and G), which are the main molecules in charge of regulating the immune system's response to pathogens as well as inflammatory responses (Halenius *et al.*, 2015). The non-classical class I HLA glycoproteins known as HLA-G antigens were initially identified by their expression at the maternal-fetal interface, which served to shield the fetus from the mother's immune system (Djurisic and Hviid, 2014). Human leukocyte antigen-G has been identified in seven isoforms, including three soluble molecules and four membrane-bound antigens (HLA-G1, -G2, -G3 and -G4) (sHLA- G5, -G6 and -G7) (Alegre *et al.*, 2014).

These molecules are thought to be powerful immunomodulators, and pathogenic diseases have been linked to their dysregulated expression (Morandi *et al.*, 2016). Two theories have been put up to explain the function of HLA-G in the immunopathogenesis of viruses during viral infection (Amiot *etal*, 2014). HLA-DR is a class II human leukocyte antigen (HLA) that is expressed on the cell surfaces of antigen-presenting cells, such as monocytes, differentiated macrophages, dendritic cells, and B cells since the initial explanation of the function of HLA-DR in immunosuppression (Volk et *al*., 1991). One-third of all human proteins-coding genes are regulated by miRNAs which are small non-coding RNAs of about 22 nucleotides in length (Hammond, 2015). Additionally, miRNAs are essential for the pathogenesis of a number of diseases, including chronic pediatric illnesses, neurological disorders, metabolic diseases, viral infections, and diseases caused specifically by parasites (Paul *et al.*, 2020). In mammalian cells infected by viruses, miRNAs have a role in the regulation of lytic and latent viral replication, (Grassmann

and Jeang, 2008). In coronaviruses, in particular, it has been demonstrated that they play a role in the immune response and viral protein production (Abu-Izneid *et al.*, 2021; Mirzaei *et al.*, 2021).

1.2. The Aim of Study:

The present study was planned to evaluate of corona infected population by assessments of certain immunological and molecular parameters (HLA-DR ,HLA-G,IL-6 , CRP and miRNA-31,192).

To complete the aim of study, the following objective should be done:

- Detection of MiRNA for each gene 31 and 192 for SARS- Cov2 patients and find out the difference between them through real-time PCR (RT –PCR) technique

- Detection of HLA types (HLA-DR and HLA-G), using ELISA technique.

- Other inflammatory immune parameters are interleukin-6 and C-reactive protein by using a fluorescence immunoassay (FIA).

Chapter Two

Literature Review

2. Literature Review:

2.1. Define of coronavirus:

The Severe Acute Respiratory Syndrome 2 Coronavirus, (SARS COV-2) is considered the virus that causes COVID-19 disease (Gorbalenya *et al*., 2020).

COVID-19 started in Wuhan, Hubei Province, China, from time to time, members of the family of coronaviruses appear to cause, infections the human, but not of the same importance as Covid 19, such as HCoVs, SARS –CoV, MERS-CoV (Elrashdy *et al*., 2020; Udugama *et al*., 2020). Reported symptoms include fever, cough, fatigue, pneumonia, headache, diarrhea, hemoptysis, and dyspnea (Adhikari *et al*., 2020).

Coronavirus (CoV) are family of RNA viruses that cause disease in humans and other animals. They can infect humans, pets, birds, bats, mice, and a variety of other wild animals' respiratory, gastrointestinal, hepatic, and central nervous systems (Su *et al.*, 2016). Coronaviruses are enveloped viruses with large single-strand positive sense RNA, genomes that belong, to the Coronaviridae family (Drosten *et al*., 2003).

2.2. Classification of Coronavirus:

Realm: Riboviria

Kingdom: Orthornavirae

Phylum: Pisuviricota

Class: Pisoniviricetes

Order: Nidovirales

Family: Coronaviridae, Sub family: Orthocoronvirinae and Torovirinae. Orthocoronavirinae is classified into four genera: (Alphacoronavirus, Betacoronavirus, Gammacoronavirus, Deltacoronavirus) (MaliK, 2020) .

The SARS-COV-2 has non-segmented, single - stranded positive RNA (+ssRNA) with a 3'-poly-A tail and 5'-cap structure, a typical CoVs genomic structure (Ashour *et al.*, 2019). Gamma-coronaviruses and Delta-coronaviruses are capable of infecting birds in addition to mammalian (Forni *et al.*, 2017), while Alpha-coronaviruses and Beta-coronaviruses appear to infect mammals and cause human respiratory and gastrointestinal infections, for examples SARS, (SARS-CoV), Middle east respiratory (MERS-CoV), and SARS CoV-2, Beta corona virus. consist of SARS- CoV, MERS CoV, Bat-SARS-like (SL) coronaviruses, human coronaviruses (HCoVs), and finally SARS-CoV-2. (Zhou *et al* ., 2020) .

2.3. Epidemiology:

The new SARS - 2 virus appeared in late 2019 in Wuhan, China in December and spread quickly, which caused the World Health Organization to proclaim a state of maximum a public health emergency, in March 2020 and that the disease has become a global pandemic (Pellegrino *et al.*, 2020). Patients have reported a high fever (above 38°C), a dry cough, lethargy, and breathing problems. The disease has been termed COVID-19 and has been connected to a seafood market in Wuhan China (Yamagishi *et al.*, 2020).

It rapidly spread to, neighboring far eastern countries, followed by the Middle East and Europe. Pneumonia, septic shock, metabolic acidosis, and bleeding are all symptoms of the condition in severe

situations (Helmy *et al*., 2020). The incubation time is anticipated to be between 5 to 14 days and varies from one patient to another depending on age and infected date (Xiao *et al.*, 2020).

The virus has infected more than 150 countries and areas around the world as of March 16, 2020. United Nations Office for the Coordination of Humanitarian Affairs reported that there has been a considerable increase in coronavirus cases in recent months (OCHA, 2020). The first case of Middle East Respiratory Syndrome, (MERS) was discovered in Jeddah, Saudi Arabia, in June 2012, and the majority of cases have occurred in the Arabian Peninsula (Zumla *et al*., 2015)

The World Health Organization (WHO) has received reports of over 2,400 cases worldwide, with over 850 deaths (WHO , 2019). MERS-CoV is a zoonotic virus that has a reservoir host in dromedary camels (Wernery *et al*., 2017; Paden *et al*., 2018). Bats are a probable original reservoir, coronaviruses similar to MERS-CoV have been found in bats, although there is no epidemiologic evidence of their function in transmission (Corman *et al*., 2014).

SARS-CoV-2 is not country-specific virus. It was extremely infectious, spreading to over 100 countries in the last two to three months and affecting over 300000 persons on the world. As of March 24, 2020, the following populations are impacted: China, Australia, Republic of Korea, Malaysia, Japan, Singapore and New Zealand, and others in the Western Pacific Region. A total of 195,511 positive cases were reported in the European Region (Italy, Spain, Germany, the United Kingdom, Norway, and so on), with 24,087 of them occurring in just one day. In a single day, there were 10,189 recorded cases and 1447 deaths (WHO, 2020).

Across Southeast Asia, there have been 1990 confirmed cases with 65 deaths. This outbreak affected a total of 27,215 people in the Eastern Mediterranean Region, with 1877 people dying as a result of it. In the Americas, there have been 49,444 reported cases and 565 fatalities, with an average of (12,428), new cases and 100 deaths per day (WHO, 2020). Coronavirus disease has quickly spread throughout Europe, Asia, North America and the Middle East, with the first cases recorded in Latin America and, Africa. According to the WHO, the Number of countries, states, and territories affected by the coronavirus had risen significantly outside of China by March 16, 2020, with 143 countries , A pandemic has been announced for Cov-19 by the Director-General of the World health organization, due to the rising levels of infection and severity (Trevor *et al.*, 2020). Tedros Adhanom Ghebreyesus, Director General of the World health organization said on March 13, 2020 the Europe has been the pandemic's epicenter (Trevor *et al.*, 2020).

2.4. Pathogenicity:

In general, the viruses depend on the cell to produce many new virus copies of the same type since the virus is obligated to parasitize. Once infecting a sensitive cell. Cell resources are harnessed for virus reproduction. A cell is considered infected if the virus attaches itself to the special receptor on the cell membrane and enters the cell. And this process is carried out by proteins specific to the virus (Krupovic *et al.*, 2019).

For SARS COV-2, its life cycle begins when (S) protein is activated by two proteins, the cellular serine protease, Transmembrane protease serine2 (TMPRSS2) and trypsin-like protease from airways (TMPRSS11D). Protein S binds itself to the Angiotensin Converting Enzyme 2 (ACE2), receptor (Wruck and Adjaye, 2020).

The ACE2 enzyme that is targeted by SARS CoV-2, is a homeostatic enzyme, which is responsible and controlling for the isometric pressure balance of extracellular fluids as well as arterial pressure in humans (Mcmurray *et al.*, 2020).

It is found mainly on the ciliated cells of the upper airway epithelium, tubular cells near the kidneys, the duodenum, small intestine, liver, Sertoli cells, Leydig cells of the testis, and glandular cells of the Gallbladder. It also present in the epididymis and cardiomyocytes, in the tissues of the heart, pancreas, seminal vesicles, and placenta (The Human Protein Atlas, 2021).

The patients infected with SARS-COV-2 may be treated differently depending on their clinical symptoms and treatment response, the stages of the disease can be divided according to severity into three stages (Sarmiento-Monroy *et al.,* 2021)

The initial stage is characterized by mild or non-specific symptoms such as fever and dry cough. It occurs at the beginning of infection or when taking some types of vaccines and it can be detected by (RT –PCR) swab, serological tests and complete blood count. At this stage, the recovery rate is high from the disease. The second stage is describing by a reduction in the anti-viral response and an increase in the proinflammatory response with viral multiplication and inflammation typically occurring in the lungs , and the third stage severe-systemic hyperinflammation, is characterized by a severe inflammatory response, also possible to add a fourth stage that includes patients recovering after infection, but showing late complications such as fibrosis of the pulmonary alveoli (Siddiqi and Mehr , 2020).

The majority of fatalities related to respiratory diseases also a severe lung damage are caused by Acute Respiratory Distress Syndrome

(ARDS), a potentially fatal lung condition when the lungs are unable to get enough oxygen (Mason *et al*., 2016).

The pathogenesis of COVID-19 can be summarized as follows: Acute Respiratory Distress Syndrome is a frequent result of severe viral pneumonia, especially pneumonia brought on by extremely Lethal coronaviruses like SARS-CoV-2. (Colafrancesco *et al.*, 2020). Individuals with fatal Human (SARS - CoV, MERS - CoV, and SARS - CoV-2), infections have mechanical ventilation is necessary due to severe respiratory distress, with histopathological results confirming ARDS (Xu *et al*., 2020).

2.5. Structure of coronavirus:

The SARS Cov-2 has a diameter of about 50-200, nanometers (Chen *et al*., 2020). And like any family coronavirus, it consists of a genome and covers that surround it found that up to 29 proteins are encoded by the Sars-CoV-2 genome, given that some segments are not expressed (Kim *et al*., 2020a). SARS COV-2 is similar to other genera of the Coronavirus family in that it consists of a single strand, unsegmented, sense positive RNA genome. There exist, as well as, similarities in the localized coding regions and non-coding regions (Tillett *et al*., 2021).

Both SARS COV-1 and SARS COV-2, which are the new addition to the human coronavirus family, include (OC43, NL63, HKU1, and MERS) which belong to the genus β-Coronavirus, and (229E and NL63) that belong to the genus of α-Coronavirus which contains a polycistronic genome, It encodes for the structural proteins that are included in the phenotype of the virus, along with the accessory proteins in the last third of the RNA strand. It also interferes with the manufacture of proteins that are not related to the formation of the structure of the non-structural proteins (nsp) virus near the N-end of the genome (Fung *et al*., 2020).

Thus, the SARS COV-2 genome consists of 29,903 nucleotides containing 16 open reading frames (Kim *et al.*, 2020b).

The viral envelope like any other membrane was made up of a lipid bilayer and a variety of proteins with structural functions, including membrane (M), envelope (E), and spike (S) (Singer *et al.*, 2014) in a ratio of E:S:M 1: 20:300 (Susan, 2020). The particle's total number of spikes is about 74. However, as shown in figure (2-2), SARS-CoV-2 has another short projection of a proteinous structure known as hemagglutinin esterase (HE) (Yousif *et al.*, 2013)

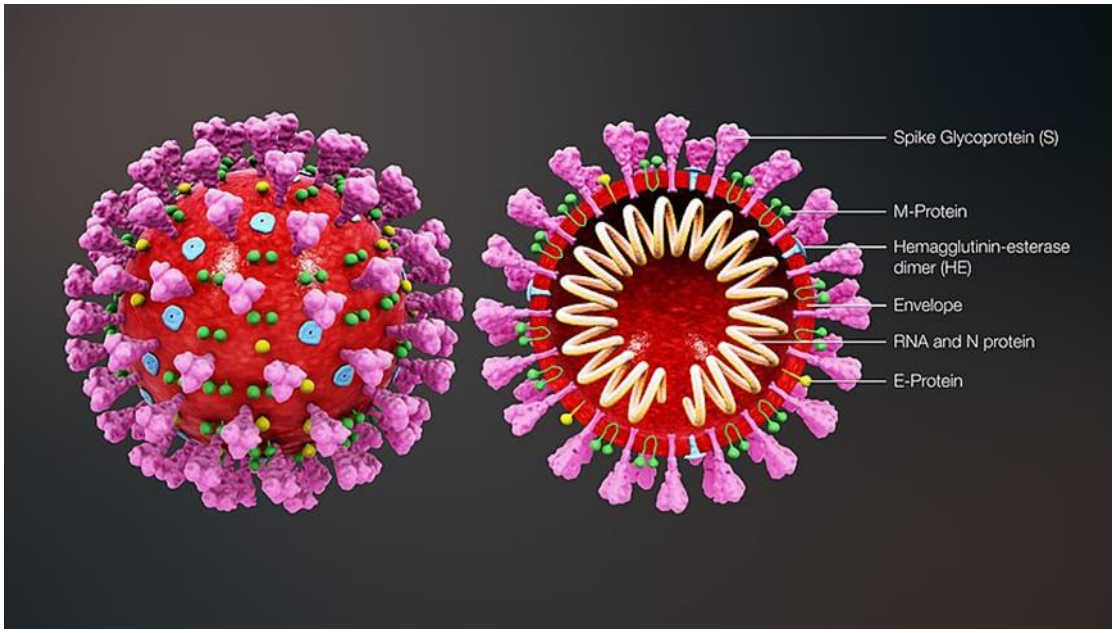


Figure (2-2): Schematic diagram of SARS-CoV-2, (Brian and Baric, 2005).

2.6. Genome Organization of coronavirus:

The size of coronavirus genome is in the range of (26 and 32 kb) and comprise 6–11. Open Reading Frames (ORFs) , That encoding 9680 amino acid polyprotein , Figure (2-3). The first ORF, comprises approximately 67% of the genome that encodes 16 nonstructural proteins (nsps), whereas the remaining ORFs encode for accessory and structural proteins. (Guo *et al.*, 2020). Hemagglutinin-esterase gene is absent from SARS-CoV-2 genome, It does however include two flanking untranslated

regions (UTRs) at the 5′ and 3′ ends of 265 and 358 nucleotides, respectively. No discernible change in ORFs and nsps was found in sequence variation between SARS-CoV-2 and SARS-CoV. The nsps contains two viral cysteine proteases, such as the papain-like protease (nsp3), the chymotrypsin-like, 3C-like, or main protease (nsp5), the RNA-dependent RNA polymerase (nsp12), the helicase (nsp13), and additional proteins that are probably involved in the transcription and replication of the SARS-CoV-2. (Chan *et al.*, 2020).

Along with nsps, four other important structural proteins are encoded by ORFs, including accessory proteins and spike surface glycoprotein (S), membrane, nucleocapsid protein (N), and envelope (E). Three transmembrane domains (TM) plus a lengthy C-terminal CT domain make up the N-terminal glycosylated ectodomain of the M protein. In contrast to the S glycoprotein, which is a fusion viral protein made up of the S1 and S2 subunits, the M and E proteins are necessary for the morphogenesis, assembly, and budding of viruses. The S1 subunit is made up of the signal peptide, the N-terminal domain (NTD), and the receptor-binding domain (RBD), and it has a 70% sequence similarity with human and bat SARS-like CoVs.(Walls *et al*., 2020).

The exterior subdomain, which is essentially in charge of how the spike interacts with the ACE2 receptor, was where the majority of the changes were discovered. The spike protein's ectodomain. The spike glycoprotein structure of SARS-CoV-2 was solved using a cloned, produced, and crystallized protein with residues (1–1208 amino acids) With an RMSD of 3.8, the spike glycoprotein structure of SARS-CoV-2 is similar to the spike protein of SARS-CoV. Additionally, the research demonstrates that the receptor-binding region (RBD) has the most structural diversity (Wrapp *et al*., 2020).

Bat SARS-like CoVs and human SARS-CoVs have 99 percent sequence similarity with the S2 subunit, which consists of two heptad repeat sections known as HR-N and HR-C that produce the coiled-coil structures around the protein ectodomain. At the junction of the S1 and S2 subunits, the S protein features a furin cleavage site (PRRARS'V) that is processed during biogenesis (Coutard *et al.* ,2020), as shown in Figure (2-3)

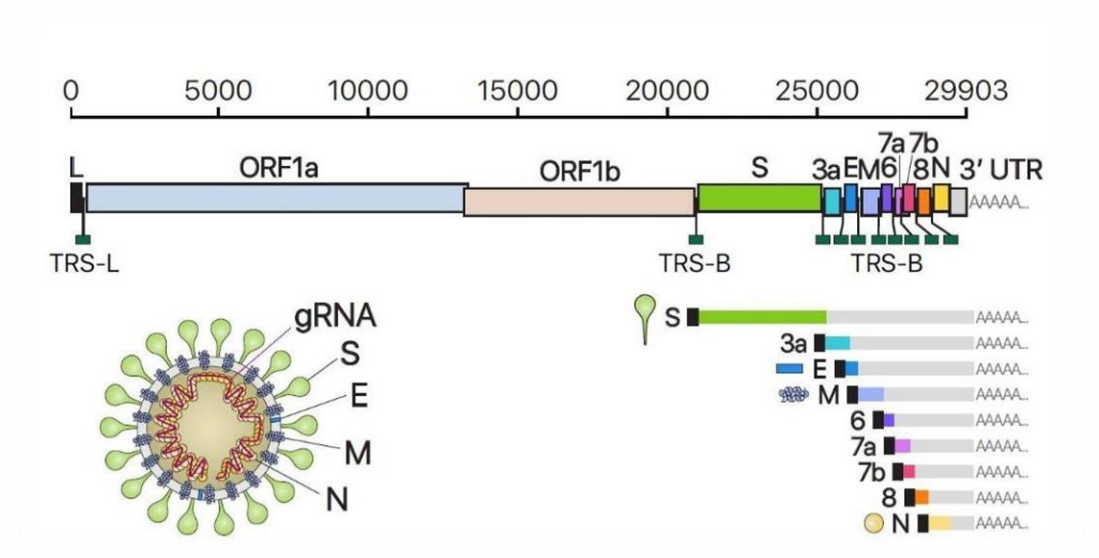


Figure (2-3): Schematic presentation of the SARS-CoV-2 genome organization (Kim *et al*., 2020a).

2.7. Human receptors of coronavirus:

Coronavirus infection (SARS-CoV-2) can be transmitted by bats and humans since it is a member of the Nidovirus order. The Angiotensin-converting enzyme 2 (ACE2) receptors, which can be found in a variety of organs, including the heart, lungs, kidneys, and digestive system, are complementary in form to the spike shape, allowing effective attachment and making it easier for the virus to reach the target cells (Rabi *et al.*, 2020) This binding occurs in the S protein domain of (SARS-CoV-2.) receptors, which is closely linked to, ACE2 of human and bat. Following

the entrance and attachment routes, the membrane of viral and the cell of host fuse (Qianqian *et al*., 2021).

The structural and accessory proteins are then generated from the sub-genomic proteins like M, S, and E, after that they are separated in the Endoplasmic Reticulum and, moved to the Endoplasmic Reticulum–Golgi Intermediate Compartment (ERGIC). In this time being, an earlier transcribed genomic material program will enter N protein, in Nucleocapsid form and progress to (ERGIC). Nucleocapsid can encounter some other structural proteins in this compartment and create small portfolio vesicles for exocytosis outside the cell as shown in Figure (2-4).(Fehr *et al*., 2015).

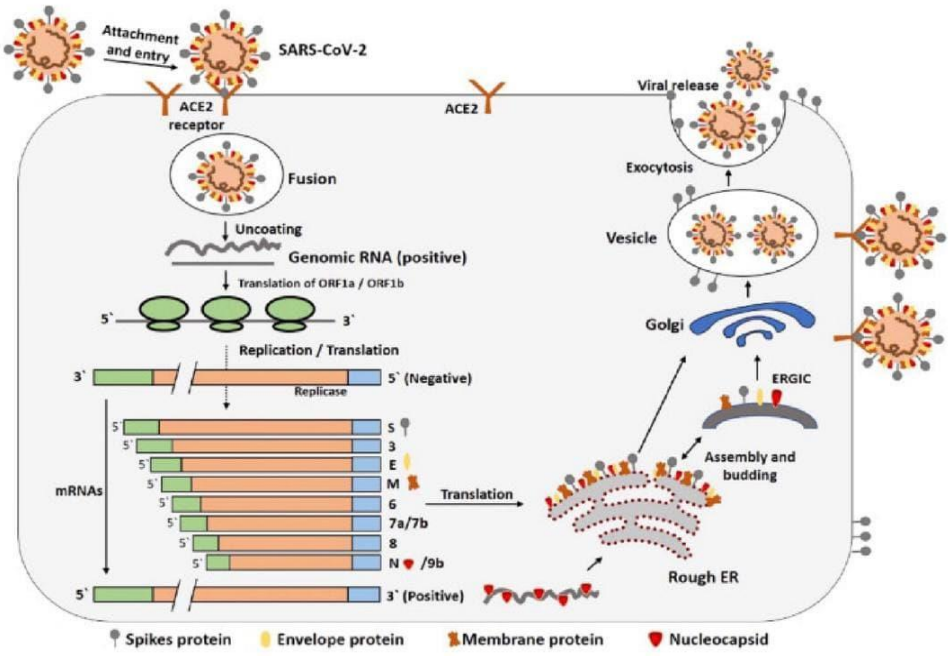


Figure (2-4): The life cycle of SARS-CoV-2 in host cells. (Fehr *et al*., 2015).

2.8. Immune response of Covid-19:

In addition to knowing the body's immune response against SARS CoV-2 and its role in the severity of infection, it is important to design an

appropriate and effective vaccine, as well as finding the appropriate treatment for the virus. Two types of immune response occur inside the body :(Huang *et al*., 2020) a weak primary response to Interferon, which allows the virus to multiply, and a severe secondary or late immune response characterized by a massive of pro-inflammatory cytokines, including interleukins IL6, IL1, TNF, MCP-1, and IL-10 proteins (Zhao *et al.*, 2020).

Further, neutrophils and macrophages that generate an immune response and a strong reaction in some cases are destructive and harmful to the patient's body (Prompetchara *et al.*, 2020)

In Order to control the Covid-19 pandemic, protect the groups at risk of infection with severe symptoms of the disease, and limit the spread of the "SARS-Cove-2" virus that causes it, our bodies must acquire immunity against this virus,(Turner *etal.* ,2021). The immune system provides this protection either by complex interaction with the virus when infected, or by responding to a vaccine. (Wang *etal*., 2021)

2.8.1 Innate immune response:

The innate immune system functions as the first line of host defense against pathogens including (SARS-CoV-2). Innate immune responses limit viral entry, replication, translation and assembly, help identify, and remove infected cells, accelerate and coordinate the development of adaptive Immunity. Cell surface, endosomal and cytosolic Pattern Recognition Receptors (PRRs) respond to Pathogen-Associated Molecular Patterns (PAMPs), to trigger inflammatory responses and programmed, cell death that limit viral infection and promote clearance (Kanneganti , 2020) .

However Excessive immune activation can lead to systemic inflammation, and severe disease. In response to innate immune-dependent viral clearance mechanisms, coronaviruses (CoVs) have evolved evasion strategies to limit host control and enhance replication and transmission (Konno *etal.* , 2020).

Innate immune cells including Monocytes , Macrophages , Dendritic cells, Neutrophils and Innate Lymphoid Cells (ILCs) ., such as Natural Killer (NK) cells, are armed with an arsenal of (PRRs), that recognize (PAMPs) or damage-associated molecular patterns (DAMPs), to induce inflammatory signaling pathways and immune responses. (Kanneganti, 2020)**.** The SARS-CoV2 infection begins when the virus binds itself by a spike protein S1 to its receptor on the cell surface called the ACE2 receptor (Scudellari, 2021). Virus injects its genome into the cell then the ribosomes make new copies of the virus, and then the Golgi apparatus and the rough endoplasmic reticulum manufacture the envelopes of the virus in order to exist outside the cell and infect other cells (Hackstadt *et al.*, 2021).

2.8.2 Adaptive immune response:

The second aspect of immunity is T cell and B cell that plays a major role in adaptive immunity. CD4 cells guide and develop the immune response to antibodies while the role of CD8 cells is to kill the virus directly (Li *et al*., 2008).

Immunogenic CD4 and CD8 T cell epitopes in SARS and MERS patients were found to localize mainly to structural proteins, particularly the S protein, Virus particles and/or cell debris affected by virus infection are recognized by dendritic cells in the lung interstitium. From there they go to the lymph nodes and present these antigens to CD4 and CD8 cells,

which have an important role in activating innate and adaptive immunity (Shin *et al.*, 2019; Hue *et al.*, 2020) .

When antigen reaches the lymph nodes, the macrophages in the lymph nodes produce cytokines, including Interferons and interleukins such as interleukin-12 (IL-12) (Rydyznski *et al.*, 2020). Dendritic cells (DCs) activate CD4 T lymphocytes in the presence of IL-12, causing them to develop into Th1 effector cells. These produce IL-2 and IFN gamma, which aid in the differentiation of CD8, T lymphocytes and B lymphocytes in to cytotoxic cells and immunoglobulin M - producing plasmocytes, (Villas-Boas *et al.*, 2020).

2.9. Cytokines & COVID-19:

The newly emerging COVID-19 is continuing to challenge medical health systems all over the world and the scenario is still getting worse. The COVID-19 poses an increasing threat to humans with a fatality rate of 6.4 % so far (WHO,2020). The COVID-19 infection is accompanied by an aggressive inflammatory response with the release of a large amount of pro-inflammatory cytokines in an event known as “cytokine storm.” The host immune response to the SARS-CoV-2 virus is hyperactive resulting in an excessive inflammatory reaction (Ruan *etal*., 2020).

Several studies analyze cytokine profiles from COVID-19 patients suggested that the cytokine storm correlated directly with lung injury, multi-organ failure, and unfavorable prognosis of severe COVID-19, (Sun *etal.*, 2020). Increased levels of pro-inflammatory cytokines in serum such as IL1β, IL6, IL12, IFNγ, IL10 and MCP1 were related with pulmonary inflammation in SARS patients (wong *et al.*, 2004).

Additionally Huang *et al* (2020) reported high levels of IL1β, IFNγ, IL10 and MCP1 in intensive care unit COVID-19 patients, which is

probably the reason for the activated T-helper-1 (Th1) cell response (Huang *et al*., 2020). Moreover, it has been found that the levels of TNF-α, IL-6 and IL-10 were correlated with the severity of COVID-19. IL-6 is an important cytokine whose production is related with various inflammatory diseases (Diao *et al*., 2020).

Subjects with (SARS-CoV-2) had high levels of IL-6 that were correlated with patient symptoms including: extensive lung damage and pulmonary inflammation. (Lu *et al*., 2020). Additionally, patients with (SARS-CoV-2) infection had low levels of suppressor of cytokine signaling-3, which regulates and stimulates the negative feedback mechanism of IL-6 (Okabayashi *et al*., 2006).

On the same line, another study reported that IL-6 levels were higher in severe COVID-19 patients and this may be used as one of the bases for predicting the transition from mild to severe infection (Wan *et al*., 2020). In particular, it has been showed that COVID-19 patients in intensive care had lower CD8+ T cell counts and their total CD4+ and CD8+ T cell counts were also negatively correlated with TNF-α and IL-6 concentrations (Diao *et al*., 2020).

In addition, recent studies showed that higher level of IL-6, CRP and also IL-10 were more significant rather than other cytokines in critical group of COVID-19 patients (Liu *et al*., 2020a). It can be suggested that immune dysregulation is a highly important point and therapeutic target for COVID-19 patients. The reasons for the large scale of the inflammatory cytokines are not clear, but it could play a crucial role in cell apoptosis associated with organ damage (Han *et al*., 2020).

Acute respiratory distress syndrome has previously been associated with genetic predisposition and inflammatory cytokines, it was found that there is a relationship between the genetic makeup of the patient and the development of HCOV disease (Meyer and Christie,

2013). Over 40 potential genes have been linked to the development or outcome of ARDS, including interleukin 10 (IL-10), ACE2, tumor necrosis factor (TNF), and vascular endothelial growth factor VEGF. Increased levels of IL-6 and IL-8 in the blood have also been linked to ARDS complications (Mason *et al*., 2016).

Cytokine storm Once the fusion is complete, the virus replicates within the host cells. This invasion of lung surface cells results in lung inflammation and a negative cycle of oxidative stress-related processes, such as increased PARP and PARG activity, ADP ribose, and TRPM2 activity (Kouhpayeh *et al*., 2020).

It has been pointed that the Antigen-Presenting Cells (APCs), such as macrophages, present SARS CoV-2 antigens to T cells after viral cell contact. This mechanism results in T cell activation and cytokine synthesis in diverse T cell subsets, such as Th17. This is followed by a huge cytokine release due to a positive response loop between cytokines, and immune cells. During SARS-CoV-2 replication, however, the viral genomic dsRNA activates Interferon Regulatory Factors (IRFs) and the TLR-3-induced NF-κB pathway, resulting in the generation of high amounts of type I IFNs and proinflammatory cytokines (Gang Li *et al.*, 2020).

Furthermore, it has been pointed that elevated free iron and hyperferritinemia in COVID-19 patients due to iron dysregulation and overload may worsen inflammatory processes that causes oxidative damage to cellular macromolecules and possibly immunogenic ferroptosis due to ROS-induced oxidative damage (Cavezzi *et al*., 2020).

Following hyper-inflammation and cytokine storms, these pathways may not only, result in an uncontrolled endothelial dysregulation of immune responses, but also pulmonary tissue damage, and multiple organ cumulated (Sarmiento-Monroy *et al*., 2021).

2.10. Immunological Parameters:

2.10.1.Association of CRP and Inflammation in COVID-19:

C-Reactive Protein can enhance the phagocytosis of phagocytes through specific CRP receptor, and remove various pathogenic microorganisms. During the process of COVID-19 pneumonia, a cytokine response storm (CRS) can be triggered, which is associated with high mortality in COVID-19 (Azar *et al*., 2020). The cytokines such as IL-6, TNF-α, stimulate hepatocyte to produce CRP (Ponti *etal*., 2020). CRP is the biomarker that most strongly correlates with COVID-19 progression, is significantly elevated during the early stage of inflammation and also prior to indications of critical findings with CT (Chan *et al*., 2020). Several retrospective comparison studies between survivors and non-survivors showed an increasing trend of acute-phase proteins, including CRP, procalcitonin (PCT), and IL-6, in non-survivors, and a stable or downward trend in survivors (Chen *et al*., 2020) CRP was verified to be independent outcome predictor and independent discriminator of disease severity, indicating that the diagnostic value of CRP for COVID-19 might be useful in clinical practice (Luo *et al*.,2020). In a multicenter retrospective study, it was reported higher level of CRP in thrombotic complication events after COVID-19 infection (Al-Samkari *et al.*, 2020).

In addition, obesity and metabolic syndrome in COVID-19 were associated with chronic systemic inflammation, including atherosclerosis and hypertension, which affected the outcomes of COVID-19 (Chiappetta *et al*., 2020). The observational study of elderly Iran patients with higher body mass index demonstrated lymphopenia, hypomagnesemia, elevated CRP and/or raised creatinine on admission were at higher risk of mortality due to the COVID-19 infection, taken together CRP might play a vital role in the process of inflammatory response, and it can be used to

assess the severity of COVID-19 and be independently associated with the risk of COVID-19 (Alamdari *et al*., 2020)

2.10.2. Association between HLA and COVID-19:

In humans the HLA system orchestrates immune regulation. The HLA system is potentially responsible for activating an immune response to (SARS-CoV-2), including the role of HLA alleles in affected individuals (Barquera *et al*., 2020). It is recognized that T-cell receptors recognize, the conformational structure of the antigen binding-grove in the HLA molecule along with the accompanying antigen peptide Thus, particular HLA haplotypes are associated with distinct genetic predispositions to disease (Horton *et al* ., 2004; Dutta *et al*.,2018; Lee and Koohy., 2020).

The repertoire of the HLA molecules composing a haplotype is thought to contribute to survival during evolution. As a result, it is advantageous to have enhanced binding capabilities of HLA molecules for viral peptides on the surface from novel viral infections, such as SARS-CoV-2, on the cell surface of antigen-presenting cells (Wieczorek *etal*., 2017 ; Dutta *et al*., 2018).

speculate that population HLA variability in a population could be correlatedwithCOVID-19 incidence since HLA plays such a crucial role in the immune response to pathogens and the development of infectious diseases. The HLA system affects clinical outcomes in multiple infectious diseases, including HIV and SARS (Lin *et al*., 2003 ; Bardeskar and Mania., 2016). For the latter, population studies observed correlations between certain HLA alleles and the incidence and severity of SARS (Lin *et al*.,2003 ; Chen *et al* ., 2006). HLA-B 07:03, B 46:01, DRB1 03:01,

DRB1 12:02 alleles were correlated with SARS susceptibility (Sanchez., 2020).

The SARS related susceptibility alleles were not shown to occur in COVID-19 patients at a significantly different level (Wang *et al.*, 2020). Host genetic variability may help to explain the multiplicity of immune responses to a virus within a community, knowing how variability in HLA can impact the progression of COVID-19, in particular, may help distinguish individuals at higher risk for the disease ,in critically ill patients results indicate down regulation of HLA-DR molecules in circulating monocytes which based on profound lymphopenia and other functional differences, create immunosuppressed conditions for host response (Benlyamani *et al.*,2020).

An in silico analysis of viral peptide-major histocompatibility complex (MHC) class I binding affinity was conducted by Nguyen *et al* (2020) which revealed that HLA-A02:02, HLA-B15:03, and HLAC 03:12 effectively presented a larger amount of peptides whereas A25:01, B46:01, C01:02 were the least efficient for of SARSCoV-2- peptide presentation. Indicating that Class I HLA molecules with a better theoretical capacity to bind SARS-CoV-2 peptides were found in patients with mild disease and showed higher heterozygosity as compared with moderate and severe disease (Iturrieta *et al.*, 2020)

2.10.2.1. Human Leukocyte Antigen-DR:

Human Leukocyte Antigen-DR is a class II human leukocyte antigen (HLA) expressed on the cell surface of antigen-presenting cells, including monocytes, differentiated macrophages and dendritic cells, as well as B cells. Since the first description of role of HLA-DR in immunosuppression. (Volk *et al*., 1991). HLA-DR expression on

monocytes has been subsequently proven to be a reliable marker for evaluating immune dysfunction and risk of secondary bacterial infections in sepsis and trauma patients (Pfortmueller *et al*., 2017)

Thus, reduced amounts of HLA-DR can also place COVID-19 patients at high risk of secondary and severe bacterial nosocomial infections. This observation is consistent with a clinical report of secondary bacterial infections and end-organ injury among COVID-19 patients requiring ICU care (Zhou *et al*., 2020) .

A recent examination of HLA variations among the world population revealed a significant impact of HLAs on the cellular immune response, in particular, on peptides from coronavirus-infected patients. HLA-B15:03 exhibited the greatest capacity to present highly conserved, shared SARS-CoV2 peptides to immune cells (Nguyen *et al*., 2020). Subsequent studies have recently confirmed that immune dysregulation in COVID-19 patients with respiratory failure is associated with a significant downregulation of monocyte HLA-DR (Giamarellos *et al*., 2020) .

2.10.2.2. Human Leukocyte Antigen –G:

The classical class I (A, B and C) and class II (DR, DQ and DP) HLA molecules are involved in mediating antigen presentation of intracellular and extracellular peptides, respectively (Wieczorek *et al*., 2017). Non-classical class I HLA molecules (E, F, and G) are other important products encoded by genes in the HLA region, and constitute the core molecules involved in controlling the immune response to infectious agents, as well as inflammatory reactions .(Halenius *et al*., 2015). HLA-G antigens are among the non-classical class I HLA glycoproteins that were first distinguished by their expression at the

maternal-fetal interface to protect the fetus from the maternal immune system (Djurisic and Hviid, 2014).

Seven isoforms of HLA-G have been recognized, including four membrane-bound antigens (HLA-G1, -G2, -G3 and -G4) and three soluble molecules (sHLA-G5, -G6 and -G7) (Alegre *et al.*, 2014). These molecules are considered potent immunomodulators, and their dysregulated expression has been implicated in several pathological conditions (Morandi *et al.*, 2016). Two theories have been put up to explain the function of HLA-G in the immunopathogenesis of viruses during viral infection. HLA-G may enhance immune escape of the virus, and this is supported by HLA-G's immunosuppressive properties. Conversely, HLA-G expression and/or secretion may reflect a robust response to inflammatory reactions that occur during viral infections (Amiot *et al*.,2014). In the context of COVID-19, the role of HLA-G in immunopathology of disease has not been well investigated. However, there have been some suggestions that HLA-G molecules may have immune-regulating effects in COVID-19 ,(Zhang e*t al*., 2020; Zidi ; 2020, De *et al.*, 2021; Rizzo *et al.*, 2021).

2.10.3.InterLeukin-6 and Development of Severe Covid-19:

Interleukin - 6 (IL-6), are associated with Covid-19 progression according to known evidence, IL-6 is superior to CRP and other markers of inflammation in predicting respiratory failure in Covid-19 (Ponti *etal*.,2020), IL-6 appears to be the most important driver of immune dysregulation and ARDS in Covid-19 (Herold *etal*.,2020). The role of systematic measurement of IL-6,CRP, and other markers of inflammation at the initial assessment during Covid-19 outbreak in long term care facilities (LTCF) and its ability to predict the severe course of disease is yet to be determine. (Han*etal*., 2020). The IL-6 is produced by stromal

cells and virtually all immune system cells in the lungs and its secretion is stimulated by proinflammatory cytokines, especially InterLeukin- 1β (IL-1β), and Tumor Necrosis Factor α (TNFα) in the early stages of the infection, it is produced by lung macrophages after stimulation of toll-like receptors (Hunter and Jones., 2015).

An important trait of IL-6 upregulation in Covid-19 is that it precedes the development of acute lung injury that implicates its usability as an early marker of severe disease (Aziz *et al*., 2020). However, there is controversy if excessive IL-6 synthesis is true a cornerstone of the pathogenesis of respiratory failure in Covid-19 or is just an epiphenomenon of increased IL-1β and TNFα in the cytokine storm (Magro ,2020).

Predominant theory is that overexpression of IL-6 have a crucial role in the incitement and propagation of the so-called cytokine storm leading to lung injury and (ARDS), It is believed that IL-6 increases the permeability of lung capillaries driving the ARDS development and also stimulates the coagulation pathway leading to thrombosis in lung circulation and increases the risk of thrombotic event suggests that patients with severe respiratory failure in Covid-19 suffer from distinct types of immune dysregulation which are mediated by IL-6 upregulation (Giamarello-Bourboulis *et al*., 2020).

This dysregulation is characterized, by high production of proinflammatory cytokines by monocytes and macrophages and CD4 lymphocyte depletion that contributes to the progression of inflammation of lung parenchyma . The direct role of IL-6 in Covid-19 pathogenesis is further supported by findings that IL-6 inhibition improves the prognosis of severe Covid-19, (Guaraldi *et al*., 2020).

2.11. Molecular Parameters:

2.11.1 .MicroRNA and role with covid-19 :

The miRNAs are short, non-coding RNAs of 22 nucleotides in length that regulate one-third of all protein-coding human genes (Hammond, 2015). Furthermore, miRNAs play a crucial role in the pathogenesis of various ailments, including viral infections, neurodegenerative disorders, metabolic diseases, chronic pediatric diseases, and parasite-specific diseases (Paul *et al.*, 2020).

Regarding the viral infection of mammalian cells, miRNAs participate in the control of lytic and latent viral replication (Grassmann and Jeang, 2008) and, specifically, in coronavirus, it has been proved that they are involved in immune response and viral protein expression (Abu-Izneid *et al*., 2021; Mirzaei *et al*., 2021).

Remarkably, miravirsen and RG-101 are the most promising miRNA-based drugs that have been widely studied in the last years to treat hepatitis C (Bonneau *et al*., 2019), and it is worth mentioning that miravirsen might have a noteworthy anti-SARS-CoV-2 effect that should be thoroughly explored (Alam and Lipovich, 2021).

Therefore, miRNA-based therapeutics are outlined as innovative strategies to mitigate viral diseases, specially COVID-19, microRNAs (miRNAs) have emerged as potential theragnostic targets that could help to elucidate the biological mechanisms underlying COVID-19 and design novel drugs against this virus (Fani *et al*., 2021).

2.11.1.1. MicroRNA-31:

MicoRNA-31 has been characterized as a tumor suppressor miRNA, with its levels varying in breast cancer cells according to the metastatic state of the tumor (O'Day *et al*., 2010). From its typical abundance in healthy tissue is a moderate decrease in non-metastatic breast cancer cell lines, and levels are almost completely absent in mouse and human metastatic breast cancer cell lines (Valastyan *et al*., 2009).

Micro RNA 31-5p has also been observed upregulated in Zinc Deficient rats compared to normal in ESCC (Esophageal Squamous Cell Carcinoma) and in other types of cancers when using this animal model (Fong *et al.*, 2016). There has also been observed a strong encapsulation of tumor cells expressing miR-31, as well as a reduced cell survival rate miR-31's antimetastatic effects therefore make it a potential therapeutic target for breast cancer(Valastyan *et al*., 2011). However, these two papers were formally retracted by the authors in 2015, mir-31 has been linked to Duchenne muscular dystrophy − a genetic disorder characterised by a lack of the protein dystrophin − as a potential therapeutic target. Duchenne muscular dystrophy is caused by mutations arising in the dystrophin gene, which impair the translation of dystrophin through the formation of premature termination codons (Cacchiarelli *et al*., 2011).

The miRNA signature was shown to be robust in the ferret model of COVID-19 and could distinguish SARS-CoV-2 infection from seasonal influenza A infection. These findings suggest that miRNA profiling may be adopted to improve COVID-19 detection and patient management. miRNA-31 including it is mature forms, miRNA (31-3p) and miRNA

(31-5p) , has a dual role both oncogenic and tumor-suppressing, being disrupted in many human cancers (Lei *et al*., 2014).

Aberrant expression of miRNA (31-5p) has been detected in various cancers and plays a significant role in tumorigenesis. Low miRNA (31-5p) expression was present in nasopharyngeal carcinoma tissues and cell lines and acted as a tumor suppressive miRNA and low expression of miRNA (31-5p), was highly correlated with tumor-node-metastasis stage (Yi *et al*., 2019).

Serum miRNA (31-5p), levels were significantly different between oral cancer patients and healthy controls and between pre- and postoperative patients. Furthermore, a miRNA (31-5p), mimic enhanced the proliferation of normal epithelial cells, and antagomiRNA(31-5p) inhibited the proliferation of oral cancer cells (Lu *et al*.,2019).

MicroRNA (31-5p) was proven to have oncogenic properties in both Colorectal Cancer (CRC), cell lines and primary colorectal tumors. In (CRC) , miRNA (31-3p) and miRNA (31-5p), dysregulation seems to have a particular role in response to treatment with anti-EGFR therapy (Lei *et al*.,2014).

MicroRNA (31-5p) was significantly down-regulated in Renal Cell Carcinoma (RCC), tissues and cell lines compared with paired adjacent normal tissues and normal cell lines, MiRNA (31-5p), downregulation was associated with poor prognosis in RCC patients. Overexpression of miRNA (31-5p) inhibited (RCC), cell proliferation, migration and invasion and cell cycl (Li *et al*., 2019). To examine the expression of four inflammatory miRNAs and their mRNA targets in blood samples from COVID-19 patients with various degrees of the disease. Also, the relative expression of these miRNAs and their mRNA targets during

hospitalization were investigated, Since some COVID-19 patients respond to medication (e.g., remdesivir and favipiravir) and some of them do not respond well, the relative expression of miRNAs and their mRNA targets is different in the two groups (Lim *et al*.,2005).

The First question is that miRNAs must be selected and investigated, from the Human miRNA pool, Although it is a very difficult and complex question, there are two main solutions (Bartoszewski *etal*.,2020). The first solution is to use a microarray to evaluate all human miRNAs. Although we can create a comprehensive list of up- and down-regulated miRNAs using this method, it necessitates an intricate and costly apparatus (Khan *etal*.,2020).

Another option is to use computational and bioinformatics tools to identify possible miRNAs. The majority of the publications on miRNAs and COVID-19 have followed the same format. (Nersisyan *et al.*, 2020). MiRNAs can be extracted from tissue or blood samples in general. (Pinilla *et al*.,2021). Importantly, miRNAs found in the blood can aid in diagnosis and prognosis. COVID-19 (Centa *et al*.,2021).

These miRNAs may originate mostly from respiratory and immunological cells. It is important to note that the blood contains a number of harmful enzymes, which might cause the targeted miRNAs to be quickly destroyed (Zhang *et al*., 2012).

It is intriguing to notice that additional RNA types, such long non-coding RNAs (long ncRNAs or lncRNAs), can also provide information on the existence of SARS-COV-2 (Wu *et al*., 2021).

2.11.1.2. The microRNA192:

Data from the miRbase, database show that there are roughly 80 different human miRNA precursors, which result in two mature miRNAs, the 5′ and 3′ strands with different seed sequences and target mRNAs that they bind, A coding gene on chromosome 11 that generates two mature transcripts, miR, (miR-192-5p) and miR-192 is the source of human miR-192 (miR-192-3p), (Bartel, 2004).

Both (miR-192-5p) and (miR-192-3p) inhibits the translation of target mRNAs, by targeting the 3′-UTR in different biological processes. (Krattinger *et al.*, 2016).

The roles of miR-192-5p have been the subject of several investigations, although little is known about the roles of miR-192-3p. MiR-192-3p is critical for adipocyte development and lipid homeostasis, according to the study of Mysore and colleagues, The first intriguing finding was that miR-192-3p in visceral adipose tissue (VAT) of obese patients is adversely linked with the levels of blood triglyceride (TG) (Mysore *et al.*, 2016).

Additional mechanistic investigations have shown that miR-192-3p targets aldehyde dehydrogenase 3 family member A2 (ALDH3A2) and stearoyl coenzyme A desaturase 1 to suppress adipocyte development in human Simpson-Golabi-Behmel syndrome (SGBS) adipocytes (SCD),Additionally, miR-192-5p and miR-192-3p work together synergistically to suppress Farnesoid X Receptor (FXR), and FXR target gene expression in cell lines generated from liver and colon cancer However, Huh7 and Caco-2 cells respond differently to miR-192 in terms of how much FXR is expressed (Krattinger *et al.*, 2016).

Underscoring their various links to one another in various clinical circumstances, they occasionally cooperate synergistically and occasionally independently the 3′-transcript's cumulative influence should not be disregarded, even if this study focuses largely on the role of miR-192-5p in human illnesses. MiR-192-5p is connected to the control of blood pressure and heart rate, according to research (Caserta *et al.*, 2016). As a result, miR-192-5p is essential for several biological functions that are essential to human physiology. Particularly, a wealth of evidence suggests that miR-192-5p controls oxidative stress, cellular growth, apoptosis, and inflammatory responses (Fuschi *et al.*, 2017).

The dysregulation of miR-192-5p may have a role in the development of human illnesses given its significance in cellular and physiological processes in humans, It is vital to note that miR-192-5p is a conserved miRNA that is abundant in the liver and plays crucial roles in a variety of hepatic illnesses, such as chronic hepatitis B (CHB), drug-induced liver damage, nonalcoholic fatty liver disease (NAFLD), and hepatocellular cancer (HCC) (Nielsen *et al.*, 2018)

Chapter Three
Materials and methods

3. Materials and methods:

3.1. Materials:

3.1.1. Equipment:

The table below lists the tools that were utilized in this investigation (3-1). **The equipment used in the investigation is shown in Table (3-1).**

NO.	Laboratory equipment	The industrial company
1	Blue pipette tips	China
2	Collection tube	Bio comma limited (Spain)
3	EDTA tube	China
4	Eppendrof tube	AFCO
5	Filter paper	China
6	Gel tube	China
7	Gilson blue tips	AFCO
8	Micropipette from 0.5-1000 micro letter	Eppendrof Research Plus (Germany)
9	PCR tube	Bio near
10	PCR tube rack	Watson Bio Lab
11	White Pipette tips	ExpellPLUS ™
12	Yellow Pipette tips	China

3.1.2. Devices:

The devices that are utilized in this investigation, as stated in the table(3-2). **The study's instruments are listed in Table (3-2).**

NO	Laboratory Devises	The industrial company
1	Cold centrifuge	Favrogen
2	Deep freezer	Japan
3	Eppendrof Centrifuge 5418	Eppendrof
4	Incubator	Memmert
5	Micro plate reader	MolecularDevices ,Liner, Aspen
6	Nano drop	Thermo Scientific™
7	Real-time cycler	Bioneer
8	Refrigerator	Vestal
9	Vortex mixer	Bioneer

3.1.3. Kits and their contents:

The kits that are utilized in the study as indicated in the table (3-3).

Kits and components used in this investigation are listed in Tables (3-3).

NO.	Kits	The industrial company	Contents
1	**Human Micro RNA kit extraction**	FAVORGEN	-RL buffer -FARB buffer -Wash buffer 1 -Washbuffer2 (concentrate) - RNase- free water

			- Filter column - FARB mini column -Collection Tube - Elution Tube
2	**Go Taq® 1-StepRT-qPCR System.**	Promega	- GoScript RT mix for 1-Step RT-qPCR, 2x -GoTaq ® qPCR master mix (50X) - 20X forward primer - 20X reverse primer - Reference dye for CXR (optional). - Nuclease-Free Water
3	**Human leukocyte antigen -G ELISA kit**	Bioassay Technology Laboratory	-Standard dosage (2400 ng/l) ELISA plate with a pre-coat Streptavidin-HRP in its typical diluent - Stop response - A substratum solution - B Substratum solution- -User instructions for Wash Buffer Concentrate-25x Biotinylated Human HLA-G Antibody - Plate sealant - zip-top bag
			- Pre-coated ELISA plate

4	**Human leukocyte antigen -DR ELISA kit**	**Bioassay Technology Laboratory**	with Standard solution - 40ng/ml. Streptavidin-HRP diluent as usual - Stop response - A substratum solution - B Substratum solution- -User instructions for Wash Buffer Concentrate-25x Biotinylated Human HLA-DR Antibody - Plate sealant - zip-top bag

3.1.4. Chemical materials:

The Chemical materials that utilized study as indicated in the table (3 -4).

Chemical materials in the table (3-4).

NO.	Materials	The industrial company
1	**Beta - markpto ethanol**	
2	**Ethanol 70٪**	Aljoud Iraq

3.2. Methods:

3.2.1. Study design:

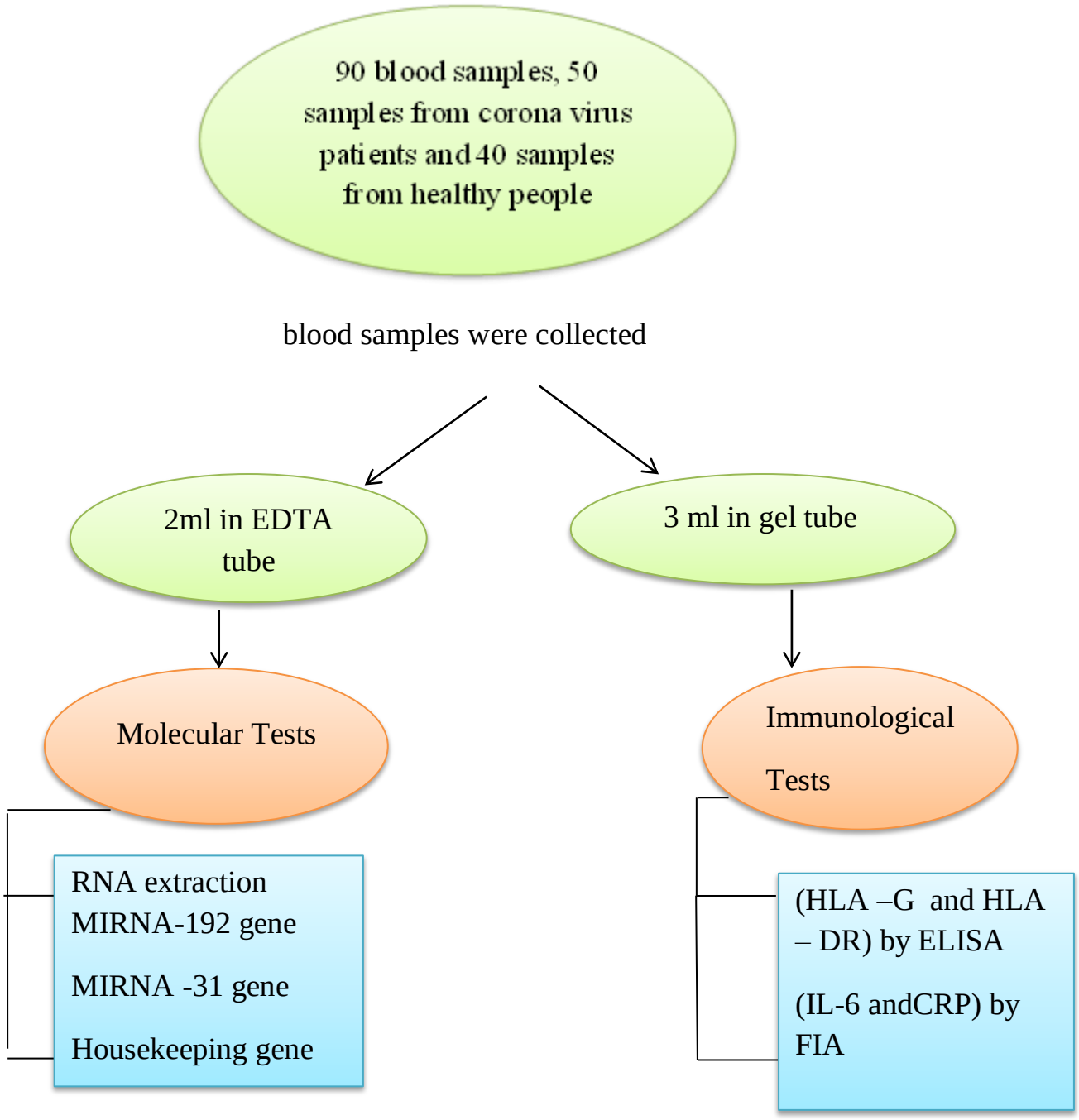


Figure (3.1): Representative diagram of the project work.

3.2.2. Sample collection:

Patients' blood samples were taken from September-2021 to -January-2022 at Kerbala Al-huseine Teaching hospital, Kerbala-middle Iraq. The hospital patients suffering from COVID-19 infections. All individuals with COVID-19 enrolled in this study and diagnosed according to the Iraqi National Guidelines for the diagnosis and treatment of COVID-19.

In addition to the interim who guidelines. Common symptoms included dizziness, headache, shortness of breath , runny nose, sore throat, diarrhea and decreased appetite. The Ethics Committee gave their approval to the project, which is a competent committee in the ministry of health and the ministry of higher, education and scientific research in iraq and informed consent was obtained from the participating patients, before collecting data and samples. Informed consent is waived for patients who are unable to obtain informed consent. blood samples were drawn from patients as follows:

Five ml of venous blood was drawn from Covid-19 patients. The blood was divided in 2ml in EDTA tube for molecular test ,3 ml in GEL tube we divide the serum into 2 eppendorf , for Immunological test

3.2.3. Ethical approval:

The formal administrative agreements were obtained before data collection which required for conducting the study as follows:

1. The initial agreement was obtained from the University of Babylon/ College of science / Higher education committee after protocol presentation.
2. The Study Protocol was accepted by the Ethical committee of the Department of Biology in Babylon university / College of science.

3. A formal requisition was sent to the Babylon and Karbala health Directorate for the agreement.
4. An official agreement was attained from the department of developing and training Babylon and Karbala health Directorate.

3.2.4. Sterilization Methods:

All instruments that were not affected by heat were sterilized in an autoclave at 121° C for 15 minutes at atmospheric pressure of 1.5 pounds per $inch^2$.

3.3. Molecular materials:

3.3.1. Primers:

The primers that utilized in the current study depicted in the table (3-5).

Primers which were utilized in the study in the table (3-5)

Gene	Nitrogen bases 5'→3'	Industrial Company	Ref.
miR- 192	F- CTGACCTATGAATTGACAGCCA	Humanizing Genomics Macrogen	Chiang *etal*. (2011). Chen *etal*. (2005).
	R- GCTGTCAACGATACGCTACGT		
miR-31	F- GCCGCAGGCAAGATGCTGGC		
	R- CAGTGCAGGGTCC GAGGT		
H.K.gene	F-GTTTTGTAGTTTTTGGAGTTAGTGTTGTGT R-CTCAACCTACAATCAAAAACAACACAAACA		

3.3.2. Total RNA was extracted from human whole blood:

Protocol:

1. Red blood cells were lysied.

2. Two hundred –three hundred *µl* were put in microcentrifuge tube (1.5 ml or 2.0 ml tube) was filled with of anticoagulant, preserved human whole blood .

3. Five volumes of the RL buffer were used combined with one volumes of the sample.

4. It was incubated on ice for 10 minutes. During incubation, there were two short vortexes.

5 . A cell pellet were made by centrifuging for 1 minute at18,000 x g ; completely discard the supernatant thereafter.

6. Six hundred *µl* of RL buffer to briefly vortex the cell pellet to suspend it.

7. Reforming a cell pellet using a centrifuge for 1 minute at 18,000 x g and then entirely discard the supernatant.

8. Three hundred fifty µl of FARb buffer and (3.5)µl of ß-mercaptoethanol to the cells pellet, Vortex vigorously for one min to suspend the cell completely.

9 . A filter column was placed in a collecting Tube, add the sample mixture, and centrifuge for two minutes at maximum speed (18,000 x g)..

10. The volume were measure of the cleared supernatant after transferring it from the collecting tube to a fresh microcentrifuge tube.

11. The vortex mixer was used a combine thoroughly one liter of ethanol that is 70% RNase-free.

12. The sample mixture was transferred with the addition of ethanol to the FARB small column after placing the column in a collecting tube.,

The flow-through was discarded away after centrifuging at full speed for one minute the FARb mini column were returned to the, collecting Tube.

13. Five hundred µl of wash Buffer 1 to the FARB Mini Column, was centrifuged at full speed for 1 min.The flow-through was discarded and the FARB Mini Column was returned back to the collection Tube.

14. To the FARb mini Column was added 750 ml of Wash Buffer 2, was centrifuged at full Speed for one minute, and then was added the results. the flow-through was disposed and was returned the FARB Mini Column to the collecting tube.

15.The step 8 for one more washing was repeated .

16. the FARB Mini Column was centrifuged at full speed for an additional 3 min to dry the FARB Mini Column.

17. A 1.5 ml Elution Tube (supplied) microcentrifuge tube should be placed over the FARB Mini Column.

18. Fourty ~ hunderd µl was injected RNase-free ddH2O into the FARB Mini Column's membrane core. For one minute, the FARB Mini Column was kept upright.

19. The RNA was eluted, the FARB micro column was centrifuged at maximum speed for one minute.

20. The RNA was Stored at -40C.

3.4. 1-step RT-qPCR system by GoTaq ®:

The user is required to provide materials equipment for real-time PCR and related tools (e.g, optical-grade PCR plates , suitable plate cover

sterile, aerosol-resistant pipette tip , nuclease-free pipettors specialized to pre-amplification operations, RNA template, and qPCR primers).

Table(3-6).General Thermocycler program

Stage	of cycles	program in standard
1- reverse transcription,	1	for 15 seconds at 37 C
2-RT inactivation	1	for 10 seconds at 95° C
3- Qpcr step		
A: Denature	40	for 10 second at 95°C
B : Anneal		for30 second at 60 °C
C : Extend		for 30 second at 72° C
4- Dissociation	1	60–95°C

3.4.1. Protocol:

Mode one-step RT qPCR (Table 3-6).

1 - A real-time was created instrument with a regular or quick setup.

2-The RNA was templated and the primer pair from the Go Taq ® 1-Step RT-qPCR System should be defrosted on ice, at room temperature, or at 37°C. Each defrosted component was combined well right away. Mix at low speed with a vortex mixer to reduce aeration. thawed reagents were kept chilled.

3.4.2. Preparation:

A. Samples of RNA (total RNA, mRNA, virus RNA, or transcript RNA [- hundre five hundred fg d ng]) in water or another diluent that is suitable with qPCR.

B. Controls and standards.

C. Primer pair: The 1X concentration ranges between 50 and 300 nm.

3- The reaction's components were mixed (Table 3-7) in a sterile, nonstick tube while it's chilled. After each addition, carefully stir. Pipette reaction amounts with caution on a dish of ice.

4- The plate was transferred from ice into the preprogrammed instrument. The run was started immediately.

5 -When the run is complete were collected the data and analyzing the results

Table (3-7). GoTaq® 1-Step RT- qPCR reaction mix.

Component	Volume	Final concentration
Go Taq ® qPCR master mix- 2X	10 μl	1 X
Go Script™ RT mix	0.4 μl	1X
for 1- Step RT-qPCR -50X		
Forward primer -20X	2μl	50–300 n m
Reverse primer -20X	2μl	50–300 n m
CXR reference dye ,optional	0.33μl/ 20μl reaction	500 n m
Nuclease - free water	to a final volume of 20μl	

3.5. Immunological Tests:

3.5.1. Human leukocyte Antigen G ELISA:

3. 5.1.1. Reagent Preparation:

Before useing all reagent should be at a temperature of room. to build a standard a 1200ng/l basic stock solution, reconstitute 120 l of the standard diluent were used with 2400 ng/l of the standard. Allow the

standard to sit with moderate agitation for 15 minutes prior to making dilutions.

By serially diluting the standard stock solution, creating duplicate standard points. (1200ng/ l), Solutions at concentrations of 600ng/l, 300ng/l, 150ng/l, and 75ng/l are produced by diluting the sample by 1:2 with standard diluent. A standard diluent has the concentration of zero (0 ng/L). Any solution that was left over had to be frozen at -20°C and utilized within a month. The proposed standard solution dilutions were as follows.

Table(3-8): Dilution of Standard Solutions for HLA-G.

ng/l 1200	No. 5- standard	Standard diluent, 120 *µl*, plus the original standard, 120 *µl*
ng /l 600	No. 4- standard	Standard,diluent120*µl*,plus120 *µl*,,Standard No.5
ng/l 300	No. 3- standard	Standard,diluent120*µl*,plus120 *µl*,,Standard No.4
ng/l 150	No. 2 –standard	Standard,diluent120*µl*,plus120 *µl*,,Standard No.3
ng/l 75	No. 1- standard	Standard,diluent120*µl*,plus120 *µl*,,Standard No.1

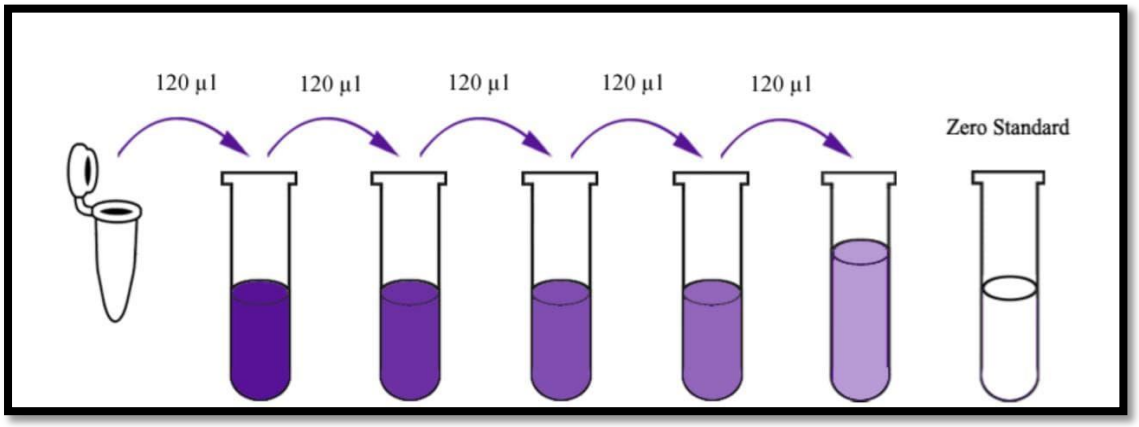


Figure (3 - 2). Standard HLA-G dilution solutions.

Washing the buffer after use. diluted 20 ml Distilled water is added to concentrate 25 times to create 500 ml of 1x Wash Buffer. When the concentrate begins to crystallize, gently agitate it until all of the crystals have disappeared.

3. 5.1.2. Procedure:

1 - As directed, preparing all reagents, standard solutions, and samples

2- It was determined how many test strips would be required. To be used, the strips were placed in the frames.

3- The standard well was filled with fifty *µl* of standard .

4- Fourty *µl* of sample should be added to the sample wells, ten *µl* of anti-HLA-G antibody should be added next, and fifyt *µl* of streptavidin-HRP should be added to the standard wells. Completely combine. Seal the plate with a sealant. 60 minutes of incubation at 37 °C.

5. The sealer was removed and wash the plate 5 times with washing buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash, decant. each well and wash 5 times with wash buffer. Blot the plate onto filter paper

6- Each well received 50 µl of the substrate solution before another 50 µl of the substrate solution was added. Plate should be incubated for, 10 minutes, in the dark, and at 37 °C.

7- Fifty *µl* were added of Stop every well's flow, and watch the blue, hue instantly become, yellow.

8- It was used a microplate reader set to 450 nm to calculate each well's optical density (OD value) within 10 minutes after adding the stop solution**.**

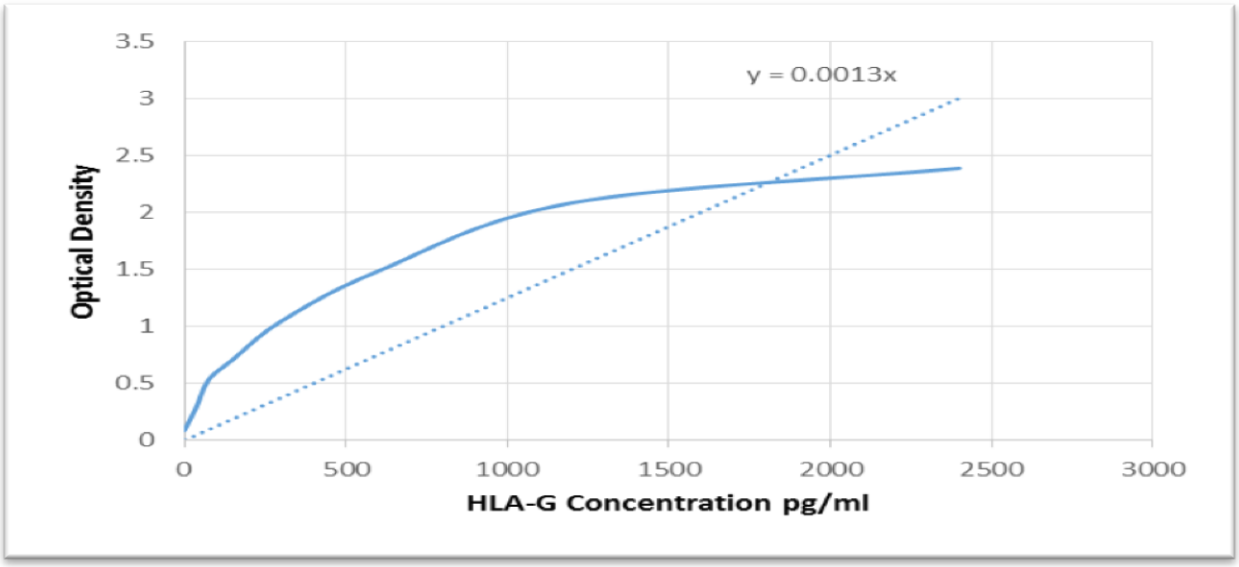


Figure (3 - 3) Standard Curve of HLA-G.

3.5.2. Human Leukocyte Antigen-DR ELISA:

The assessment of HLA-G was done by ELISA principle and method as like as HLA-DR , with the specific standard curve in which that mentioned in the Figure (3 - 4)

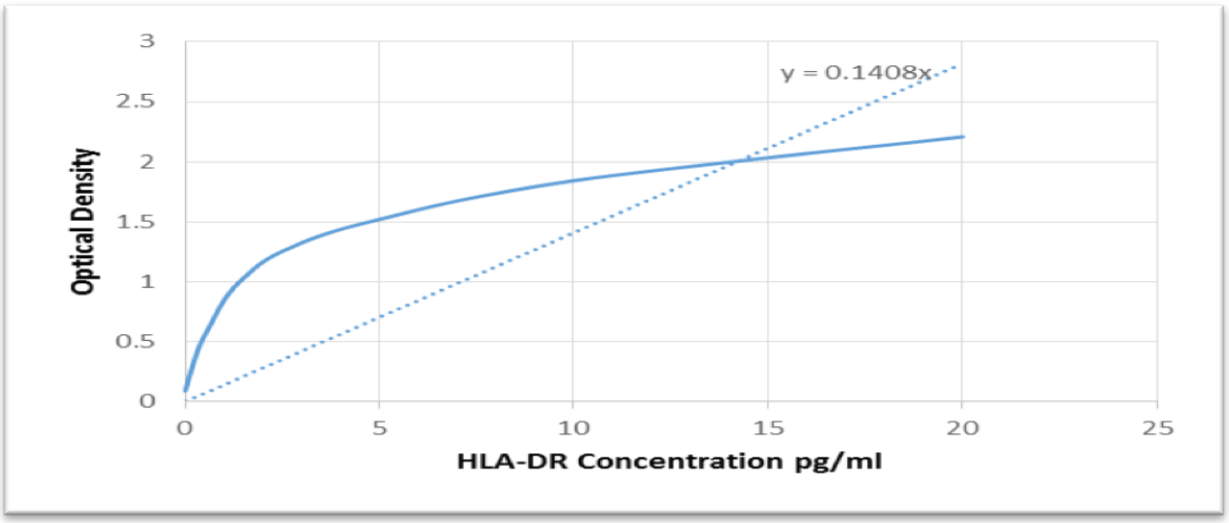


Figure (3 - 4) Standard Curve of HLA-DR.

3.6. IL-6 Guantity detection via fluorescence immunoassay (FIA):

3.6.1. Principle:

The antigen-antibody complexes formed by the antibody detection in the buffer, binding to the antigens in the sample, and migrating onto the nitrocellulose matrix are detected by the other immobilized antibodies on the test strip. This test employs a sandwich immunodetection approach. The sample will have more antigens. Result in an increase in antigen-antibody complexes, which in turn will cause detector antibodies to provide a greater fluorescence signal, which will then be analyzed by an instrument for ichroma testing to determine the quantity of Interleukin-6, in the sample.

3.6.2. IchromaTM IL-6's component parts:

Cartridge Box, detector tube, Detector diluent, ID chip 1, capillary tube.

3.6.3. Test Procedure:

1- One hundred fifty µL of the detector diluent were used a pipette to a detector tube containing granules. When the granule form is completely dissolved in the tube, it becomes detection buffer.

2- It was used a pipette, add 35 mL of sample serum, to a detection tube.

3- I were covered the detection tube's lid and thoroughly mixed the sample by shaking for 20 min.

4- 75 µL of the sample mixture was added to the cartridge's sample well.

5-Before placing the device into the holder., was waitde 12 minutes for the Cartridge to reach room temperature. Following the end of the incubation phase , scan the cartridge with samples right away.

6- The sample was inserted -loaded cartridge into the ichromaTM test device's cartridge holder to scan it.

7-Started was the scanning procedure by tapping the 'START' button on the ichromaTM test device.

8- The ichromaTM testing device will begin scanning the inserted sample cartridge right away.

9- The test outcome was observed for ichromaTM tests on the instrument's LCD, screen.

3.7. Fluorescence Immunoassay (FIA) for the quantification of CRP:

3.7.1. Principle:

The test employs immune detection using a sandwich approach; Antigen-antibody complexes are created when the detector antibodies in the buffer bind to antigens in the sample and, the other immobilized antibodies on the test strip by moving onto the nitrocellulose matrix. The sample will include more antigens, which will produce more antigen-antibody complexes. , which in turn will cause detector antibodies to produce brighter fluorescence signals, which will then be analyzed by an instrument for ichromaTM testing to determine the quantity of CRP in the sample.

3.7.2. IchromaTM CRP's component parts:

Cartridge box, cartridge number 25, ID chip 1, and use instructions Box 25 of the Sample Collector, which contains the detection buffer.

3.7.3. Test procedure:

1- The sample was inserted an empty collector to make a hole in a detection buffer's top.

2- A sample was drawed of 10 µL serum using a sample collector.

3- It was combined the detection buffer and sample into one.

4- The container was shake at least ten times before the sample comes out by inversion. The sample and buffer combination must be utilized 30 seconds quickly.

5- The cap was removed the constructed tube's top. Before putting the reagent to a cartridge, dispense two drops onto the paper towel.

6- Only two droplets of the combination to the cartridge's sample wisely.

7-Before placed the device into the holder, cartridge has to be given three minutes to acclimate to room temperature. the incubation period is over, scan the cartridge that holds a sample as soon as possible.

8- The sample loaded cartridge was inserted - into the ichromaTM test device's cartridge holder to scan it. Before squeezing the cartridge completely within the cartridge holder , be sure it's oriented correctly. For this purpose, a distinctive arrow is marked on the cartridge.

9-To begin the scanning procedure, press the "Select" or "START" button on the equipment for ichromaTM testing.

10-The ichromaTM was instrumented will begin the sample-loaded cartridge is being scanned right away.

11- The ichromaTM test was instrumented display screen will show the test findings.

Chapter Four
Literature Review

4. Results and Discussion:

4.1. Demographic distribution of Patients with Sars Cov2 infection:

The, total group of patients have 50 (55 .6%) persons were newly with virus Sars-Cov2 infection, in comparison with 40 (44.4%) healthy persons as a control group at P. Value (0.037) as shown in figure (4 – 1) .

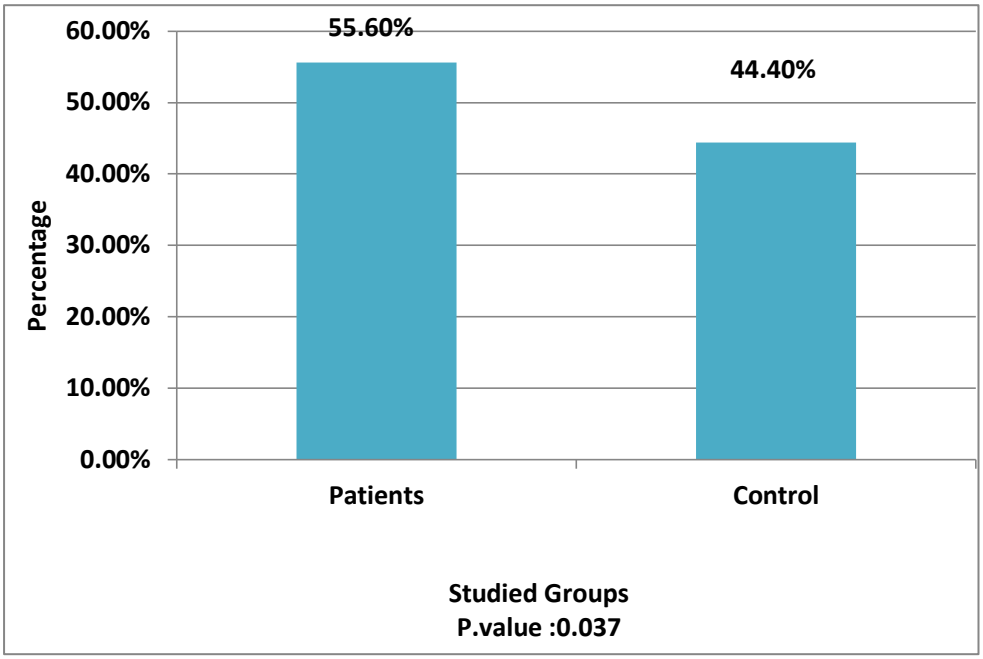


Figure (4 -1) Studied group of patients and control .

4.1.1. Sex distribution of infected patients:

The male patients have higher percentage (52%) than female (48%) of SARS –Cov2 infected patients enrolled in the present study as shown in Figure (4 -2)

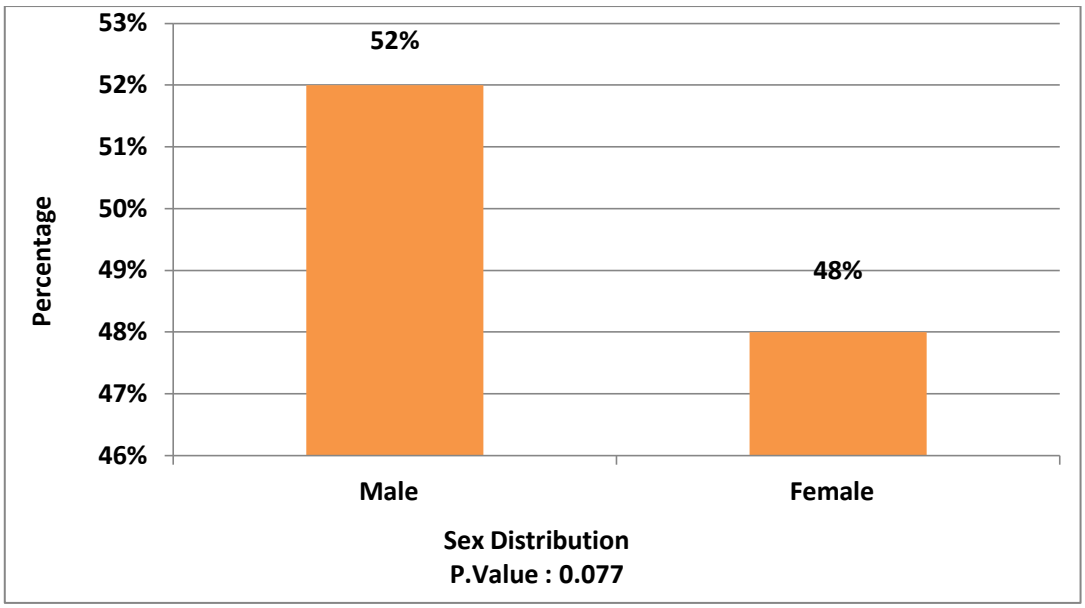


Figure (4 -2) Sex distribution of infected patients .

The relationship between the age and sex distribution and the COVID-19 epidemic features supported earlier findings that women are more vulnerable to the disease ,the findings serve as a reminder that, for better clinical treatment, male patients—especially those over 65—need to get more attention (Dingtao *et al* ., 2021)

However, in previous COVID-19 infection modeling methods, the impact of gender has been disregarded, the model is calibrated using actual data and is used to evaluate the impact of speculative contact scenarios, all of which start at a rate of 10 new infections per million every day inhabitants (Esteve *et al.*, 2020) .

In relation to other study done by Jaillon *et al.*(2019) , they stated that , despite the fact that sample included more women, the critically ill

category was still overwhelmingly made up of elderly men, indicating that elderly men are more likely to turn into critically ill patients. They reported that, in case contacts the median female contacts (56.3% percent) were evaluated 51.5% of them having a median age of 53 (34-64 years); female contacts; and SARS CoV-2 found positive age, 50 (30 - 61 years) (56.8percent). The percentage of infected people who experienced symptoms ranged from 18.1% among individuals under the age of 20 to 64.6% among those who were 80 years or older. The majority of contaminated contacts (1948 : 2824 people, or 6 9.0 percent) did not experience respiratory problems or fevers higher than or equal to 37.5 °C, Only 26.1% of those who were infected under the age of 60 exhibited respiratory symptoms or fevers higher than or equal to 37.5 °C; those who were infected above the age of 60 developed critical illness at a rate of 6.6%. 53.7% of patients were female. After SARS-CoV-2 infection, female patients have a lower risk of developing a critical illness than male patients (Poletti *et at.,* 2021).

At working ages, sex ratios show that women have higher infection risks than males, however this is reversed as people get older men and women have different death rates across all age categories, Older men are always at increased mortality and infection risks, and even The incidence of illnesses and death in old age are significantly affected by slight variations in contact rates during working and young age (Dudel *et al.*, 2020) .

In relation to the studies, sex is another source of variation in COVID19 mortality, males at greater risk than women due to huge sex differences that may be related to sex hormones such testosterone and estrogen (Dowd *et al.,* 2020) . that appears to be crucial in adjusting the body's immunological reaction and the existence of additional risk

factors, such as diabetes, hypertension, and cardiovascular illnesses, which afflict more men than women (Caramelo, 2020). Gender is related to infection rates and their effects in COVID-19, and contact rates according to gender can simulate this relationship, All policy choices must take gender specific routes of transmission into account. Therefore, current and statistics on touch behaviors by gender, illnesses, and mortality are crucial for fighting Emerging viruses like SARS COV-2 (Achim *et al.*, 2022) .

4.1.2. Age groups distribution of infected patients:

The adult patients have more susceptibility to infected with Sars–Cov2virus at age (> 60 ,38%) followed by 50 – 59 Years (22 %) . in comparison with young age patients .

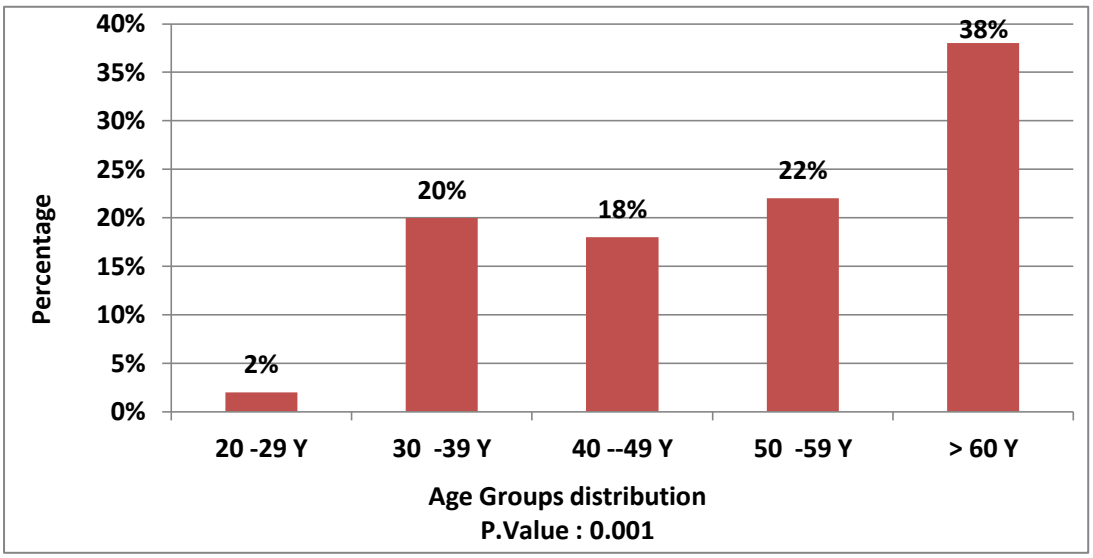


Figure (4 - 3) Age groups distribution of infected patients.

In comparison with other study which stated that high infection and mortality rates highlight the interplay of poor healthcare, poverty, geographical disparity, economic and social risk factors (Egede *et al.*, 2020).

Given that older people tend to be frailer and have poorer immune systems than younger or middle-aged people, older age has been highlighted as a risk factor to be affected, which is mostly owing to the older population's greater frequency of chronic diseases, Analysis by age in China indicates a relatively low incidence of cases in individuals under the age of 20 (China, 2020).

It is not clear whether this is because young people are less likely to be infected, or less likely to develop serious symptoms and seek medical care and be tested (Scott and Dylan ;2020). A follow-up to a retrospective study in China found that children are just as susceptible to infection as adults, In terms of the number of deaths, countries that test more have smaller age distributions of cases, compared to the larger population (Ward and Dan., 2020).

Women contribute more to the rise of infections in the young and middle ages since they have more contacts, One factor that may aid in the transmission of the disease and lead to gender-specific infection rates and fatality outcomes are gender disparities in contact rates(Kulu *et al* ., 2020).

4.1.3. Chronic diseases in association with SARS –Cov2 Infection:

The most group of infected patients in the present study have no other chronic disease at the time of study , the diabetes and hypertensive were show in a group of patients (18%) for each disease , 10% of studied patients have both diseases at the same time diabetes melitus and hypertensive (D.M and TH) only (6 %) have Pulmonary Thrombosis .

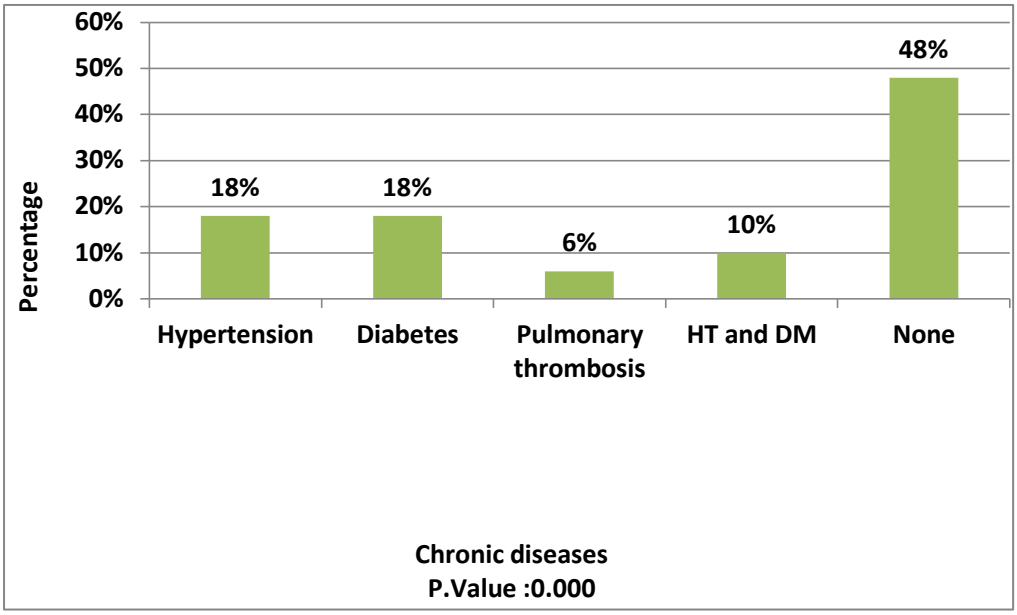


Figure (4 - 4) Chronic diseases in association with infection .

In comparison with other study (U.S, 2020), stated that , older adults and those with underlying conditions such as diabetes, heart disease, respiratory disease, high blood pressure, and immunosuppressed patients are at greater risk of illness and serious complications, along with the Centers for Disease Control and Prevention has advised that, they should stay home as much as possible in areas of community outbreak. (WHO , 2020)

4.1.4. Association of Smoking and SARS-Cov2 infection:

The smoker patients have lower percentage (14%) than non - smoker (86%) as in

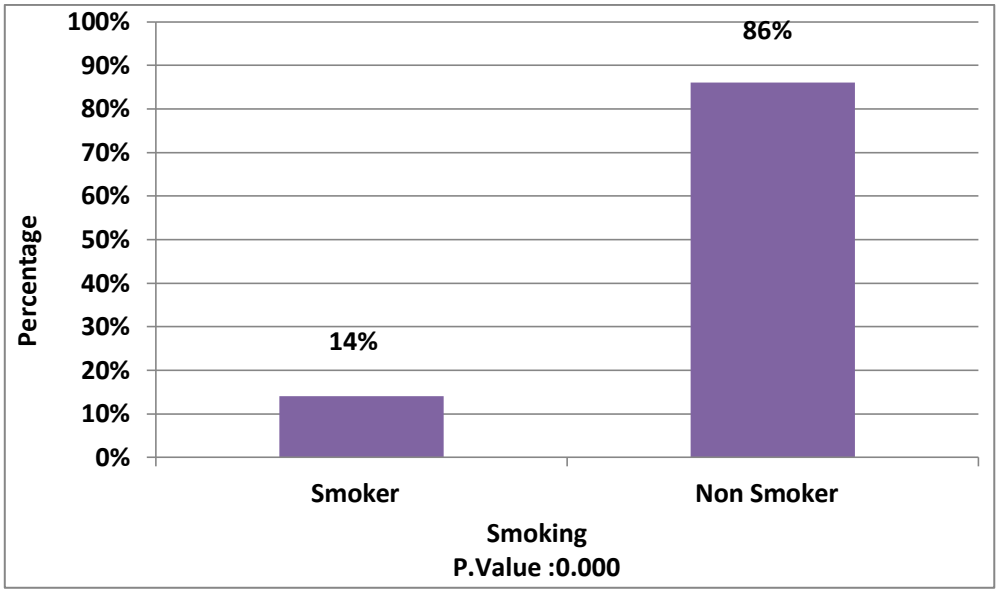


Figure (4 -5) Association of Smoking and SARS-Cov2 infection.

In relation to other study, (Vardavas and Nikitara., 2020) mentioned that Smoking tobacco is a significant established risk factor for serious disease from numerous respiratory illnesses, including death. Smoking's effects on COVID-19 are debatable at the moment (Harapan *et al.*, 2020).

Early research indicates that people with greater likelihood of developing COVID-19 negative health effects if, they were smoking in the past, increases significantly in comparison to non-smokers, is linked to increased rates of ICU admission, using ventilators and eventually results in mortality (s) because smoking increases the chance of developing respiratory infections by suppressing the immune system (Han *et al.*, 2019). So, it is possible to infer a relationship between smoking and deteriorating COVID-19 additionally, smoking was known to increase the likelihood that COVID-19 patients would develop a more severe condition (WHO, 2020).

4.2. Relationship of immunological parameters (CRP, IL-6 and WBC) with Sars-Cov2 Infected patients:

4.2.1. Sex distribution and CRP, IL-6 and WBC:

The result of Table (4- 6) shows increase of all studied immunological parameters (IL-6 , CRP and WBC) among female patients in comparison with male at P. Vale (< 0.05) , the overall result might be refer to that rapid enhancement in immunological response among female patients infected with Sars-Cov 2 virus .

Table (4 - 1) Sex and CRP, IL-6 and WBC.

Parameters		**Sex**	**N**	**Mean**	**Std. D.**	**P. Value**
CRP	Patients	Male	26	23.62	12.23	0.000
		Female	24	30.25	16.54	
	Control	Male	23	1.374	0.778	
		Female	17	1.384	0.397	
IL- 6	Patients	Male	26	8.80	5.93	0.001
		Female	24	16.97	6.85	
	Control	Male	23	1.72	0.42	
		Female	17	1.89	0.67	
WBC	Patients	Male	26	8.54	2.657	0.700
		Female	24	7.387	2.777	
	Control	Male	23	7.66	0.855	
		Female	17	7.75	1.986	

The study done by (Zhu *et al* ., 2020) found that many COVID-19 patients had elevated CRP levels, which is consistent with other research. Furthermore, in this study, aggravated cases had significantly higher CRP levels than CRP may be a disease-related blood marker in non-severe individuals, according to this. Aggravation in COVID-19 patient . Overproduction of inflammatory cytokines is one potential reason for C-reactive protein rises in COVID-19. The development of C- reactive

protein is stimulated by cytokines like IL-6 and tissue destruction (Vasileva *et al*., 2019).

During viral infections, particularly SARS CoV-2, lymphocytes are essential for preserving immunological homeostasis. (Channappanavar *et al*., 2014). Lymphopenia can predict outcome in COVID-19 patients, according to several cohort studies. (Li *et al.*, 2020; Qin *et al*.,2020). Additionally, eosinopenia was discovered in a few investigations to be connected to elements that had poor prognostic value. Therefore, differentiation of peripheral white blood cells might be a sign of immunologic dysfunction at the beginning of a disease.. (Wei *et al*., 2021)

4.2.2. Age groups distribution and CRP, IL-6 and WBC:

The old age groups of patients at >60 years have higher level among all (CRP , IL-6 **,** and WBC) .

Table (4 –2) Age Groups distribution and CRP, IL-6 and WBC.

Descriptive		**N**	**Mean**	**Std. Deviation**	**LSD Value**
CRP	20 -29 Pat	1	3.26	.	2.41
	30 -39 Pat	10	11.13	1 .20	
	40 --49 Pat	9	30.73	3 .56	
	50 -59 Pat	11	15.62	2.29	
	> 60 Pat	19	39.79	4 .69	
	20 -29 Con	17	1.29	0.17	
	30 -39 Con	8	1.35	0. 17	
	40 -49 Con	3	1.59	0.46	
	50 -59 Con	5	1.43	0.53	
	> 60 Con	6	1.31	0.15	
IL- 6	20 -29 Pat	1	5.55	.	2.32
	30 -39 Pat	10	8.74	4.62	
	40 --49 Pat	9	12.07	8.36	
	50 -59 Pat	11	7.95	5.44	
	> 60 Pat	19	18.26	10.10	
	20 -29 Con	17	1.90	0.66	

	30 -39 Con	8	1.82	0.14	
	40 -49 Con	3	1.55	0.52	
	50 -59 Con	5	1.69	0.62	
	> 60 Con	6	1.66	0.60	
WBC	20 -29 Pat	1	8.30	.	3.12
	30 -39 Pat	10	7.99	2.01	
	40 --49 Pat	9	6.76	3.13	
	50 -59 Pat	11	7.22	1.64	
	> 60 Pat	19	8.39	3.23	
	20 -29 Con	17	7.63	1.12	
	30 -39 Con	8	7.54	0.30	
	40 -49 Con	3	6.93	0.47	
	50 -59 Con	5	7.98	2.08	
	> 60 Con	6	8.11	1.61	

In the other result might be refer to that increased in IL-6 and CRP among patients in age > 60 years as an immunological response as well as physiological , because the age effective among such group of patients. The median age of patients in 32,583 laboratory-confirmed cases of Covid-19 in Wuhan (China) were 56.7 years, and older individuals had a greater chance of severe or critical disease (Pan *et al*., 2020).

In individuals with severe COVID-19, increased total leukocyte count and differential neutrophil count were more frequently seen. comparable results in serious COVID-19 patients (Yuan *et al*., 2020).

Lymphopenia was shown to be highly related with serious disease in this investigation. The similar finding was reached by Tan *et al* investigation, which involved 90 hospitalized patients (Tan *et al*., 2020). Particularly throughout infancy and puberty, physiological development is known to influence laboratory test findings to some extent (Loh and Metz ,2015). as a kid approaches puberty, serum alkaline phosphatase activity rises and reflects fast bone development, it

subsequently falls as the child approaches adulthood Similarly, as people age, their total and differential WBCs also alter (Zierk *et al*., 2017).

The context of age and sex dependent dynamics should be used when interpreting total and differential WBCs (Zierk *et al.,* 2015). Until now, there have been few reports of age-dependent variations in WBCs in Chinese children. The current study, which was based on the Pediatric Reference Intervals in China study, examined age-dependent variations in total and differential WBCs in healthy children aged 0 to 18 years (Ni *et al.*, 2018).

4.2.3. The infection patients and CRP, IL-6 and WBC:

The result of the below table shows increased levels of IL- 6 and CRP in the infected patients after comparison with patients and healthy population as in **Table (4 – 3) The infection patients and CRP , IL-6 and WBC**.

Descriptives		**N.**	**Mean.**	**Std. deviation.**	**P. Value**
CRP	Patients	50	26.38	5.79	.000
	Control	40	1.34	0.41	
IL- 6	Patient	50	12.72	2.32	.003
	Control	40	1.66	.89	
WBC	Patients	50	7.76	2.67	.675
	Control	40	8.06	2.27	

In comparison with other study Patients with COVID-19 showed a considerable rise in CRP, with values averaging 20 to 50 mg/L. (Chen *et al*., 2020 ; Gao *et al*., 2020 ; Mo *et al*., 2020) . In individuals with severe COVID19, elevated CRP values of up to 86 percent were seen (Chen *et*

al ., 2020). Compared to mild or non-severe patients, those with severe disease histories had much higher levels of CRP(Chen *et al*., 2019)

Apparently in one research, individuals CRP concentrations for patients with more severe symptoms were generally 39.4 mg/L, whereas those with lesser symptoms were typically 18.8 mg/L.. Levels of CRP were first reported in the severe category must be more than the moderate group, 12 In a different research, individuals with severe conditions had a mean CRP concentration was substantially higher (46 mg /l) than that with non-severe conditions (23 mg /l) (Guan *et al*., 2020).

4.2.4. Other diseases and CRP, IL-6 and WBC:

The previous diabetes present in Sars-Cov 2 infected patients shows increase in IL-6 level in comparison with other conditions , while more CRP production was shown in complicated patients in which have Pulmonary Thrombosis, WBC have more count in hypertensive condition associated with Sars-Cov 2 infection rather than other studied conditions, These results were mentioned in table (4- 4) .

Table (4- 4) Other diseases and CRP, IL-6and WBC .

Descriptives		**N**	**Mean**	**Std. D.**	**LSD Value**
CRP	Hypertension	9	14.97	6.86	2.36
	Diabetes	9	17.31	3.52	
	Pulmonary thrombosis	3	19.39	5.19	
	HT and DM	5	69.10	8 .73	
	None	24	26.03	9.36	
	Control	40	1.35	0.25	
IL- 6	Hypertension	9	18.24	12.94	3.94
	Diabetes	9	19.12	4.01	
	Pulmonary thrombosis	3	14.25	2.04	
	HT and DM	5	8.79	2.01	
	None	24	8.88	2.82	
	Control	40	1.79	0.54	

WBC	Hypertension	9	8.81	2.96	2.65
	Diabetes	9	7.32	1.40	
	Pulmonary thrombosis	3	5.48	2.46	
	HT and DM	5	7.82	3.97	
	None	24	7.81	2.65	
	Control	40	7.67	1.39	

In comparison with other People with COVID-19 who had a high WBC count exhibited more severe disease and mortality, according to the studies of. (Zhu *et al.,* 2020). Lower WBC counts, according to a number of further researches or more severe illness and mortality in COVID-19 were related to higher WBC counts with lower lymphocyte levels in particular. (Zhang *et al*., 2020) .This may be due to the fact that when the body fights the virus, levels of some WBCs, including neutrophils, rise while lymphocyte levels stay low (Yang *et al*., 2020)

4.2.5. Smoking and CRP, IL6 and WBCs:

The result of table (4 - 5) shows increase IL-6 and CRP level among non-smoker patients infected with Sars-Cov2 in comparison with smoker patients as well as control population.

Table (4- 5) Smoking and IL-6, CRP and WBC .

Descriptive		**N**	**Mean**	**Std. D.**	**LSD Value**
CRP	Smoker Patient	7	21.60	6.43	2.13
	Non Smoker Patient	43	27.16	8.10	
	Smoker Control	15	1.34	0.41	
	Non Smoker Control	25	1.35	0.03	
IL-6	Smoker Patient	7	11.20	2.56	2.43
	Non Smoker Patient	43	12.97	2.66	
	Smoker Control	15	1.66	0.89	
	Non Smoker Control	25	1.87	0.01	

WBC	Smoker Patient	7	7.46	1.99	3.35
	Non Smoker Patient	43	7.81	2.78	
	Smoker Control	15	8.06	2.27	
	Non Smoker Control	25	7.44	0.02	

This result might refer to that the smoking might impaired the immune activity and lead to low level of Both IL-6 and CRP , with noted that such parameters mostly associated with acute attach of diseases and conditions. IL-6 is a multifunctional cytokine that regulates immunological and inflammatory responses. It has been found to play a key role in the development and progression of coronavirus pneumonia.(Sun *et al*, 2020).

Circulating IL-6 concentrations in COVID-19 patients with hyper-inflammatory syndrome have been associated with disease severity, the frequency of acute lung injury, and the requirement for mechanical ventilation, indicating that monitoring IL-6 levels can assist inform therapeutic choices. (Liu *et al.*, 2020).

In recent studies, researchers found that those with a history of smoking had a higher risk of getting severe COVID-19 progression compared with those who never smoked (Patanavanich and Glantz, 2020). Determining the relationship between total white blood cell count and absolute neutrophil counts after smoking cessation. After 7 weeks, there was a significant decrease in white blood cell indices in continuously abstinent subjects compared with continuing smokers (Abel *et al*., 2005).The most often seen neutrophilia, lymphocytosis, monocytosis, and basophilia were related with tobacco induced leukocytosis, which was defined by a slight rise in the total white blood

cell count. Leukocytosis improved after quitting smoking.,(Caleb *et al.*, 2021).

4.3. Relationship of HLA types and SARS-Cov2 Infected patients:

To evaluation of certain types of HLA-types in relation to Sars-Cov2 infection , as an examples for Class 1 choosing (HLA-G) , and HLA-DR from Class II.

4.3.1. Relationship of Sex and HLA types(HLA-DR and HLA-G):

There is no noticeable difference between males and females in the values of HLA-DR, while there is a clear increase in the values of HLA-G in male patients than in females, and there is an increase in values between patients and healthy subjects. This result was mentioned in table (4 –6)

Table (4 - 6) Relationship of Sex and HLA-Types.

Parameters		Sex	N	Mean	Std. D.	P. Value
HLA–DR	Patients	Male	26	3.27	1.113	0.000
		Female	24	3.35	1.045	
	Control	Male	23	3.82	1.98	
		Female	17	5.53	2.76	
HLA–G	Patients	Male	26	628.961	104.680	0.034
		Female	24	564.455	134.188	
	Control	Male	23	601.2040	217.669	
		Female	17	599 .62	169 .78	

Patients with COVID-19 who have low levels of HLA-DR are also at an increased risk of developing serious and secondary nosocomial infections

due to bacteria, This finding is in line with a clinical study that COVID-19 patients requiring ICU care had secondary bacterial infections and end-organ damage (Elliott *et al*., 2021) .Previous research has revealed that sex significantly affects how illnesses turn out and is linked to fundamental variations in how the immune system reacts to infection (Fischer *et al* ., 2015 ; Klein and Flanagan, 2016), For instance, men are more likely than women to get TB and hepatitis A (Guerra and Abad, 2013).

4.3.2. Relationship of Age groups and HLA types (HLA - DR and HLA- G):

In relation to age groups distribution of HLA studied types (HLA-DR and HLA - G) ,The result of table (4 - 7) shows that The young age groups have lower level of HLA-DR in comparison with adult age groups , the Mean ± SD , of young age groups (30 -39 and 40 -49 was revealed that 2.89 ± 0.65 and 2.59 ± 0.64) respectively , the control age groups were have higher level that patients especially at adult age groups or in patient at more than 50 years old , the LSD value (1,07) . While the result of HLA-G show that the higher level were spotted at adult age groups (40 -49 yeas , at 606.66 ± 176.32) in comparison with other age groups as well as control groups, with note that higher level in control groups at adult age group (50 - 59 year , at 989.07 ±199.86), at LSD value 54.8.

Table (4 - 7) Age groups distribution and HLA types.

Age Groups and HLA		N	Mean	S.D	LSD Value
	20 -29 Pat	1	3.46	.	
	30 -39 Pat	10	2.89	0.65	
	40 --49 Pat	9	2.59	0.64	
	50 -59 Pat	11	3.82	1.68	

HLA –DR	**> 60 Pat**	**19**	**3.58**	**0.74**	**1.07**
	20 -29 Con	**17**	**4.15**	**2.36**	
	30 -39 Con	**8**	**4.53**	**2.70**	
	40 -49 Con	**3**	**4.81**	**2.36**	
	50 -59 Con	**5**	**5.93**	**2.27**	
	> 60 Con	**6**	**4.53**	**1.95**	
HLA –G	**20 -29 Pat**	**1**	**416.15**	**.**	**54.8**
	30 -39 Pat	**10**	**573.52**	**148.93**	
	40 --49 Pat	**9**	**606.66**	**176.32**	
	50 -59 Pat	**11**	**578.60**	**164.14**	
	> 60 Pat	**19**	**574.93**	**32 .55**	
	20 -29 Con	**17**	**469.23**	**40.80**	
	30 -39 Con	**8**	**749.90**	**271.17**	
	40 -49 Con	**3**	**469.23**	**76.37**	
	50 -59 Con	**5**	**989.07**	**199.86**	
	> 60 Con	**6**	**606.92**	**174.82**	

In comparison with other studies, patients were suffering from the Coronavirus infection in 2019 (COVID-19) who need to be hospitalized in an intensive Care unit, (ICU) Patients who recovered from acute sepsis or trauma have reported similar findings and later develop life-threatening immune suppression (Venet *et al*., 2018) .

The immune suppressive characteristics of HLA-G support the idea that it may facilitate viral immune escape on the other hand, HLA-G secretion or expression may signify a strong sensitivity to be inflammatory processes that take place when viruses are infected (Amiot *etal*., 2014).

The significance of HLA-G in illness immunopathology has not been well researched in relation to COVID-19 HLA-G molecules, however, may influence COVID-19's immune system in ways that regulate it, according to certain theories (Fraga-Silva *et al*., 2021 ,Rizzo *et al*.,2021, Zidi .,2020 , Zhang *et al*.,2020).

4.3.3. The infected groups and HLA types (HLA-DR and HLA-G):

The infected patients with SARS cov2 virus have reduced in HLA – DR expression in comparison with patients as well as in control (3.31 ± 1.07 , 4.56± 2.90) at P. Vale < 0.05 . While the patients have higher level of HLA-G than infected patients and healthy control at mean ± SD (597.99±217.51, 663.5±179.71) respectively at P Value <0.05) .

Table (4 - 8) The infected patients and HLA Types.

infected patients and HLA		**N**	**Mean**	**S.D**	**P. value**
HLA –DR	Patients	50	3.31	1.07	0.001
	Control	40	4.56	2.90	
HLA –G	Patients	50	597.99	217.51	0.006
	Control	40	663.5	179.71	

In comparison with other study , (Nguyen *et al*., 2020 , Giamarellos *et al*., 2020). The relationship between immunological dysregulation and a considerable downregulation of monocyte HLA-DR in COVID-19 , patients breathing difficulties has recently been verified by other investigations.

Viral infections may increase the expression of many immunological suppressive receptors, including Human leukocyte antigen G (HLA G) , Maturation of dendritic cells (DCs)., the formation of B cell antibodies, and Natural killer (NK) and T cells immunological responses are all inhibited by HLA-G receptor signaling, such as that caused by interaction with ILT-2 or ILT-4 (Aifen and Wei , 2021).

HLA G can cause severe immunological suppression, which helps SARS-CoV-2 avoid being attacked by the immune system. We address the immunopathological features of HLA- G receptor signaling in SARS CoV-2 infection here, despite the little evidence In regard to the. HLA-clinical G's significance in SARS CoV-2 infection (Wilder *et al*., 2020) . It may improve knowledge of the course of COVID-19 illness may improve and provide possible immunotherapies to fight against infection with SARS CoV-2 (Aifen and Wei , 2021). As a result, the host's antiviral immune system becomes susceptible due to differential modification in the expression of HLA antigen brought on via viral infection (Garcia , 2020).

4.3.4.Other diseases associated with HLA types (HLA-DR and HLA-G):

Some patients were choosn to the present study, have other chronic diseases rather than Sars-Cov 2 infection, mostly as hypertension, Diabetes , pulmonary thrombosis and certain groups have both diabetes and hypertensive at the same time . The level of HLA –DR was reduced in patients having pulmonary thrombosis than other conditions (HT , DM , and in patients have both HT and DM) at mean ± SD (2.55 ± 0.89) at LSD value 1.12 . While The HLA –G level were more elevated in patients with pulmonary thrombosis than other conditions at mean ± SD (953 ± 224.43) as shown in table (4 - 9) .

Table (4 - 9) Other diseases and HLA Types.

Other diseases and HLA types		**N**	**Mean**	**Std. De.**	**LSD Value**
HLA – DR	Hypertension	9	3.69	1.10	1.12
	Diabetes	9	3.78	1.53	
	Pulmonary thrombosis	3	2.55	0.89	

	HT and DM	5	3.16	0.58	
	None	24	3.12	0.90	
	Control	40	4.55	1.47	
HLA – G	Hypertension	9	614.52	126.84	52.4
	Diabetes	9	615.28	104.34	
	Pulmonary thrombosis	3	953.58	224.43	
	HT and DM	5	496.30	105.31	
	None	24	562.05	69.44	
	Control	40	580.73	121.61	

In comparison with other study , The most distinctive comorbidities of 32 non-survivors from a group of 52 intensive care unit patients with coronavirus disease 2019 (COVID-19) in the study by (Yang *et al*., 2020.) were cerebrovascular diseases (22%) and diabetes (22%). Another study (Guan *et al*., 2020) consisted of 1099 individuals with confirmed COVID-19, 173 of whom had severe illness and comorbidities of hypertension (23%) diabetes mellitus (16%) coronaryheart disease (5%) and cerebrovascular disease (2%) Thirty percent of the 140 patients in a third trial who were hospitalized with COVID-19 also had hypertension, and 12 percent had diabetes (Zhang *et al*., 2020). In this study, the HLA-DRB1 and HLA-DQB1 allele frequencies were compared in Korean patients with idiopathic pulmonary arterial hypertension (IPAH) and in healthy controls to see if there was any correlation between IPAH's clinical features and any particular HLA alleles (Yoon *et al*., 2007).

The prevalence of type 1 diabetes varies among populations according to different HLA genotype patterns (Noble and Erlich ,2012) and Relative to other genes, they have the biggest influence. HLA class II DR, DQ, and DP correlations with type 1 diabetes (Noble and Johnson. , 2011; Hanscombe *et al.*, 2018).

For instance, it was discovered that the Caucasian populations' high-risk HLA haplotypes, DRB103:01-DQB102:01 and DRB04:01-DQB103:02, were rare in Japan and Southeast Asia, but the Japanese and Korean people' susceptibility HLA haplotypes, DRB104:05-DQB104:01 and DRB109:01-DQB103:03 (Kawabata *et al*., 2002).

4.3.5. The effect of Smoking on HLA types (HLA-DR and HLA -G):

The smoker patients have lower level of both HLA DR , and HLA G in comparison with non-smoker patients and control , at mean ± SD (2.70 ± 0.42 , 456.15 ± 121.23) respectively of HLA DR and HLA G .as listed in table (4 – 10) .

Table (4- 10) The effect of smoking on HLA types.

Smoking and HLA types		**N**	**Mean**	**Std. D.**	**LSD Value**
HLA –DR	Smoker Patient	7	2.70	0.42	1.17
	Non Smoker Patient	43	3.41	1.11	
	Smoker Control	15	5.33	2.90	
	Nonsmoker Control	25	4.08	2.09	
HLA –G	Smoker Patient	7	456.15	121.23	38.7
	Non Smoker Patient	43	621.08	126.68	
	Smoker control	15	679.95	177.71	
	Nonsmoker Control	25	534.40	160.84	

There was a significant difference in risk between the two extremes for Multiple Sclerosis; smokers with HLA-DRB1'15 and no HLA-A'02 had a 13-fold higher risk than non-smokers without these genetic risk factors (OR 12.7, 95 percent CI 10.8-14.9), Smoking status has a significant impact on the risk of MS linked with specific HLA genotypes, and vice versa. Given that HLA molecules' role in presenting peptide antigens to T

cells, the relationships that have been shown clearly imply that smoking affects the risk of MS via affecting adaptive immunity (Hedstrom *et al.*, 2017).

4.4. Correlation between studied parameters among SARS-Cov2 infected patients:

4.4.1. Correlation between CRP and IL-6 level:

The figure (4 - 6) demonstrates a connection between IL-6 and CRP level in response to infection with SARS –Cov2 virus. This result might refer to that acute attach of infection lead to the increased production of both IL_6 and CRP in the same time. This result was mentioned in Figure (4-6)

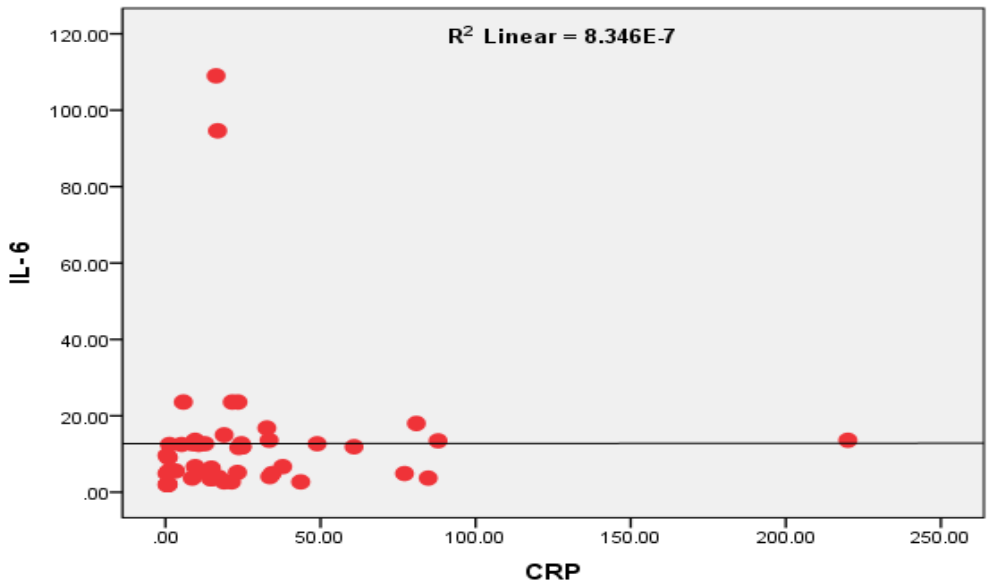


Figure (4-6) Correlation between CRP and IL-6 level

4.4.2. Correlation between IL- 6 and Total WBC count:

The figure (4 - 7) shows that there is a negative association between IL-6 and total WBC count in response to infection with SARS –Cov2 virus.

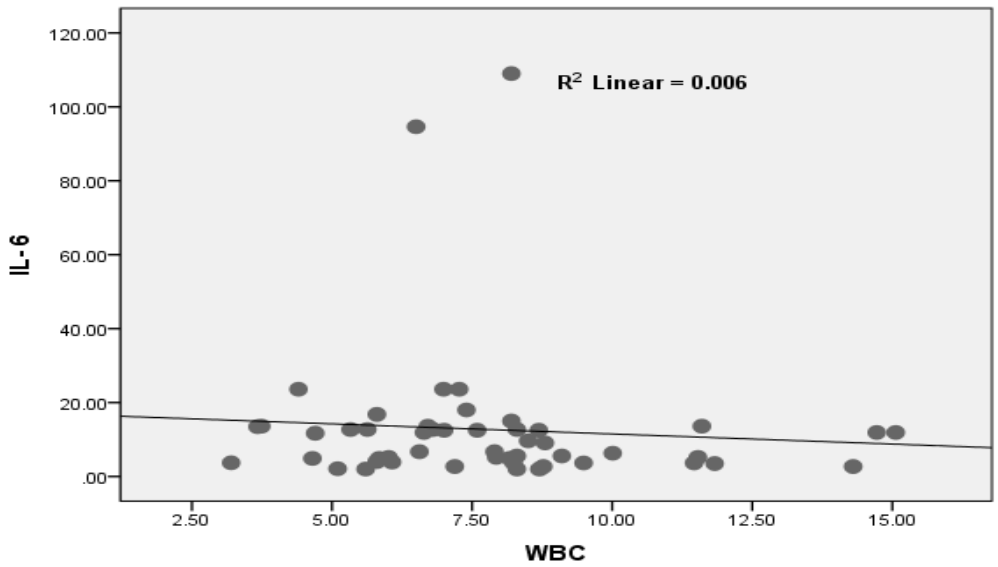


Figure (4 - 7) Correlation among IL- 6 and total WBC count .

In light of the fact that the WBC count overall has a substantial connection with bad health consequences, such as death, researching this hypothesis will assist to enhance knowledge the relationship between IL-6 and WBC as well as the physiological causes for the increase in IL-6 levels. (Gabriel *et al.,* 2002). Significant correlations between IL-6 levels, and total WBC, neutrophil, monocyte, eosinophil counts, as well as lymphocyte and basophil counts, were found. After taking age, race, and smoking history into account, these relationships were still quite significant stepwise increases were seen in the Total WBC, neutrophils, monocytes, and eosinophils in four IL-6 quartiles, in descending order. Additionally, IL-6 levels and total and differential WBC counts were variably correlated with age, race, and cigarette smoking (Leng *et al.*, 2005) .The increase of serum IL-6, decrease of lymphocytes, and increase of neutrophils were noticed in patients over 60 years old.(Li *et al* ., 2020)

4.4.3. Correlation between CRP and total WBC count:

As like as IL-6**,** the result correlation of CRP with total WBC count shows a negative correlation between CRP and total WBC count in

response to infection with SARS –Cov2 virus. This result might be refer to that acute attach of infection lead to increased production of CRP with reduction of WBC count, as shown in figure (4 - 8).

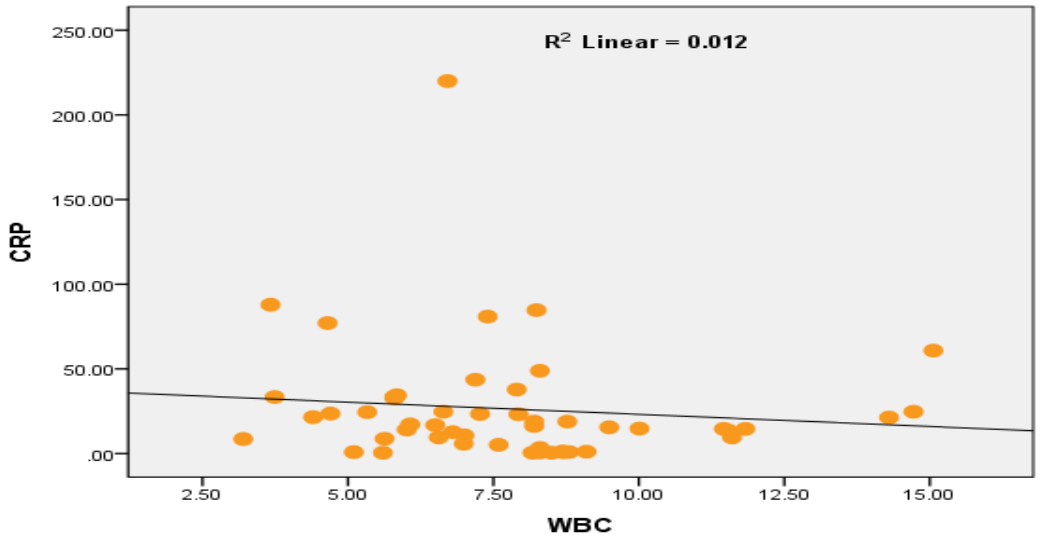


Figure (4 -8) Correlation between CRP and total WBC count.

4.4.4 Correlation between HLA-DR and IL- 6:

The figure (4-9) shows HLA DR expression is more prevalent which might lead to reduce IL-6 level , and there is a negative correlation between them during infection with SARS–Cov 2 virus . The result might be referring to that HLA - DR more associated with infection at second or more weeks from onset.

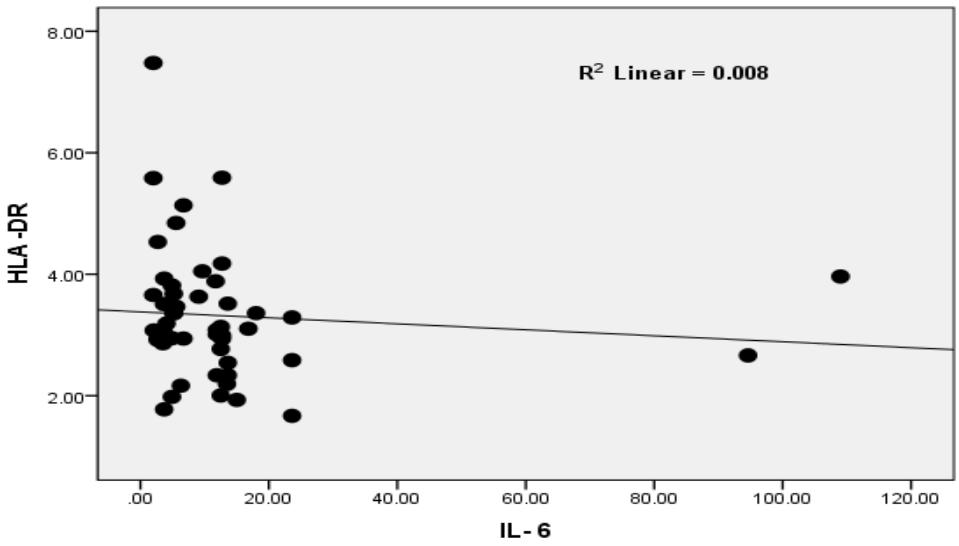


Figure (4 -9): Correlation between HLA-DR and IL- 6 .

Observational evidence accruing throughout the COVID-19 pandemic has identified several factors associated with COVID-19 severity, including older age, male sex, cardio metabolic comorbidities (eg, hypertension and diabetes) and non-white ethnicity (De *et al* ., 2020 ; yang *et al.*, 2020) .

4.4.5. Correlation between HLA-DR and CRP:

As like as IL- 6 the result shows that enhanced expression of HLA DR might lead to reduce CRP level , and there is a negative correlation between them during infection with SARS –Cov 2 virus , as like as IL-6 , the CRP level were associated with HLA-DR after one week of SARS-Cov2 infection , HLA-DR might be suggested as diagnostic test

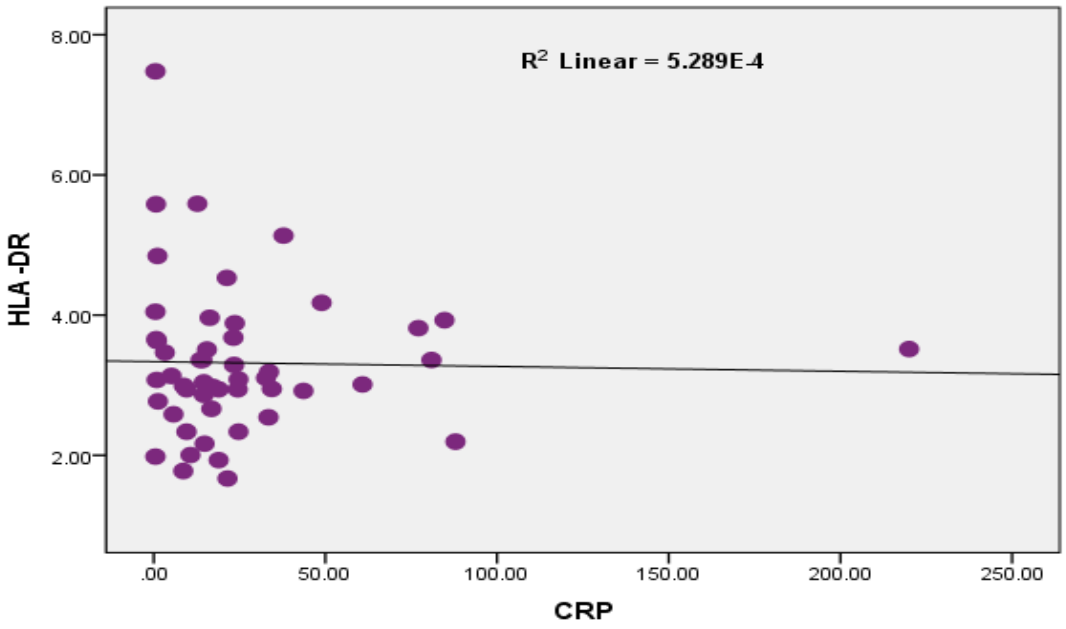
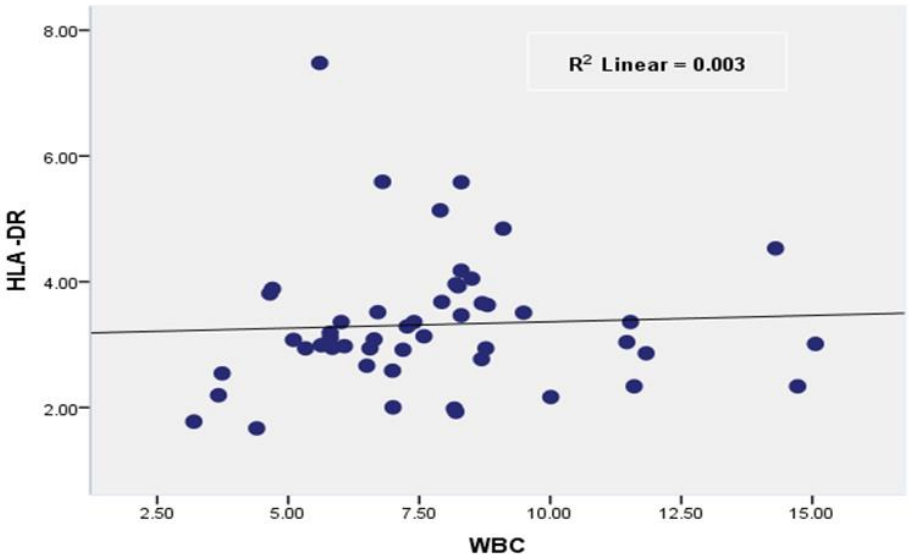


Figure (4- 10) Correlation between HLA-DR and CRP .

4.4.6 Correlation between HLA-DR and WBC:

The figure (4 -11) shows higher HLA-DR expression which might lead to increase WBC , and there is a positive correlation between them during infection with SARS –Cov 2 virus . The total WBC count is one of the most important parameter which refers to the primary response to different infection from the first hours of happened.



Figure(4 - 11) Correlation between HLA-DR and WBC.

4.4.7. Correlation between HLA-G and IL- 6:

The figure (4-12) shows increase expression of HLA Class 1 (HLA-G) might lead to reduce IL-6 level, and there is a negative correlation between them during infection with SARS –Cov 2 virus.

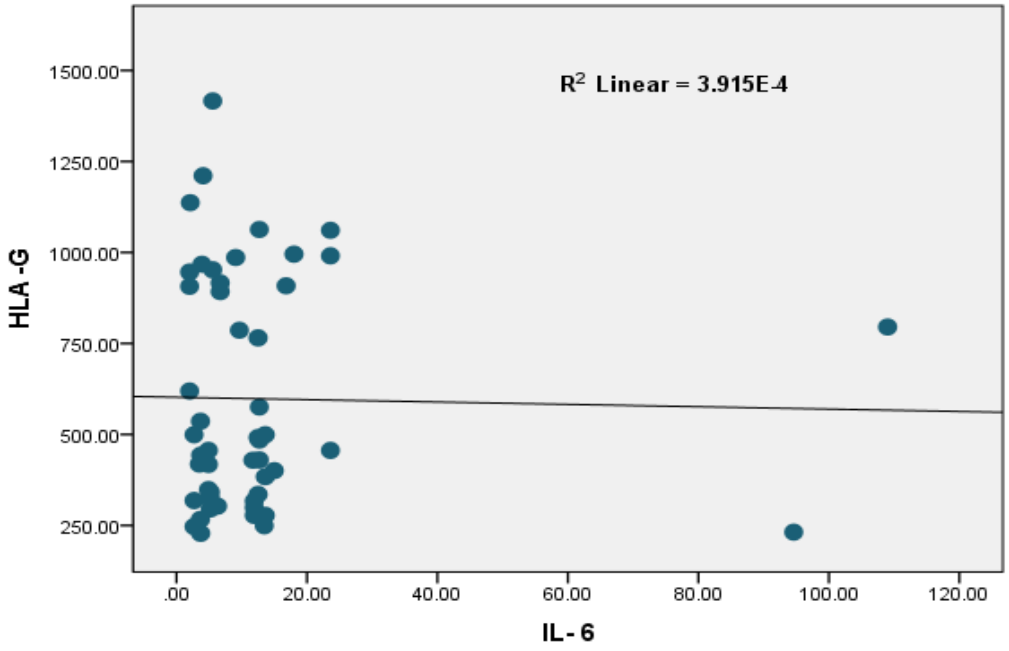


Figure (4- 12) Correlation between HLA-G and IL-6.

In relation to other study, the weakness of traditional T cell detection of infected cells is the warded by HLA class I and II antigens. The identification is one approach effectively used viruses to evade the immune system, as the creation of non-classical HLA classI antigen HLA-G. Almost all immune cell subsets variably express the ligand for immune inhibitory receptors. Consequently, the vulnerability of the host antiviral immune system is caused by differential modification in HLA antigen expression by viral infection. (Lin *etal*., 2007; Carosella *et al*., 2008) . The effects of HLA-G/receptor signaling on synergistic suppression are well known. Apoptosis and senescence of cells are both induced, as well as the suppression of cell growth and differentiation. This may be responsible for the severe decline or possibly depletion of

NK cells, T cells, macrophages, B cells in COVID-19 patients are immune competent cells. (Amiot *et al*., 2014).

The following aspects are anticipated investigation needed: (a) HLA G expression has been linked to the course of certain infectious illnesses showing the severity, prognosis, virus load of individuals with COVID-19 is correlated with circulating soluble HLA-G levels and cell surface HLA-G levels (b) In individuals with severe COVID-19, IFN- γ, IL-6, and IL-10 cytokines significantly up regulate HLA-G expression. (Persson *et al*., 2020 ; Ragab *et al*., 2020) .

4.4.8. Correlation between HLA-G and CRP:

The figure (4-13) shows increased expression of HLA Class 1 (HLA-G) might lead to highly reduce in CRP level , and there is a negative correlation between them during infection with SARS –Cov 2 virus. This result might refer to the acute attach of infection lead to increase expression of Class 1 HLA (HLA-G) with progressive reduction of CRP level.

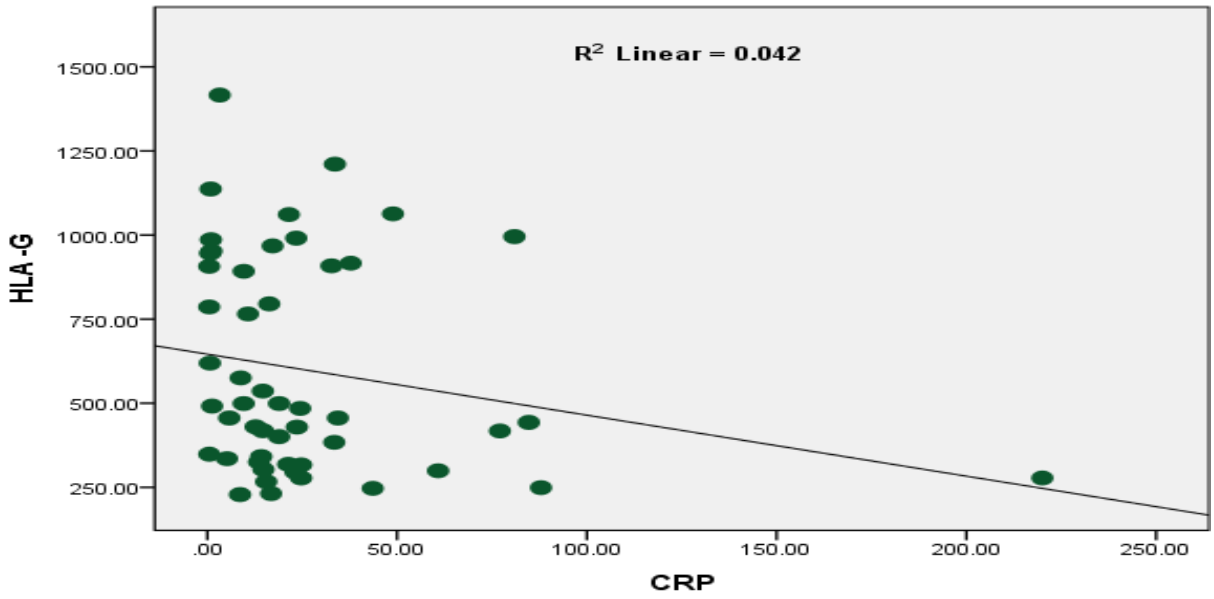


Figure (4- 13) Correlation between HLA-G and CRP

One of the clinical parameters, serum C-reactive protein (CRP), (Chen *et al.*, 2020) has been identified as a significant marker that changes in extreme COVID19 patients Up to 86 percent of individuals with severe COVID-19 had high CRP levels (Wang *et al.*, 2020). Patients with severe illness had CRP levels that were noticeably greater than those with moderate or less severe disease. (Chen *et al.*, 2020).

4.4.9. Correlation between HLA-G and WBCs:

The figure (4-14) shows increased expression of HLA Class 1 (HLA-G) might lead to reduce in Total WBC count , and there is a negative correlation between them during infection with SARS –Cov2 virus. This result might be refer to that acute attach of infection lead to increased expression of Class 1 HLA (HLA-G) and decreased in WBC count.

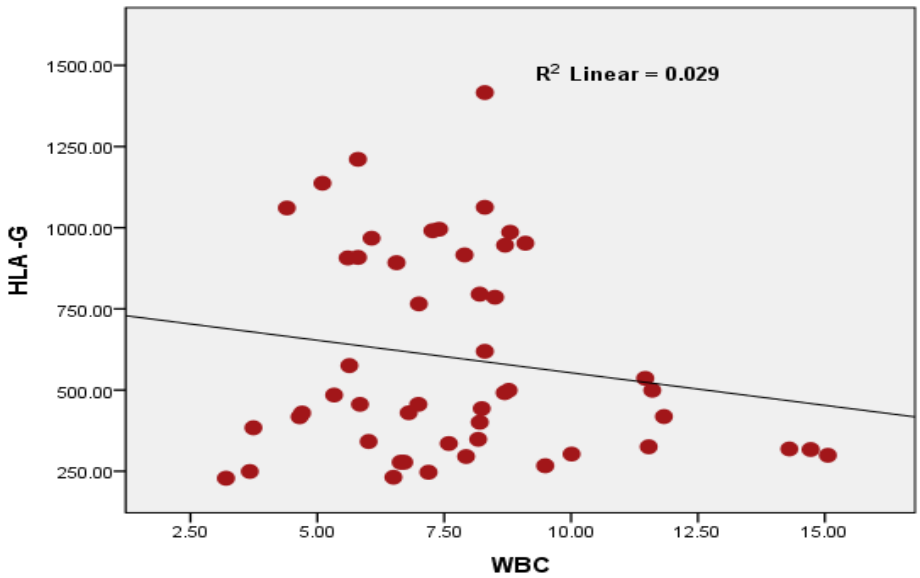


Figure (4 - 14) Correlation between HLA-G and total WBC

A disorder known as lymphocytopenia or lymphopenia affects those who have low lymphocyte counts this happens when your body doesn't produce enough of these blood cells.It is also contagious, as seen in AIDS

patients, for example.(National Institutes of Health). Low white blood cell counts can be a symptom of conditions including HIV, cancer therapy, and uncontrolled diabetes Lymphocytopenia infections that recur often Non-resolving infections infections unusual or uncommon fevers infected bladders, mouth ulcers skin conditions, stuffy nose or sinus infections (National Institutes of Health).

4.4.10. Correlation between HLA-DR and HLA-G:

The figure's outcome (4 -15) display that direct relationship or positive relationships between HLA- Class II (HLA- DR) and HLA- Class I (HLA-G) , this result might be refer to that increased in HLA-DR associated with increased in HLA-G , both of them are most important in regulation of immune response at both humeral and cellular activity against viral infections especially at Sars –Cov2 infection. The HLA-DR was have more ability to interferance with covid -19 infection.

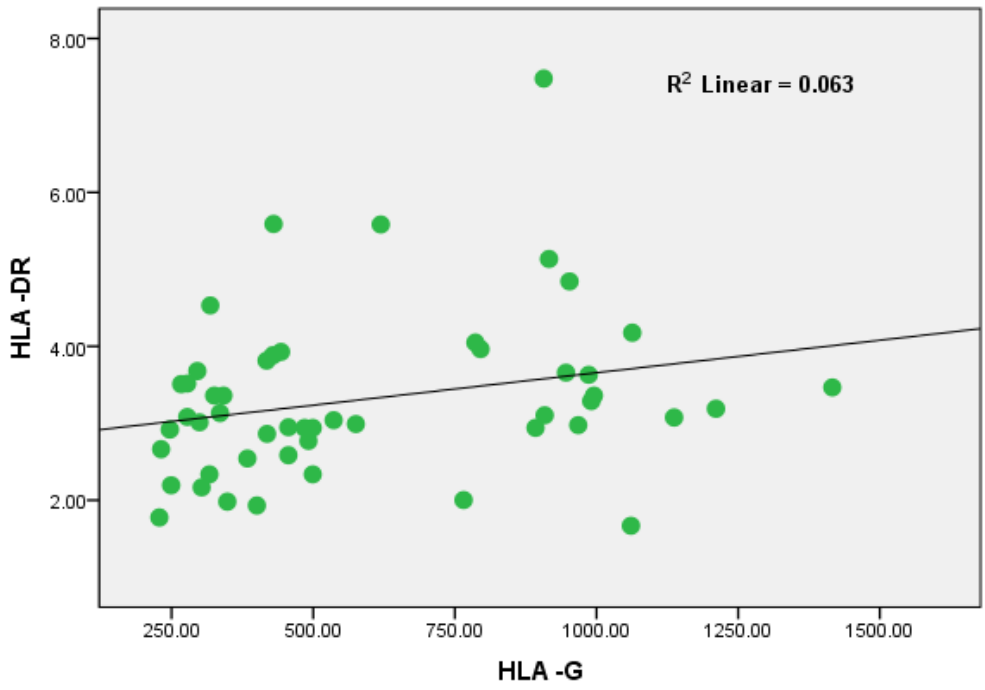


Figure (4 -15) Correlation between HLA-DR and HLA-G .

The fundamental and clinical roles of HLA- G in, COVID-19 development and prognosis may be further established with further data. Aspects that are anticipated tobe investigated comprise: HLA-G expression has been linked tothe development of certain infectioue illnesses. (Li *et al*., 2013 ; Amiot *et al*., 2014).

In COVID-19 patients the serious, , prognosis, Virus load are correlated with the levels of circulating soluble HLA G and cell surface HLA G In individuals with severe HLA-G expression is increased by cytokines such IFN-g, IL-6, and IL-10 in COVID-19.is greatly elevated. (Persson *et al*., 2020 ; Ragab *et al*., 2020) . In these cells, HLA-DR expression is downregulated. ratios of neutrophils to lymphocytes, CD3 to neutrophils, and CD4 to neutrophils, and Patients had a considerably higher neutrophil/CD8 ratio than controls. Patients with SARS CoV-2 infection had a considerably lower total number of CD3, CD4, CD8, CD19 cell People with more severe impairment and a more advanced stage of the illness showed a considerable decrease in CD8+T and CD4+T count along with HLA DR cell expression and an apparent impairment in cellular immunity (Asmaa-Nafady *et al.*, 2022)

4.5. The sensitivity and specificity of studied parameters:

The figure (4 – 16) shows that the distribution of different data were used in the present study , and the more sensitive and specific one is IL-6, followed by CRP and WBC. The table (4 - 11) shows different variables with area and significant value.

Table (4-11) ROC curve table of analysis data.

Area Under the Curve					
Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
HLA –DR	.444	.061	.357	.324	.563

HLA –G	.495	.063	.929	.372	.617
IL- 6	.904	.035	.000	.834	.974
CRP	.769	.059	.000	.654	.884
WBC	.522	.069	.719	.388	.656
The test result variable(s): HLA -DR, HLA -G, IL- 6, CRP, WBC has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.					
a. Under the nonparametric assumption					
b. Null hypothesis: true area = 0.5					

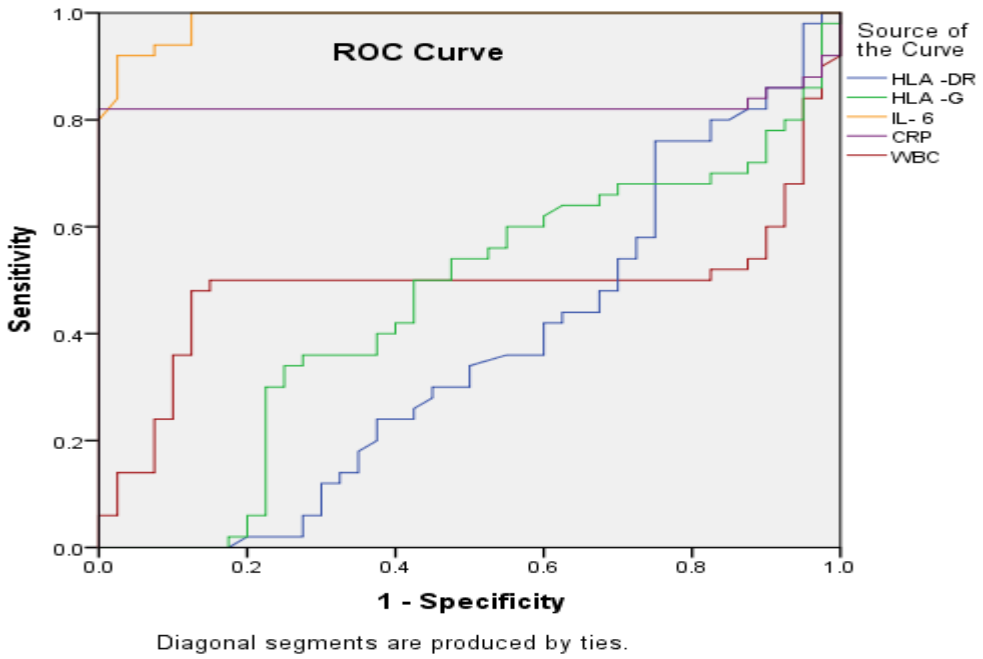


Figure (4 - 16) ROC curve of different analysis parameters

IL-6 and CRP is the more specific and sensitive in diagnosis infection with SARS Cov 2 in comparison with other parameters used in the present study. In COVID 19 individuals an increased blood concentration of cytokines that cause inflammation, aberrant immunologic abnormalities were found by analyzing the clinical characteristics. (Huang *et al.*, 2020).

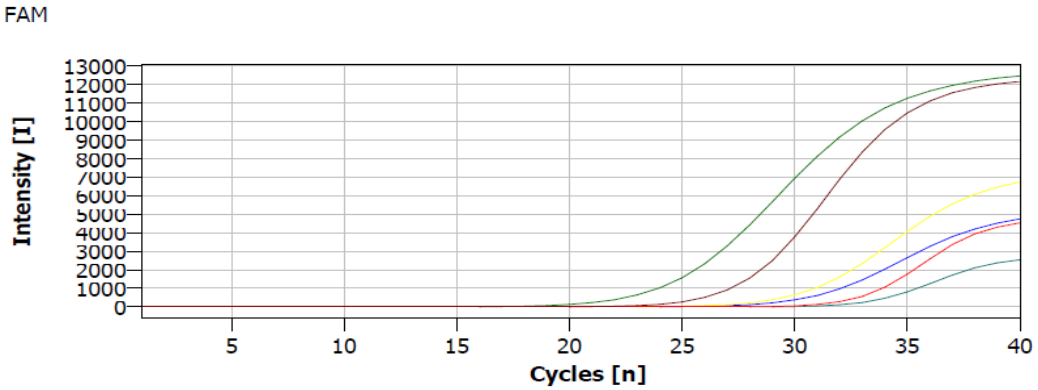
Tumor necrosis factor (TNF), interleukin 2 receptor (IL-2R), interleukin 6, interleukin 8, and interleukin 10 serum concentrations were all considerably greater in dying individuals compared to their recovered

counterparts. (Chen *et al.*, 2020a). Additionally, in comparison to moderate instances, these cytokines' concentrations were much greater extreme instances. , It implies the need for IL-6 identification for early severity prediction. (Chen *et al.*, 2020b ; Gao *et al.*, 2020).

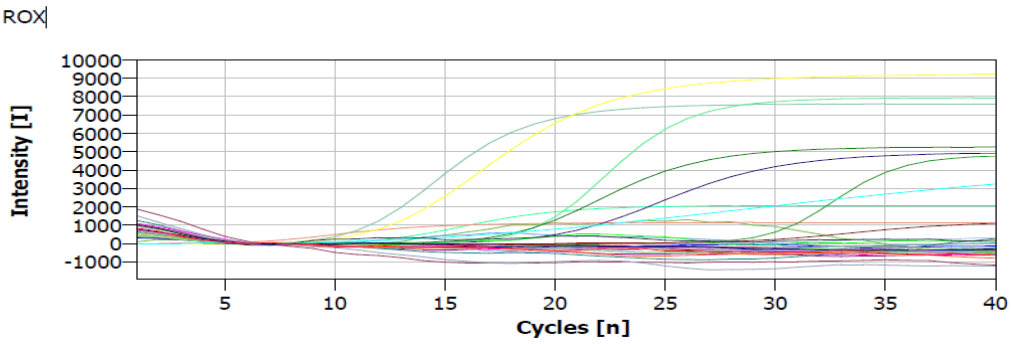
Host-directed treatments with the goal of reducing excessive and abnormal host immune responses are hypothesized to be helpful (Zumla *et al.*, 2020).

4.6. Molecular study and Micro RNA results:

The SARS CoV-2 virus is among a group of infected patients using quantitative RT-PCR, the evaluation of miRNA was performed. It should be mentioned that only a limited quantity of patients were chosen, and the results demonstrate its FAM CT value suggested positive miRNA, as opposed to gene ROX. H. K., also known as housekeeping genes, are widely used as a control miRNA to standardize amounts of mRNA between various substances or genes . However, these genes can vary under specific circumstances at mild to high fluorescence in



A : Positive for the miRNA target.



B : H.K gene testing positive as a control

Figure (4-17) : miRNA quantitative RT PCR in coronavirus-positive people.

miRNA 192 had a positive RT-PCR result of 84 percent, according to the total findings of the examined genes, vs 16 percent with a negative result or no CT value., Despite the fact that 36% of people are positive for miRNA-31. Outcome compared to a 64 % negative result and a 100% H.K. gene, as shown in .

Table (4-12): The RT PCR result of miRNA genes (192 , 31).

Gen type	RT- PCR	No.	Percantage
miRNA 31	Positive	9	36 %
	Negative	16	64 %
miRNA 192	Positive	21	84 %
	Negative	4	16 %
H.K. Gene Control	Positive	18	100 %
	Negative	0	0 %

Host miRNAs are essential for viral replication and infection. Viral miRNAs can change the host's transcriptome in an exchange way Given the significance during infection of miRNAs in host pathogen interactions and their relevance to the activity of various immunological equilibrium throughout the host and immune cell types , these small molecules may be investigated as targets for antiviral therapy. (Iqbal *et al.*, 2020).

This data may indicate the expression of miRNA 192 was greater in Covid-19 patients , Additionally, it was shown that expression of miRNA-31 varied significantly, and the overall For several additional medical conditions, a predicted survival rate issues that may be producing minimal gene expression,The profiles of miRNA might indicate to possible therapy options by revealing cellular processes implicated in disease severity. (Tribolet *et al.*, 2020).

targeting and security are generally Toxicology and expense are the main problems of miRNA-based therapy. It may be a potent and effective technique to target a number of critical miRNAs in the battle Virus-fighting, but additional research is required to recognize before clinical studies, some miRNAs can start. (Nihad *et al* ., 2021).

4.6.1. The CT value of the House Keeping gene, MiRNA 31, and 192:

Because the frequency of CT values and miRNA levels are inversely correlated, infected COVID-19 patients have higher levels of miRNA-31 than MiRNA-192 and H.K. gene. , The analyzed patients' high levels of this gene are indicated by the miRNA-31's low CT value (31.04) and by the miRNA-31's elevated CT value (33.12) shows a reduced amount in individuals with curries following the SARS CoV-2 viral infection. Despite the fact that in the H.K. gene CT value was (24 .39), the optimal control, (H. K. gene) The CT value ought to fall between 20 and 30 . .The miRNA under examination's CT value, is shown in

Table (4-13): The result CT value of studied miRNA.

CT value of RT – PCR		**No.**	**Ct Mean**	**Std. Deviation**	**P. Value**
miRNA-31	Infected	5	31.04	1.77	0. 000

	Control	4	33.12	(1.30)	
miRNA192	Infected	16	33.42	(2.37)	0. 000
	Control	5	31.90	(1.30)	
H . K gene		18	24.39	(4.16)	

In COVID-19 patients who responded to treatment, a number of particular miRNAs and their mRNA targets returned to normal expression. The has miR 31-3p expression revealed a substantial negative correlation. a better, understanding for the phrase of inflammatory miRNAs in the blood of COVID-19 patients that are up- and down-regulated. The data obtained could help with COVID-19 diagnosis and prognosis. (Reza *et* al., 2021).

Investigation of the prognostic utility of the miRNA panel, It also relates to two important discoveries: (1) Unstable COVID-19 induces different molecular changes a miRNA profile in circulation; and (2) In the clinically severe phase, miRNAs, especially a signature composed of Both The prognostic indicators miRNA 192-5p and miRNA 323a-3p are useful for patients. (Bonneau *et al*., 2019).

4.6.2. MiRNA quantitative CT value evaluation in individuals with coronavirus:

Using H.K. gene serving as a control gene and miRNAs 31 and 192 as markers, 25 samples were chosen for molecular analysis. The results of miRNA 31 revealed that 5 patients had positive results, whereas 13 had negative results or a Ct value of 0. The control samples included 4 people had positive results in the counter and 3 had negative results. 16 patients with positive results for miRNA 192, and 3 control samples. as listed in table (4-14).

Table (4-14) The results of miRNA RT-PCR in the groups under study

RT – PCR result		**reaction.**	**n.**	**mean.**	**Std. deviation**	P. value
miRNA-31	Patients	+ ve	5	31.04	1.77	0.000
	Patient	- ve	13	No Ct	No Ct	
	Control	+ ve	4	34. 76	2. 94	
	Control	- ve	3	No Ct	No Ct	
Total			25	9. 13	15. 02	
miRNA 192	Patient	+ ve	16	31 .68	1. 76	0. 141
	Patient	- ve	4	No Ct	No Ct	
	Control	+ ve	3	34. 36	2. 99	
	Control	- ve	2	No Ct	No Ct	
Total			25	21. 26	16. 38	
H.K. gene		+ ve	18	24 .39	4. 16	

Significantly altered miRNAs are implicated in the control of immunological and inflammatory processes at many levels, including the creation of cytokines and chemokines T cell development, differentiation, and activation are regulated by(miR 16-5p, miR 192-5p, and miR 451)a. as well as B cell development, differentiation, and activation, The circulating miRNA profile is impacted by the severity of COVID-19,In severely ill COVID-19 patients, Analysis of plasma miRNAs suggests that an effective method for patients at risk categorization. (David *et al*., 2021).

As COVID-19 grade increased, has miR 126-3p, miR 31-3p, and miR 29a-3p relative expression decreased while the relative expression of their mRNA targets increased. Patients with COVID-19 who did not respond to treatment during their hospital stay showed this trend. (Baud *et al* ., 2020).

The severity of COVID-19 is correlated with certain circulating miRNA patterns. Plasma miRNA profiles are a unique approach for the early detection of vital status decline in ICU patients Six miRNAs were downregulated in severely sick patients between non-survivors and survivors. a two miRNA-based signature (miR-192-5p and miR-323a-3p). (Grasselli *et al* ., 2020)

4.6.3. Relationship between miRNAs 31 and 192:

The picture's outcomes show a distinct relationship between miRNAs - 31 and 192, which may indicate that higher production of miRNA 192 caused miRNA 31 to rise in some subgroups of SARS CoV-2 patients and curry patients.

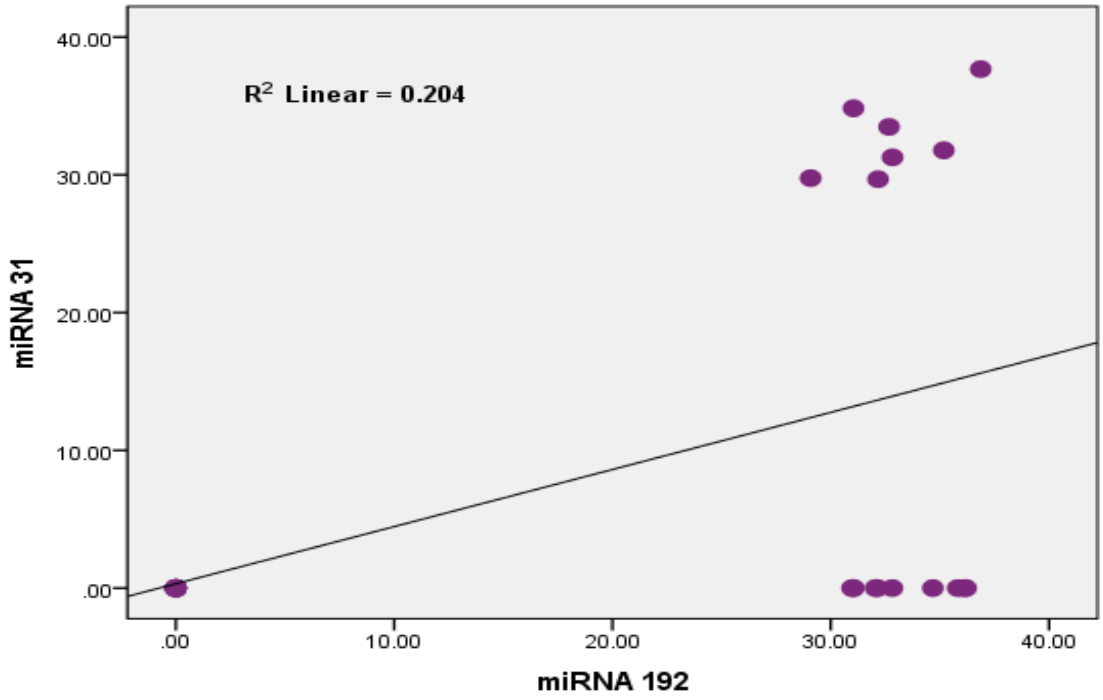


Figure (4-18): relationship between miRNAs 31 and 192

4.6.4. Correlation between the H.K. gene and miRNA 31:

Being a human gene control, the H. K. gene was employed. The housekeeping genes are clearly associated with miRNA-31, according to

the picture's results. The data would suggest that in some subgroups patients who had curries and those who had SARS CoV-2, increased production of miRNA 31 increases the H. K. gene.

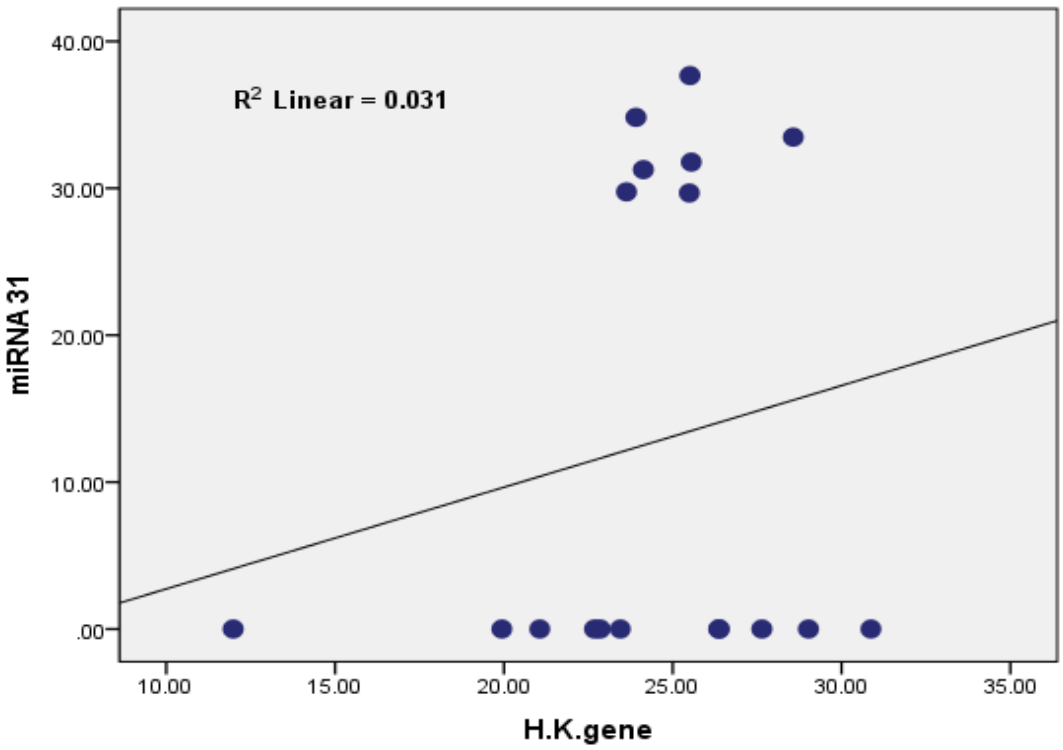


Figure (4-19): Correlation between the H.K. gene and miRNA 31 .

The findings demonstrate that H. K. gene, used a human control gene and miRNA 192 , have a bad relationship. This might imply that some subgroups of Sars-Cov2 patients and curried patients experience lower levels of the H. K. gene due to greater production of miRNA 192. . Figure shows the result (4-19)

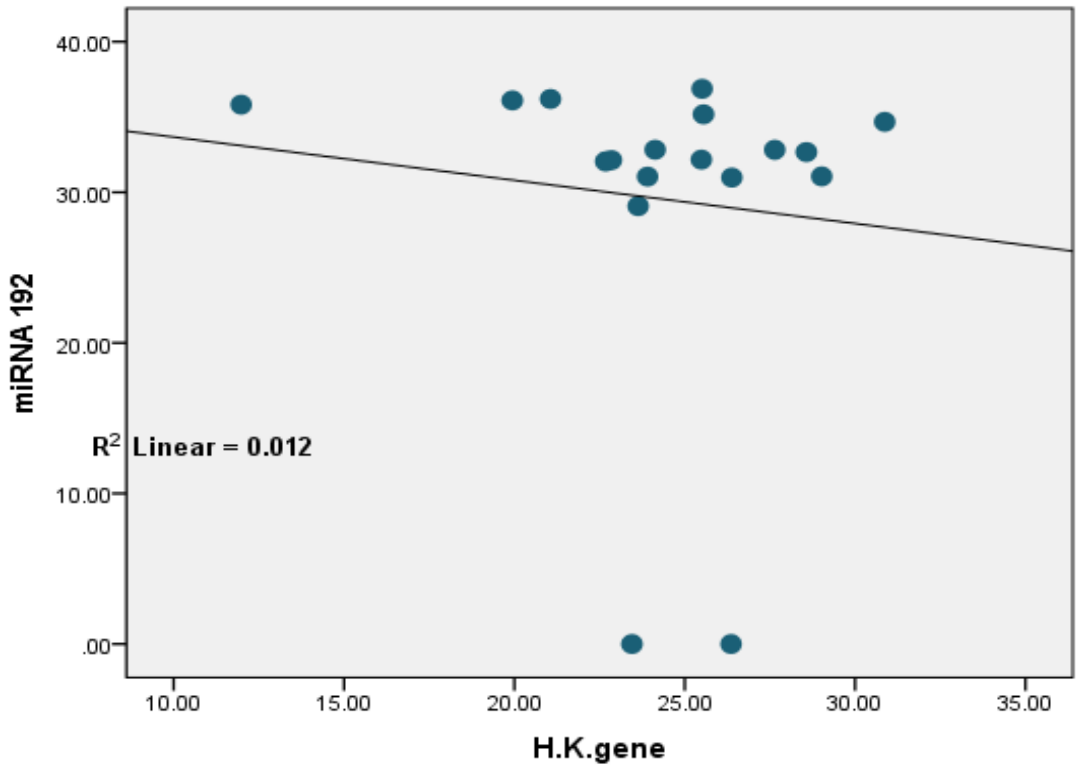


Figure (4-20): correlation between the H.K. gene and miRNA 192 .

4.6.5. Pearson Correlation of MiRNA and HLA typing:

By using a Pearson correlation model in SPSS statistical program, the result show that there is a direct correlation between both HLA-DR, HLA-G and miRNA-31, and miRNA-192, in comparison with negative correlation with H. K. gene. This result refered to that increase of HLA – DR , HLA-G level that will lead to increase in miRNA (31 and 192) gene expression. H.K. gene reduced expression in relation to HLA-DR and G, although it was used as a control miRNA in the present study. while the IL-6 has negative correlation with miRNA-31, and positive correlation with miRNA-192. There is a significant positive comparison between miRNA-31 and miRNA-192. These results were listed in table (4-15).

Table (4-15): Correlation between MiRNA and studied HLA typing

Correlations		HLA –DR	HLA –G	IL- 6	miRNA 31	miRNA192	H.K. gene
HLA –DR	Pearson Correlation	1					
	Sig. (2-tailed)						
HLA –G	Pearson Correlation	-.069-	1				
	Sig. (2-tailed)	.515					
IL- 6	Pearson Correlation	-.154-	-.055-	1			
	Sig. (2-tailed)	.148	.607				
miRNA 31	Pearson Correlation	.346	.390	-.151-	1		
	Sig. (2-tailed)	.091	.054	.472			
miRNA192	Pearson Correlation	.338	.382	.046	.452*	1	
	Sig. (2-tailed)	.098	.059	.829	.023		
H.K. gene	Pearson Correlation	-.098-	.085	.153	.175	-.109-	1
	Sig. (2-tailed)	.699	.736	.545	.487	.666	

*. The 0.05 level of significance for correlation (two-tailed) is reached
* * . The mark (-) denotes a negative correlation before the value.

Conclusion and Recommendation

Conclusion:

1-The correlation between the composition of age and sex and the epidemic characteristics of Sars-Cov2 infection, confirmed previous points that females are more susceptible to COVID-19 than males.

2- Age more than 60 years have more susceptible to infected with covid-19, rather than young age and adult.

3- The previous diabetes present in Sars-Cov 2 infected patients shows increase in IL-6 level in comparison with other conditions such as hypertension and pulmonary thrombosis .

4-The more sensitive and specific one is IL-6 , followed by CRP and WBC,so that IL-6 and CRP are the more specific and sensitive in diagnosis of SARS-COV2 infection in comparison with other parameters

5- The HLA- Class II (HLA-DR) , More predicted work in female rather than male at the infected population , and might be associated with active immune mechanism. Rather than Class I (HLA-G) .

6- Increasing of HLA-Class II and Class I among infected covid-19 patients has the most regulation of immune system at both humeral and cellular immunity .

7- Increasing interluken-6 at acute stage of covid-19 infection might be enhancement of HLA-G , while decrease of HLA-DR.

8- Infection with Sars-Cov2 , might induce expression of miRNA - 192 as well as miRNA - 31 among selected groups of covid 19 patients .

Recommendation:

1-Further study need of the detected other immunological parameters such as relationship with COVID-19.

2-Investigate other types of miRNAS.

3-Detection the association of HLA typing with COVID-19.

4-Viral miRNA and HLA typing could be dependent as additional biomarker for COVID-19.

5-Further study including other types of HLA needed and important in this fiel.

6- Study the association of other cytokines such as with genetic assay.

7- Investigate the relationship of other immunological parameters such as with susceptibility the infected.

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الخلاصة

ينتمي فيروس كورونا (SARS COV-2) الى فيروسات الحمض النووي الريبي RNA التي تسبب المرض للإنسان والحيوانات الأخرى عن طريق دخول الجسم من خلال مستقبلات الإنزيم المحول للأنجيوتنسين 2 (ACE2) الموجودة في عدة أعضاء مثل القلب والرئتين والكلى والجهاز الهضمي. الفيروسات الكبيرة التي تسمى الفيروسات التاجية تكون كروية ، ومغلفة ، وتحتوي على امتدادات تبرز من السطح بهيئة اشواك بالإضافة إلى امتلاكها جينوم من نوع حامض نووي رايبي أحادي االشريط موجب.

وخلال المدة الزمنية من ايلول 2021 إلى كانون الثاني- 2022 تم جمع تسعين عينة دم ، وتم جمع 50 عينة دم من المرضى المصابين ب19- Covid الراقدين في مستشفى الحسين التعليمي في محافظة كربلاء ، و40 عينة من أفراد أصحاء كمجموعة ضابطة. تراوحت أعمارهم بين 20-80 سنة. تم تشخيص الاصابة ب Covid-19 باستخدام تقنية تفاعل البوليميراز المتسلسل في الوقت الفعلي (RT-PCR).

وبينت النتائج أن نسبة الاصابة بفيروس SARS –COV2 في الذكور (52٪) أعلى مما عليه الإناث (48٪) . وكان اغلب المصابين في هذه الدراسة ليس لديهم أي مرض مزمن اثناء مدة الدراسة ،في حين كان بعض المصابين يعانون من داء السكري وارتفاع ضغط الدم بنسبة (18٪) لكل مرض ، و 10٪ من المصابين قيد الدراسة لديهم كلا المرضين في في نفس الوقت (السكري وارتفاع ضغط الدم) ،وكانت نسبة المصابين الذين يعانون من التجلط الرئوي حوالي(6٪). كانت نسبة المصابين المدخنين أقل من غير المدخنين بحوالي (14٪)و (86٪)على التوالي.

تمت دراسة بعض المتغيرات المناعية في امصال 50 مريضاً و 40 شخصاً سليماً باستعمال فحصي الامتزازالمناعي المرتبط بالإنزيم (ELISA) و المناعي المتالق(FIA).

كانت هناك زيادة في مستويات انترلوكين6 والبروتين التفاعلي C في المصابين اكثر مما عليه في الاصحاء،وكانت مستويات انترلوكين6 والبروتين التفاعليC وخلايا الدم البيضاء في الاناث المصابات اعلى مماعليه في الذكور المصابين.كانت هناك زيادة في مستويات انترليوكين 6 في المصابين الذين يعانون من داء السكري بينما اظهر البروتين التفاعلي C تراكيز اعلى في المصابين الذين لديهم تجلط رئوي، وكان عدد خلايا الدم البيض اعلى في المصابين الذين يعانون من ارتفاع ضغط الدم بالمقارنة مع الامراض المزمنة المناعي المرتبط بالانزيم انها اعلى في الاصحاء مما عليه في مصابيي كوفيد-19..

كانت النتيجة عند قياس مستويات HLA(HLA-DR,HLA-G) بواسطة فحص الامتزازالمناعي المرتبط بالانزيم أعلى في مجموعة الضابطة بالمقارنة مع مجموعة المصابين. لم تكن هناك فروق ملحوظة في مستوى HLA-DR بالنسبة للمصابين بين الجنسين بينما كانت هناك زيادة واضحة في مستوى HLA-G في الذكورالمصابين بالمقارنة مع الاناث المصابات.

كان أعلى مستوى ل HLA-DR في المصابين ضمن الفئة العمرية (50-59) بتركيز 3.82 نانوغرام. ، بينما أظهرت نتيجة HLA-G أن المستوى الأعلى تم رصده في الفئة العمرية للمصابين (40-49 عامًا ، عند 606.66) مقارنة بالفئات العمرية الأخرى. المرضى المدخنين لديهم مستوى أقل من HLA -DR و HLA-G مقارنة مع غير المدخنين من المرضى والمجموعة الضابطة ، بمتوسط (2.70 ، 456.15) نانوغرام، على التوالي .

قيمت جينات microRNAs في المرضى المصابين بفيروس SARS CoV-2 والأفراد الأصحاء ، وكشفت النتيجة أن الجين 192- microRNA إيحابي بنسبة 84 ٪ مقارنة مع 16 ٪ كانت النتيجة سلبية ، في حين أن الجين الآخر 31 - microRNA لديه 36 ٪ إيجابي نتيجة مقارنة بـ 64٪ كنتيجة سلبية ، بالإضافة إلى 100٪ من جين housekeeping gene ، كانت أعلى قيمة Cycle threshold لجين 31- MIRNA مقارنة مع حينات microRNA-192 وكذلك housekeeping gene في المرضى المصابين.

تشير قيمة Cycle threshold المنخفضة للقيمة الحدية للدورة (34.76) لجين microRNA-31إلى مستوى عالٍ من هذا الجين في المصابين قيد الدراسة ، نظرًا لوجود علاقة عكسية بين رقم قيمةCycle threshold ومستوى microRNA. بينما كانت قيمة Cycle threshold للجينhousekeeping gene (24.39).

هذه الدراسة بينت أن الشيخوخة تعد عاملا رئيسيا لجعل الناس أكثر عرضة للاصابة ب كوفيد 19 .يبدو أن IL-6 هو السايتوكين الحركي الأكثر أهمية في احداث الاضطرابات المناعية وومتلازمة الضائقة التنفسية الحادة في مصابيCovid-19 .يتوقع أن الفئة الثانية HLA- DR أكثر فعالية في الإناث المصابات مما عليه في الذكور المصابين وقد يرتبط بآلية المناعة النشطة بدلاً من الفئة الأولى (HLA-G)، وقد يكون سببا للاستجابة المناعية السريعة في الاناث المصابات ب Covid-19.

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كلية العلوم للبنات

تحديد بعض المعايير المناعية المرتبطة ب miRNA 31,192 بين المصابين بعدوى Covid-19

رسالة مقدمة الى
مجلس كلية العلوم للبنات – جامعة بابل
كجزء من متطلبات نيل درجة الماجستير في علوم الحياة

من قبل

هديل صلاح مهدي المسعودي

بإشراف

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اختصاصي مناعة سريريه الدكتور
رحيم طعمه عبيس المعموري

ربيع الأول 1444هـ

تشرين الأول 2022 م