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Immunological and Molecular Study of Single Nucleotide Polymorphism for Some Genes Associated With Covid-19 Patients in Babylon Province

A Submitted To The Council of the College Of Science for women Of Babylon University In Partial Fulfillment Of The Requirements For The Degree Of Master In Biology

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بِسْمِ اللهِ الرَّحْمنِ الرَّحِيمِ

﴿قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ ﴿٣٢﴾﴾

صدق اللّٰهُ العظيم

البقرة (٣٢)

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 my great parents..

To my dear wife, who supported and trusted me and My beloved kid (Adam)..

To all my family, my friends and all people whom i loved..

To everyone who aided me in every possible way to make this work see the light..

I dedicate this work..

Mustafa

Acknowledgment

At first of all, thanks to Allah the most gracious and the most merciful, who gave me the ability and desire to achieve this study.

I would like to express my deepest appreciation and very honest gratitude to my supervisor, ***(Dr. Ishraq Abdul Ameer Saleh Al-Maamori)*** *for her guidance, support, interest and her encouragement.*

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My sincere thanks to all staff of Merjan Hospital City for presenting all the facilities to finish this work.

My special and sincere thanks to patients who i take the samples from and may Allah heal them and have mercy on those who died because of this epidemic.

My sincere thanks are also to my family, friends and to anyone who helped and supported me by his pray for success in my work.

Mustafa

Summary

Coronaviruses (CoVs) are a group of related RNA viruses that cause illnesses in human and vertebrates. They can communicate a disease to the respiratory, gastrointestinal, hepatic, and central nervous system of human, which are enveloped viruses that own large-sized single-strand positive sense RNA genomes. This virus can enter the human body by its Angiotensin-converting enzyme 2 (ACE2) receptors which are found in various organs which is complementary in shape to the spike shape which allows successful attachment and makes it easier for the virus to enter the target cells.

A total of 90 blood samples were collected, 50 of which were from people infected with SARS-CoV-2 and 40 samples healthy non infected with SARS-CoV-2 that aged (20-60 years) from October/2021 to January/2022. All patients were examined by RT-PCR at Marjan Teaching Hospital. Blood samples from all COVID- 19 patients and control used to be collected, then DNA used to be extracted and analyzed for *ACE* gene (I/D), *IL-4* gene (PCR/RFLP) to determine genotypes and alleles frequencies with (PCR) and by means of gel electrophoreses used to be examined. The result found that I/D Allele Frequencies for Patients, which frequencies found DD, ID and II genotypes of *ACE* gene found 42%(21), 38%(19) and 20%(10) respectively. The D allele frequency was once 59%, also I allele frequency from 41%. Showing frequencies from DD, ID, also II genotypes of *ACE* gene in the control show 32%(13), 25%(10), 43%(17) found D allele frequency about 41% and the I allele frequency can 59%, also the results of *IL-4* genotype show critical variety between alleles in persistent and control, the CC was showed up in 52.5%(21) control while it was 24%(12) in patients, TT genotype was more successive in patients 56% (2) than control 35%(14).

CT genotype was more continuous in control 12.5%(5) than patients 20%(10). Some immunological parameters were studied on serum of 50 patients and 40 control by using AFIAS, Fuji and enzyme linked immunoassay sorbent (ELISA) techniques. The result found high levels of CRP, Ferritin, D-Dimer, LDH, PCT, IL-4, IL-6, IgG and IgM (25.76mg/L, 276.58ng/mL, 503.97ng/mL, 236.48IU/L, 299.95pg/mL, 122.89pg/mL, 13.17pg/mL, 12.75 and 1.77) and low levels of WBC, Eosinophil count and Platelets (7.7658Cells/cumm, 0.2302Cells/cumm and 171.68Cells/cumm) respectively than control (1.49mg/L, 147.61ng/mL, 222.24ng/mL, 134.37IU/L, 226.40pg/mL, 95.05pg/mL, 2.07pg/mL, 0.93, 0.08, 7.9835Cells/cumm, 0.3427Cells/cumm and 269.49Cells/cumm) respectively. The statistical analysis was done by using SPSS, ANOVA and Chi – Square .

Gene differentiation of SARS-CoV-2 with allelic discrimination by real time PCR, The presence of S gene mostly in first few days of infection while E gene present in the end time of infection over 1-2 week of onset of infection as well as N gene but in less degree. Genotype D/I in the ACE (I/D) polymorphism related of some infectious high effect on COVID-19 severity. The patients that receive 2 dose of protective Corona vaccine before infected with SARS-CoV-2 revealed mild symptoms rather than newly infected population. Decreased of platelets, increased of (CRP, Ferritin, D.Dimer and LDH) as diagnostic markers of SARS-CoV-2 infected patients, and increased of immunological parameters in response to SARS-CoV-2 infection such as (IL-6 , IL-4, PCT, IgG and IgM). There was no differences in WBC, and eosinophil count at both patients (at all ages) and control, while reduced the platelets count in (> 60 years) comparison with control while there were no differences in hematological parameters among both male and female of SARS-CoV-2 infected patients**.** Elevated IL-6 was revealed at patients with high fever

and moderate cough also patients without cough, while IL-4, PCT and IgM were increased in patients with mild fever and patients without cough, while IgG was elevated in patients with moderate fever and mild to moderate cough. IL-6 has negative correlation with PCT, IL-4, IgG and IgM, while IL-4 has positive correlation with PCT and negative correlation with IgG and IgM. PCT level elevated in comparison with SARS-CoV-2 IgG antibody, while no relationship between PCT and SARS-CoV-2 IgM antibody. The immunity status of patients infected with SARS-CoV-2 virus was gradually activation during time of exposure to infection by elevation of IgG instead of IgM.

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List of Abbreviations

NO.	Abbreviations	Terms
1	**A**	Adenine
2	**AB**	Antibody
3	**ACE**	Angiotensin-converting enzyme
4	**AFIAS**	Automated fluorescent immunoassay system
5	**ARDS**	Acute respiratory distress syndrome
6	**B cell**	Bone marrow cell
7	**Bat-SL-CoVZC45**	Bat SARS-like coronavirus ZC45
8	**Bat-SL-CoVZXC21**	Bat SARS-like coronavirus ZXC21
9	**BMI**	Body mass index
10	**BP**	Base pair
11	**BSA**	Bovine serum albumin

12	**BsmF1**	Bacillus stearothermophilus F1
13	**C**	Cytosine
14	**C- Terminal**	Carboxyl terminal
15	**CD**	Connector domain
16	**CD4**	Cluster of differentiation
17	**CDC**	Center for diseases control
18	**cDNA**	Complementary deoxyribonucleic acid
19	**COI**	Cut off index
20	**COVID-19**	Coronavirus disease-2019
21	**CoVs**	Coronavirus
22	**CRP**	C-Reactive protein
23	**CSF**	Colony stimulating factor
24	**CT**	Cycle threshold line
25	**CTD**	C terminal domain
26	**CXCL-10**	C-X-C chemokine ligand 10
27	**D**	Deletion
28	**DB**	Detective buffer
29	**DCs**	Dendritic cells
30	**DH_2O**	Deionizes Water
31	**DNA**	Deoxyribonucleic acid
32	**E**	Envelop
33	**ECs**	Endothelium cells
34	**EDTA**	Ethylene diamine tetra acetic acid
35	**ELISA**	Enzyme linked immune sorbent assay
36	**ERGIC**	Endoplasmic reticulum-Golgi intermediate compartment
37	**ESE**	Exonic splicing enhancer
38	**F**	Forward
39	**FABG**	β-ketoacyl-ACP reductase
40	**Fc**	Fragment of crystallization
41	**FEU**	Fibrinogen Equivalent Units
42	**G**	Guanine
43	**GF**	Growth factor
44	**GGT**	Gamma-glutamyl transferases
45	**HBV**	Human hepatitis virus
46	**HCoV-229E**	Human coronavirus-229E
47	**HCoV-HKU1**	Human coronavirus-HKU1
48	**HCoV-NL63**	Human coronavirus-NL63
49	**HCoV-OC43**	Human coronavirus-OC43
50	**HR1**	Heptad repeat 1
51	**HR2**	Heptad repeat 2
52	**HRP**	Horse reddish peroxidase

53	**HSF**	Human splicing finder
54	**HWE**	Hardy-Weinberg equilibrium
55	**I**	Insertion
56	**IC**	Internal control
67	**ICR**	Infection critical rate
68	**ICU**	Intensive care unit
69	**ID chip**	Identification chip
70	**IFR**	Infection fatal rate
71	**IgA**	Immunoglobulin alpha
72	**IgD**	Immunoglobulin delta
73	**IgE**	Immunoglobulin epsilon
74	**IgG**	Immunoglobulin gamma
75	**IgM**	Immunoglobulin mu
76	**IL-1β**	Interleukin-1 beta
77	**IL-4**	Interleukin-4
78	**IL-6**	Interleukin-6
79	**INF**	Interferon
80	**INF-γ**	Interferon-gamma
81	**ISR**	Infection severe rate
82	**KDa**	Kilo Dalton
83	**KLH**	Keyhole limpet hemocyanin
84	**LDH**	Lactate dehydrogenase
85	**M**	Membrane
86	**MERS-CoV**	Middle east respiratory syndrome - coronavirus
87	**MIN**	Minutes
88	**MMWR**	Morbidity and Mortality Weekly Report
89	**mRNA**	Messenger Ribonucleic acid
90	**N**	Nucleocapsid
91	**N- Terminal**	Amine terminal
92	**NA**	Nucleic acid
93	**NAD**	Nicotinamide adenine dinucleotide
94	**NADH**	Nicotinamide adenine dinucleotide hydrate
95	**NAT**	N-terminal acetyltransferase
96	**NC**	Negative control
97	**NEB**	New England biolab
98	**NTB**	Nitrotetrazolium blue
99	**NTD**	N terminal domain

100	**OD**	Optical density
101	**OR**	Odd ratio
102	**ORFs**	Open reading frames
103	**P. value**	Probability value
104	**PBS**	Phosphate buffer saline
105	**PC**	Positive control
106	**PCR**	polymerase chain reaction
107	**PCT**	Procalcitonin
108	**PD Buffer**	peritoneal dialysis buffer solution
109	**PP1a**	Polyprotein 1 a
110	**PW Buffer**	preheating of domestic water buffer
111	**QC card**	Quality control card
112	**R**	Revers
113	**RAS**	Renin angiotensin system
114	**RBD**	Receptor binding domain
115	**RDRP**	RNA dependent RNA polymerase
116	**RFLP**	Restriction fragments length polymorphism
117	**RFU**	Relative fluorescence unite
118	**RNA**	Ribonucleic acid
119	**RNAi**	Ribonucleic acid interference
120	**RNP**	Ribonucleoprotein
121	**RPM**	Round per minutes
122	**IL-4 rs**	Interleukin-4 Receptor Signaling
123	**RT**	Room temperature
124	**RT-PCR**	Real time- polymerase chain reaction
125	**S gene**	Spike gene
126	**S1**	Sub unit 1
127	**S2**	Sub unit 2
128	**SARS-CoV**	Sever acute respiratory syndrome - coronavirus
129	**SARS-CoV-2**	Sever acute respiratory syndrome - coronavirus-2
130	**SEC**	Seconds
131	**SNPs**	Single nucleotide polymorphism

132	**SsRNA**	Single strand Ribonucleic acid
133	**T**	Thymine
134	**T cell**	Thymus cell
135	**TBE**	Tris borate EDTA
136	**Th1**	T-helper cell 1
137	**TM**	Transmembrane
138	**TMPRSS2**	Transmembrane protein serine protease 2
139	**TNF-α**	Tumor necrosis factor-Alpha
140	**USA**	United states of America
141	**UTR**	Un-translated region
142	**UV Light**	Ultra-Violet light
143	**Vs.**	Verses
144	**VSR**	Viral suppressor of RNAi
145	**W Buffer**	Wash buffer
146	**WHO**	World health organization
147	**Y**	Year
148	**α-CoV**	Alpha- coronavirus
149	**β-CoV**	Beta- coronavirus
150	**γ-CoV**	Gamma- coronavirus
151	**δ-CoV**	Delta- coronavirus
152	**2019-nCoV**	2019- novel coronavirus

Introduction

1.Introduction

Coronaviruses were first isolated from chickens in l937 and known as coronaviruses because of the coronaviruses' crown shaped particles, coronaviruses can cause multi-system infections in a variety of animals, previously, there were six major types of coronavirus that can infect humans, including two highly lethal coronavirus, SARS-CoV and MERS-CoV, and four coronavirus that cause mild upper respiratory disease, namely HCoV-OC43, HCoV-229E, HCoV-NL63 and HCoV-HKU (Chang *et al*.; 2022). The pneumonia outbreak was caused by a novel coronavirus (Wu *et al*.; 2020), the virus is the seventh member of the human coronavirus family (Zhu *et al*.; 2020, Kobayashi *et al*.; 202l).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) originated in nature, and there are two most likely natural ways of origin: the first is that it evolved into a pathogenic state in animals and then passed to humans, and the second is that animals spread viruses that do not cause disease to humans, which then evolved into the pathogenic viruses that cause pandemic in humans (Andersen *et al*.; 2020, Hull *et al*.; 2021).

Coronaviruses are spherical, enveloped viruses of approximately 120 nm in diameter, containing a helical symmetry nucleocapsid, with a single-stranded RNA genome of positive polarity, non-segmented, 29.9 kb in size, and a GC content of 38% (Chan K. *et al*.; 2020). Its genome is composed of 13 open reading frames (ORFs) (Lu *et al*.; 2020) encoding 7096 amino acids that constitute four structural proteins spike (S), envelope (E), membrane (M), and nucleocapsid (N) and 15 non-structural proteins, in addition to eight accessory proteins that perform numerous functions in the processes of virus replication and assembly (Wu *et al*.;

2020). The researchers by Chang *et al*.; 2022, also investigated the virus's spike proteins, which showed that the SARS-CoV-2 had a similar structure to the human SARS-CoV.

The spike protein of SARS-CoV-2 has a variety of conformation states, revealed the high-resolution structure and distribution of trimmers on the virus surface, and found that most of the spike proteins were in a pre-fusion state (Walls *et al*.; 2020, Ke *et al*.; 2020). In addition, on the surface of SARS-COV-2, the Receptor Binding Domain of spike protein exists in many different states, and this helps to understand the interaction between neutralizing antibodies and viruses (Chang *et al*.; 2022). Yan *et al*.; 2020, identified the complex structure of ACE2 full-length protein and the viral S protein receptor binding domain, which marks an important advance in understanding how SARS-CoV-2 infects human cells. Xu X. *et al*.; 2020, pointed out that the binding receptor protein of Spike protein of SARS-CoV-2 in human body is ACE2 protein. Wrapp *et al*.; 2020, also pointed out that SARS-CoV-2 has a high affinity for human ACE2, which may make the virus easy to spread from person to person. They noted that ACE2 bound to SARS-CoV-2 with approximately 10-20 times more affinity than ACE2 bound to SARS-CoV (Chang *et al*.; 2022).

IL-4 cells are generated from Th2 cells. These interleukins block the pathway of Th1 immune response and trigger the reactions of Th2 cells. It was found that in cases of autoimmune conditions and cases of hyperactive immunity reactions, there is an increased production of Th1 cells. In cases of high intense care, Th2 cells were found to have been present in high amounts in COVID-19 patients (Prompetchara *et al.*; 2020). Interleukin-6 (IL-6) is one of the main mediators of inflammatory and immune response initiated by infection or injury and increased levels of IL-6 are found in more than one half of patients with COVID-19 (Zhang *et al*.; 2020). Levels of IL-6 seem to be associated with inflammatory response, respiratory failure, needing for mechanical ventilation and/or intubation and mortality in COVID-19 patients (Herold *et al*.; 2020). In a meta-analysis including nine studies (total 1426 patients) reporting on IL-6 and outcome in COVID-19, mean IL-6 levels were more than three times higher in patients with complicated COVID-19 compared with those with non-complicated disease, and IL-6 levels were associated with mortality risk (Aziz *et al*.; 2020).

2. Aims of study

The study aim to immunological and molecular investigation of some associated parameters in patients with SARS-CoV-2 infection, and to achieve the above aim the following objectives were concluded:

1. Detection single nucleotide polymorphisms of some related genes such *ACE* (I/D) and *IL-4* (rs11209032) using Polymerase Chain Reaction-insertion/deletion (PCR-I/D) and Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP).
2. The gene distribution of specific genes (S gene, N gene and E Gene) of SARS-CoV-2 was done by using Real-Time PCR for nasopharyngeal swab.
3. Compare the variation in levels of some immunological markers Interleukin-4 (IL-4), Interleukin-6 (IL-6), C-reactive protein (CRP), Procalcitonin (PCT), Ferritin, D- Dimer and SARS-CoV-2 antibody (IgM and IgG) between the SARS-CoV-2 patients and control groups for both gender.

Chapter One

Literature Review

1. Literature Review

1.1. Coronaviruses (CoVs)

The name "coronavirus" is derived from Latin corona, meaning "crown" or "wreath", itself a borrowing from Greek κορώνη korṓnē, "garland, wreath" (Merriam-Webster. 2020). The name was coined by June Almeida and David Tyrrell who first observed and studied human coronaviruses (Tyrrell *et al*.; 2002). The name refers to the characteristic appearance of virion (the infective form of the virus) by electron microscopy, which have a fringe of large, bulbous surface projections creating an image reminiscent of the solar corona or halo (Tyrrell *et al*.; 2002).

Coronaviruses constitute the subfamily Orthocoronavirinae, in the family Coronaviridae, order Nidovirales and realm Rboviria (Fan *et al*.; 2019). They are enveloped and have a non-segmented, single-stranded, positive-sense ribonucleic acid (ssRNA+) as their nuclear material (Mahendra *et al*.; 2020).

Of the four coronavirus genera (α,β,γ and δ), human coronaviruses (HCoVs) are classified under α-CoV (HCoV-229E and NL63) and β-CoV (MERS-CoV, SARS-CoV, HCoV-OC43 and HCoV-HKU1). SARS-CoV-2 is a β-CoV and shows fairly close relatedness with two bat-derived CoV-like coronaviruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21 (Malik 2020). Middle East Respiratory Syndrome (MERS-CoV), which first appeared in 2012 and is still circulating in camels (Dong *et al*.; 2020); and SARS-CoV-2, that first reported in Wuhan, China in December 2019 (Xu *et al*.; a2020).

1.2.History of SARS-CoV-2

In Southern China in the autumn of 2002, SARS-CoV epidemic was began and spread to 29 countries or regions, resulting in 8096 cases and 774 deaths (Hon *et al*.; 2003). SARS' etiologic agent (an unidentified coronavirus; SARS-CoV) was isolated and its genome sequenced in record time (Marra *et al*.; 2003, Cherry and James. 2004). The mini pandemic was brought under control within 7 months of its onset (Cherry and James. 2004).

In Saudi Arabia, Jeddah, in June 2012, the first case of Middle East Respiratory Syndrome (MERS) was discovered, 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 (Killerby and Marie. 2020). MERS-CoV is a zoonotic virus that has a reservoir host in dromedary camels (Paden *et al*.; 2018).

At the end of 2019, an increasing number of patients with pneumonia from unknown causes in Wuhan (Hubei, China), with a population of 11 million, has caused concern from the local hospital (WHO, 2020). On December 29, 4 cases were linked to the Huanan seafood market (The 2019-nCoV Outbreak), in which nonaquatic live animals were also sold, including several species of wild animals, the local Center for Disease Control (CDC) discovered additional patients associated with the same market after the investigation and reported to the China CDC on December 30, 2019 , the World Health Organization was informed of the unknown causes of cases of pneumonia by China CDC (WHO, 2020).

During the first outbreak in Wuhan, the virus was usually referred to as "coronavirus" or "Wuhan coronavirus" (Fox 2020) or "Wuhan virus" (WHO, 2020). A few weeks later, In January 2020, WHO recommended "novel coronaviruses-2019" (not -2019) as the temporary name for the virus. This was according to the WHO guidelines for 2015 against the use of geographical locations (such as Wuhan), animal species, or groups of people in the names of diseases and viruses (Hui 2020).

On February 11, 2020, the International Virus Classification Committee adopted the official name "SARS-CoV-2" severe acute respiratory syndrome Coronavirus-2 (Paul, 2020). To avoid confusion with SARS, the World Health Organization sometimes refers to SARS-CoV-2 as the "COVID-19 virus" in public health communications (Morgan, 2020). The World Health Organization (WHO) on March 11 declared COVID-19 a pandemic, pointing to the over 118,000 cases of the coronavirus illness in over 110 countries and territories around the world and the sustained risk of further global spread, the World Health Organization has announced a pandemic (Jamie, 2020).

1.3.Classification of Coronavirus

Coronaviruses are members of Coronaviridae family, and Orthocoronavirinae subfamily of the Nidovirales order. Depending on genetic and antigenic criteria, coronaviruses are classified into four genera: alphacoronavirus (α-CoV), betacoronavirus (β-CoV), gammacoronavirus (γ-CoV), and deltacoronavirus (δ-CoV) (Lu *et al.*; 2020). Bats and mice act as reservoirs for alpha and beta coronaviruses, while birds serve as reservoirs for gamma and delta coronaviruses (Li *et al.*; b2020). SARS-CoV-2 belongs to the subgenus Sarbecovirus of the genus Betacoronavirus, according to phylogenetic analysis as shown in Figure (1-1) (Lorusso *et al*.; 2020).

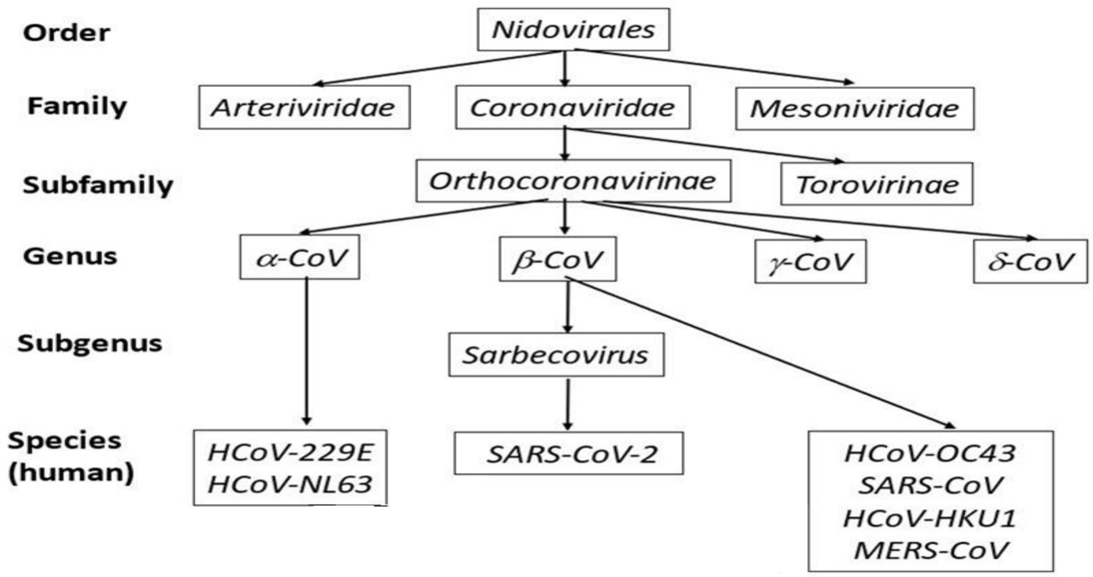


Figure (1-1): Classification of Coronaviruses (Lu *et al*.; 2020).

1.4. SARS-CoV-2 -Structure

The structure of the viral envelope as any membrane consisted of a lipid bilayer and a various number of structural proteins, these are membrane (M), envelope (E), and spike (S) in a ratio of E:S:M 1: 20:300 as shown in figure (1-2) (Susan 2020). The coronavirus surface S glycoprotein is a ~600 kDa trimmer, one of the largest known class 1 fusion proteins (Du *et al*.; 2020). Located on the outer envelope of the virion, it plays a critical role in viral infection through recognition of the host cell receptors and by mediating the fusion of the viral and host cell membranes (Zost *et al*.; 2020). S also has been shown to elicit a strong immune response, making it the primary target for the recently developed vaccines for SARS-CoV-2 necessary to stem the COVID-19 pandemic (Robbiani *et al*.; 2020).

The SARS-CoV-2 S gene encodes a ∼1300 amino acid precursor protein which is then activated through proteolytic cleavage into an amino (N)-terminal S1 subunit (∼700 amino acids), and a carboxyl (C)-terminal S2 subunit (∼600 amino acids) with a single transmembrane (TM) region anchor (Letko *et al*.; 2020). The S1 and S2 subunits form a heterodimer, that in turn oligomerize into a trimmer resulting in the formation of the surface spike on the virion (Cai *et al*.; 2020). The S1 subunit consists primarily of an N-terminal domain (NTD) and a receptor binding domain (RBD), as well as two C-terminal domains (CTD) (Nathan and Peijun. 2022). S2 consists of a fusion peptide, heptad repeat (HR1), central helix region, connector domain (CD), heptad repeat 2 (HR2), and the transmembrane region (Cai *et al*.; 2020).

Along with M, the coronavirus E protein is one of the major membrane components in SARS-CoV-2 (Nathan and Peijun. 2022), E is a small, 8.5 kDa protein consisting of 75 amino acid residues. In coronaviruses, E is a cationic selective viro-porins, forming a channel across the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) membrane (Nathan and Peijun. 2022). In SARS-CoV, E mediates the budding and release of viruses (Mandala *et al*.; 2020). Deletions of E have been shown to attenuate the virus, while mutations abolishing channel activity reduce pathogenicity and this provides a target for potential antiviral drug development as well as a potential vaccine candidate in SARS-CoV-2 (Nathan and Peijun. 2022).

The major protein component of the SARS-CoV-2 inside of the virion is the nucleocapsid (N) protein, and it is responsible for binding the genomic RNA within the virion and packaging it into the ribonucleoprotein (RNP) complex (Nathan and Peijun. 2022). N proteins have a variety of functions beyond packaging, with the SARS-CoV-2 N protein found to interfere with RNA interference (RNAi) and function as a viral suppressor of RNAi (VSR) in cells (Mu *et al*.; 2020).

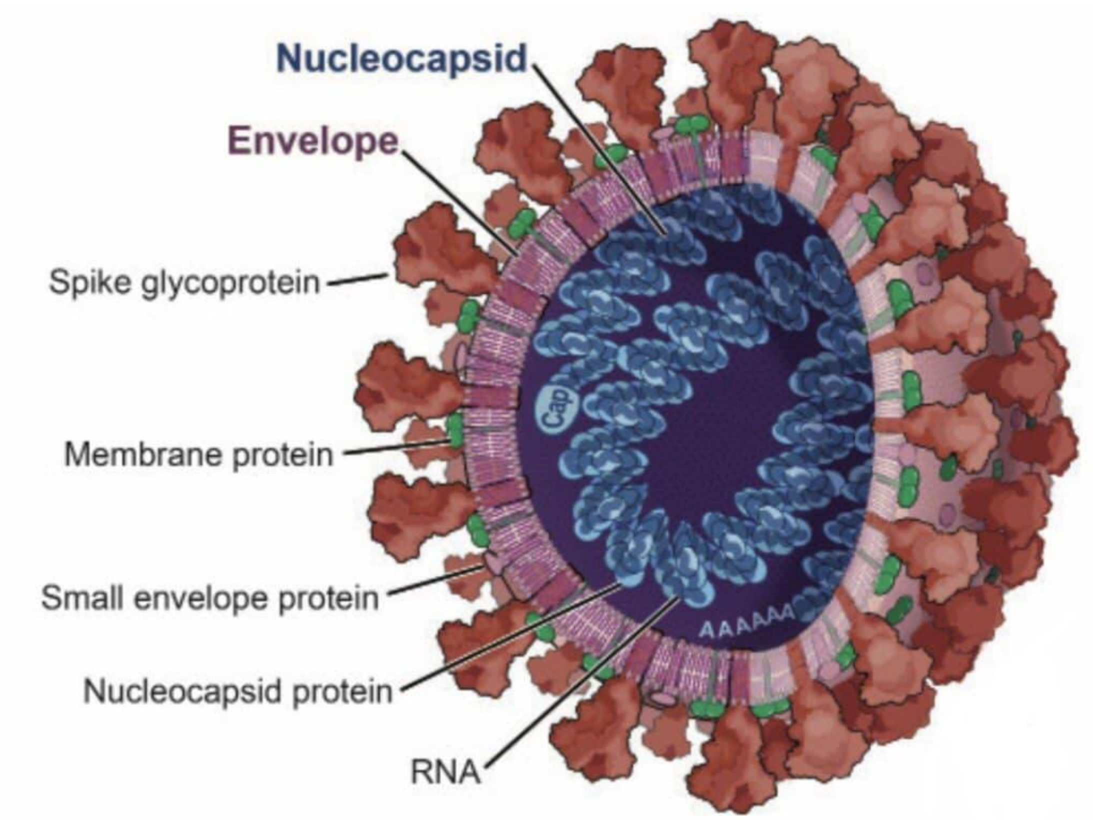


Figure (1-2): Structure of Coronaviruses (Cornelia *et al*.; 2020).

1.5. SARS-CoV-2 Genome

Coronaviruses are RNA viruses with a genome made up of a single stranded, positive–sense RNA with a size ranging from 26 to 31 kilobases (Knapp, 2020). The sequence of the coronavirus genome is in the following order; started with 5′-leader-UTR-then replicate/transcriptase-spike (S)-envelope (E)-membrane (M)-nucleocapsid (N) finally with-3′UTR-poly (A) tail (Decaro *et al*.; 2011) as shown in figure (1-3).

The genome of SARS-CoV-2 is similar to other coronaviruses that comprise of ten open reading frames (ORFs). The first ORFs (ORF1a/b), about two-thirds of viral RNA, are translated into two large polyprotein pp1a and pp1ab (Chan *et al.*; 2020), which encode 16 nonstructural proteins, including proteases, RNA-dependent RNA polymerase (RdRp), RNA helicase, primase, and others, that form the viral replicase complex, a platform to propagate viral mRNAs. These nonstructural proteins are all potential targets for therapies, which would in theory work against all coronaviruses (Kim *et al*.; 2020, Hoffmann *et al.*; 2020, Lan *et al*.; 2020).

The remaining portion of the genome includes interspersed open reading frames for the structural proteins, as well as a number of accessory proteins generally nonessential for replication in tissue culture but capable of suppressing immune responses and enhancing pathogenesis (Lu *et al*.; 2020).

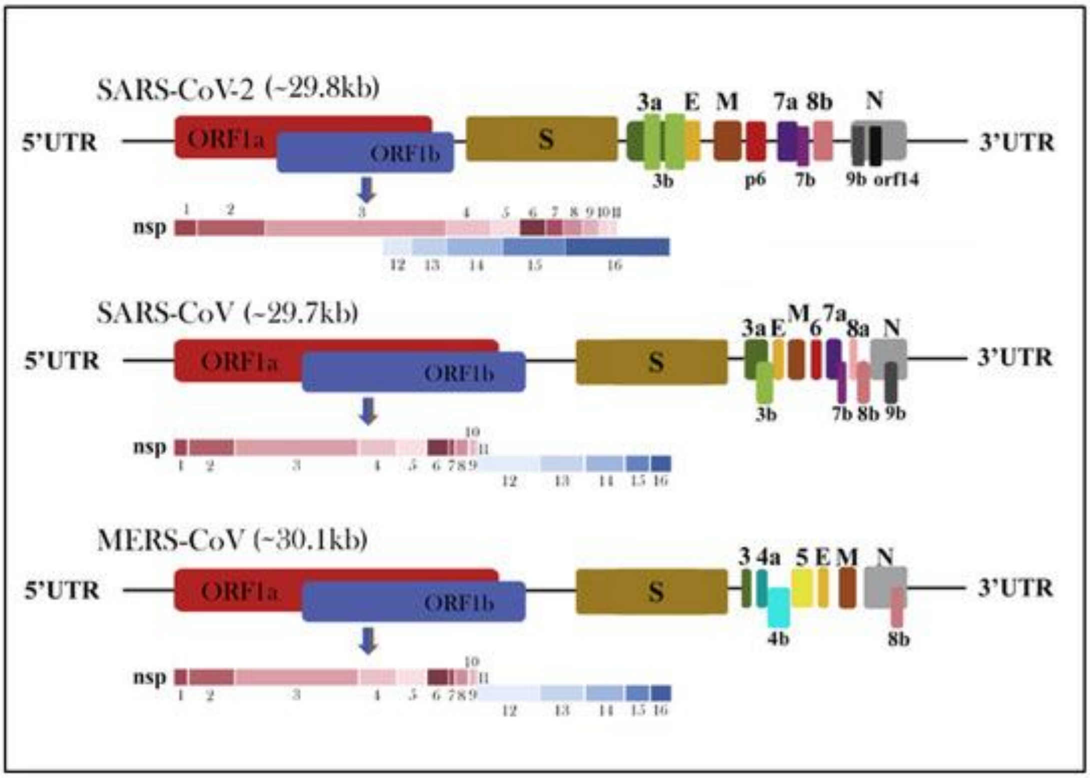


Figure (1-3): SARS-CoV-2 Genome (Hossein *et al.*; 2020).

1.6. SARS-CoV-2 entry to the host cell

Coronavirus spike proteins are key determinants for virus attachment and entry into target cells (Cornelia *et al*.; 2020). The receptor for both SARS-CoV and SARS-CoV-2 is angiotensin-converting enzyme 2 (ACE2) (Hoffmann *et al*.; 2020, Lan *et al*.; 2020) a cell-surface enzyme contributing to control of blood pressure. SARS-CoV cell entry is independent of ACE2 catalytic activity (Cornelia *et al*.; 2020).

Entry involves 2 spike protein subunits, which mediate distinct functions, the S1 subunit mediates ACE2 attachment through the receptor-binding domain and the S2 subunit, containing the fusion peptide and transmembrane domains, drives fusion of viral and host cell membranes (Cornelia *et al*.; 2020).

To be activated for fusion, the spike protein must be cleaved at 2 sites directly at the cell membrane, through endosomes, or both (Cornelia *et al*.; 2020). The sequence of the cleavage sites, one located at the border of S1 and S2 subunits, the other (S2´) within S2 just upstream of the fusion peptide, provide substrates for a variety of cellular proteases and determine cleavage efficiency (Cornelia *et al*.; 2020).

The route of infection thus depend on the proteases available in different cell types and the protease cleavage sites (Cornelia *et al*.; 2020). This is also demonstrated by involvement of the cellular serine protease TMPRSS2 (transmembrane protein serine protease 2) and activities of furin and endosomal cathepsins B and L in SARS-CoV-2 entry as shown in figure (1-4) (Hoffmann *et al*.; 2020).

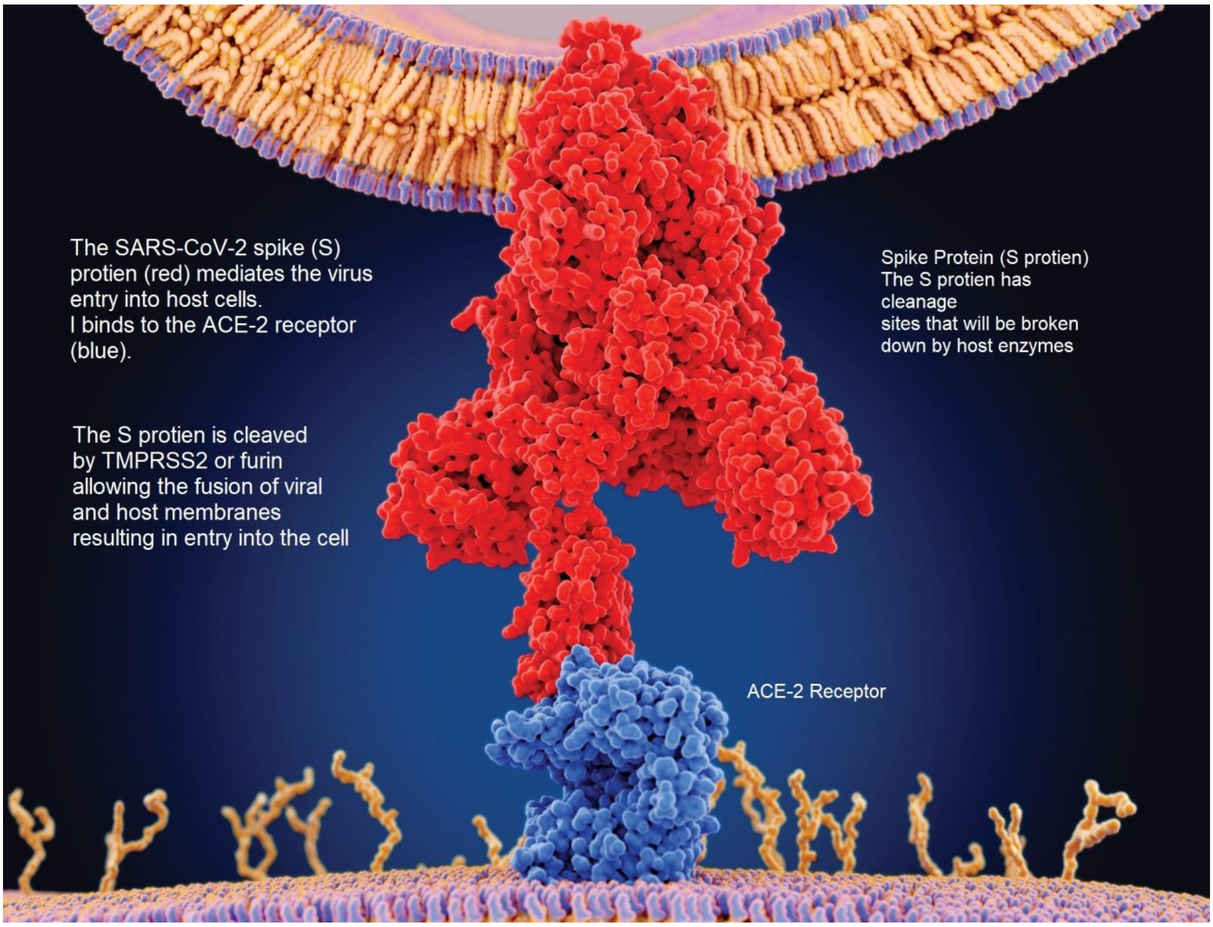


Figure (1-4): S protein binding with the Angiotensin- converting enzyme 2 (ACE2) (Sanil Rege. 2020).

TMPRSS2 activity is also involved in viral spread and pathogenesis in SARS-CoV-infected and MERS-CoV-infected mouse models (Iwata-Yoshikawa *et al*.; 2019). Host proteases that cleave the S protein are also potential targets for antiviral drugs (Cornelia *et al*.; 2020). A higher rate of SARS-CoV-2 infections compared with SARS-CoV infections may be at least partially explained by a higher affinity of spike protein for ACE2 (Lan *et al*.; 2020).

The sequence divergence in both the receptor binding domain and cleavage domains in the spike protein between SARS-CoV-2 and the bat virus highlight how only a few changes are needed to adapt an animal virus to humans (Zhang *et al*.; 2020).

After fusion, the implanted SARS-CoV-2 later the genomic material will be released into the cytoplasm, and it will become nucleoplasmically localized (Qianqian *et al*.; 2021). The genetic material of this virus would be mRNA, which is prepared for translation into a protein (Qianqian *et al*.; 2021).

This virus's genomic material has been supplemented by around 14 open reading frameworks (ORFs), every one encodes different set of structural and non-structural proteins that aid in the virus's survival and virulence. By contributing to the ribosome frame shifting event, the genetic parts that encode non-structural proteins first convert to ORF1a and ORF1b to create two great superimposed proteins, pp1a and 19 pp1ab, during the transformation stage (Marquardt *et al*.; 2020).

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 (Masters *et al.*; 2006, Fehr and Perlman, 2015) as shown in figure (1-5).

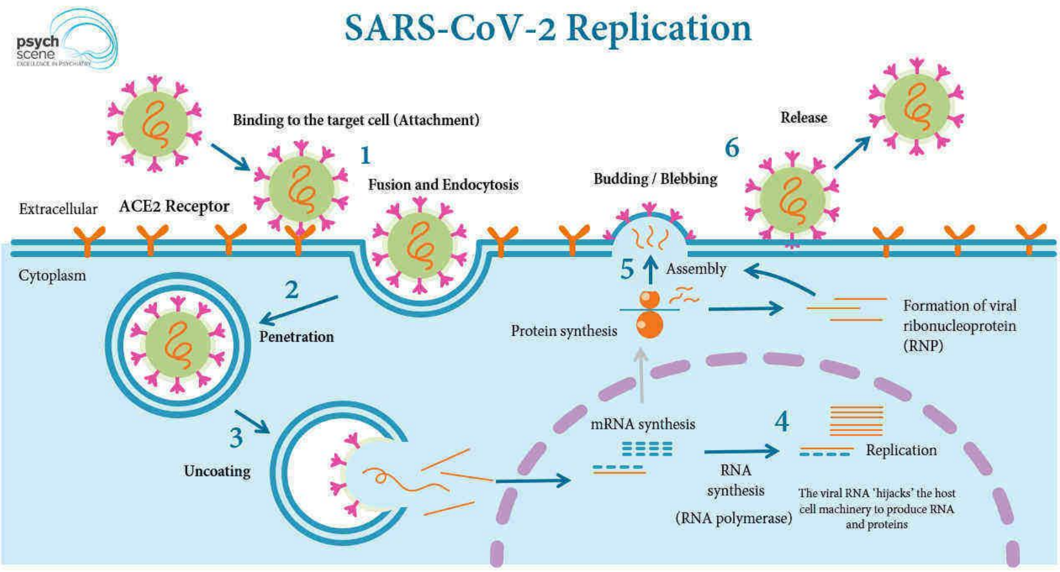


Figure (1-5): SARS-CoV-2 entry and replication and exit from the host cell (Sanil Rege. 2020).

1.7. Transmission Among Animals

In horseshoe bats (genus *Rhinolophus*), two separate research groups Lau *et al.*; 2005, Li *et al.*; 2005 identified new coronaviruses were discovered to be compatible with human SARS-CoV, called SARS-CoV-linked viruses or SARS-like coronaviruses.

According to these studies by Lau *et al.*; 2005 and Li *et al.*; 2005, bats may have acted as a natural host for SARS-CoV, while civets just served as an intermediary.

Another research by Ge *et al*.; 2013 and Hu *et al*.; 2017, revealed the coexistence of multiple SARS-CoVs in bat species that inhabit a cave in Yunnan province, China.

In light of the above, it is of interest to consider the current evidence of recombination observed in the case of SARS-CoV-2. The SARS-CoV-2 virus was hypothesized to have emerged as a result of a recombination event between strains of beta-coronaviruses endemic to certain species of bats and pangolins (Zhang *et al*.; 2020). however this theory has invited intense debate as regards convincingly proving the proximal origin of the virus (Seyran *et al*.; 2021). Bats are an effective reservoir for CoV recombination and evolution due to their high level of CoV recombination (Banerjee *et al*.; 2019).

1.8. Transmission from Animals to Human

SARS-CoV-2's zoonotic origin in Wuhan, China, can be directly linked to the wet animal market, where a large majority of people who contracted the virus were exposed to it at some stage (Rothan and Byrareddy, 2020). There have been many attempts to identify the primary host or intermediate carriers from which the virus could have spread to humans.

According to new findings, SARS-CoV-2 and bat coronavirus share more than 95% genomic similarity, suggesting that bats are the most likely hosts of the current (Perlman, 2020, Zhou *et al*.; 2020). There are a few other animal hosts have been recognized as virus reservoirs, in addition to bats.

Ji *et al*.; 2020 considered snakes to be a virus reservoir with the ability to infect humans, while Lam *et al*.; 2020 discovered SARS-CoV-2-linked coronaviruses in pangolins (*Manis javanica*).

The opportunity of minks serving as intermediary hosts for SARS-CoV-2 was mentioned. It's thought that the infection spread from bats to civets and then to people living nearby the site, that the spread was caused by infected animals being traded in the wild (Ahmad *et al.*; 2020, Lu *et al.*; 2020)**.**

Several factors influence the likelihood of animal-to-human transmission, including disease dynamics in an animal host, virus exposure level, and human inhabitants susceptibility (WHO, 2020).

Many of these factors have broken down into 3 main phases that show how viruses spread. The primary phase describes the pathogen pressure on human hosts is characterized by the amount of virus that interacts with humans, which is managed by virus diffusion from animal host, then after the virus trying to survive, growth and dissemination external animal host (WHO, 2020). In the following phase, human and vector activity determine the likelihood of viral exposure, the way of entrance, and the virus amount. Genetics, the physiological and immunological situation of the human host, as well as phase two factors influencing likelihood and harshness of infection, all affect the final stage (Plowright *et al.*; 2017).

1.9. Transmission Among Humans

The transmission of SARS-CoV-2 occurs primarily through respiratory droplets and aerosols generated during coughing or sneezing, which may land on the nose, mouth or eyes (Li *et al.*; 2020).

A live SARS-CoV-2 has already been found in patients feces in recent studies by Gu *et al.*; 2020 and Holshue *et al.*; 2020, demonstrated the virus's persistence throughout the gastrointestinal tract and modifying gastrointestinal signs, reappearance, and transmission via the fecal-oral way. However, it is unclear if consuming virus-infected food would result in infection and transmission (Wu *et al*.; 2020).

SARS-CoV-2 transmission from one person to another can happen as a result of prolonged and unprotected contact with an infected person, according to Ghinai *et al*.; 2020, implying persistent pathogen pressure leading to infection and disease.

Up to now, a person diagnosed with SARS-CoV-2 has served as a chief infection vector, with droplets in the respiratory system serving as the chief mode of diffusion, followed by aerial droplets and close contact, before fusing with the cell membrane, the virus binds to host receptors and initiates infection (Jin *et al*.; 2020).

In the case of SARS-CoV-2 human-to-human transmission, the receptor-binding domain (RBD) of virus spikes binds with the Angiotensin-converting enzyme 2 (ACE2) receptor of the possible host cell, according to studies by Jaimes *et al*.; 2020 and Wan *et al*.; 2020.

1.10. *IL- 4* Gene

Understanding the role of genetic variants in the course of respiratory infections might help in the recognition of possible candidates for further analysis in patients affected by SARS-CoV-2, however, data related to polymorphism of cytokine genes in SARS-CoV-2 infection is very limited thus, this current review brings important thoughts on how polymorphisms in cytokine genes could be essential for disease outcomes in COVID-19 and for that, Adriana *et al*.; 2021 discuss crucial findings and mechanistic aspects of polymorphism and cytokine gene expression and pinpoint some possible associations amongst cytokine gene polymorphisms, cytokine storm, and the risk of Severe Acute Respiratory Syndrome.

Although the genes encoding cytokines, as well as, their receptors are relatively conserved in their exons, non-silent mutations in the coding regions can result in loss, functional change, or overexpression of these proteins (Adriana *et al*.; 2021). The complex remodeling of the immune system resulting from infection by SARS-CoV-2, possibly related to polymorphisms in the cytokine genes, may explain the pro-inflammatory status recognized by cell exhaustion and the cytokine storm characteristic of dysfunctional immunity and acute inflammation of respiratory syndrome also the progressive increase of cytokines in pro-inflammatory status and its association with these polymorphisms needs to be clarified to gain a deeper understanding of disease progression in different parts of the world (Adriana *et al*.; 2021).

Regarding the modulatory cytokines, a study conducted by Patarčić *et al*.; 2015 performed meta-analyses of studies on tuberculosis, influenza, respiratory syncytial virus, SARS-CoV-1, and pneumonia, One single-nucleotide polymorphism from the IL4 +1059 C/T gene was significant for pooled respiratory infections (rs2070874). IL4 has previously been described to have a pivotal role in shaping the nature of the immune response, by promoting and stimulating both T-cell and B-cell differentiation and modulation (Adriana *et al*.; 2021). It provides a balance between Th1 and Th2 response, biased to the latter, and therefore alteration of its function may substantially affect immune response also most commonly reported such alteration is associated with an increased risk of atopy and allergies (Patarčić *et al*.; 2015).

1.11. *ACE* Gene

Angiotensin converting enzyme (ACE) genotypes are known to be associated with development of acute respiratory distress syndrome (ARDS) and resultant mortality, the ACE gene consist of two variant alleles; insertion (I) and deletion (D) polymorphisms (Aunga *et al*.; 2020). The allelic distribution of the ACE gene within a population follows the Hardy-Weinberg equilibrium, with three distinct genotypes: II, ID, and DD (Saab *et al*.; 2007).

The entry of SARS-CoV-2, the agent that causes COVID- 19, into the cell occurs by binding viral spike proteins to angiotensin- converting enzyme 2 (ACE2) receptors of the host membrane, it was suggested that increased susceptibility to COVID- 19 infection is associated with the expression of the target ACE2 receptor in the epithelium exposed to the virus (Wan *et al*.; 2020).

Low infection and complication level is present among children, serum ACE levels in children are higher than in adults and ACE2 receptor gene expression in the nasal epithelium, which is the first point of contact for SARS- CoV- 2, was age- dependent, lowest in younger children, and increasing with age into adulthood (Sevim *et al*.; 2021). It was also suggested that the lower risk among children is due to the lower expression of the ACE2 receptor (Bunyavanich *et al*.; 2020).

A counter- regulatory relationship between ACE2 and ACE located on opposite axes in the renin- angiotensin system (RAS) has been reported, also ACE plays an important role in converting angiotensin I to angiotensin II and ACE2 is a negative regulator of the RAS and counterbalances the function of ACE and the lungs consider as a primary organs for ACE receptor expression and generating circulating angiotensin II (Sevim *et al*.; 2021).

RAS plays a role in the pathogenesis of pulmonary hypertension and fibrosis, which are common chronic lung diseases, also recent studies by Kuba *et al*.; 2006 show that RAS also plays an important role in acute lung diseases, particularly acute respiratory distress syndrome (ARDS).

A common 287 base pair insertion/deletion (I/D) polymorphism has been reported by Sevim *et al*.; 2021 in intron 16 of the ACE gene and is known to be associated with serum levels of circulating ACE and serum ACE concentrations were reported to be significantly higher in subjects with the D/D genotype compared to the I/D and I/I genotypes. Considering the opposite effect between ACE and ACE2, decreased ACE2 receptor gene expression is strongly related to an increase in ACE expression, so it could be hypothesized that having a D allele for ACE I/D polymorphism affects the clinical course of the COVID- 19 by decreasing the ACE2 receptor level (Sevim *et al*.; 2021).

Changes in the ACE2 receptor gene expression level, genetic variations play a very important role as well as environmental factors, one of the many polymorphisms identified in the ACE2 receptor gene, rs2106809 and rs2285666 are particularly remarkable and a bioinformatics tool called Human Splicing Finder (HSF) (v.3.0) predicts that the rs2106809 polymorphism, intronic single- nucleotide polymorphism (SNP) found in the ACE2 receptor gene, might create an intronic- exonic splicing enhancer site (ESE) (Sevim *et al*.; 2021). Chen *et al*.; 2018 was suggested that the splicing efficiency of the ACE2 receptor gene may be influenced by the creation of these enhancer motifs. Liu *et al*.; 2016 was also found that ACE2 rs2106809 CC or CT genotype carriers had higher circulating ACE2 receptor levels compared with TT genotype carriers.

The G8790A (rs2285666) polymorphism is at the fourth base of the third intron and situated in the intron adjoined to the exon, suggesting, this locus could alter messenger RNA (mRNA) alternate splicing and affect ACE2 receptor gene expression, Kramkowski *et al*.; 2006 has also reported that this polymorphism showed a strong linkage disequilibrium with the other SNPs (rs1978124 intron 1 and rs714205 intron 16) in the ACE2 receptor gene.

1.12. Immunological Parameters

1.12.1. C- Reactive protein (CRP)

COVID-19 is a novel infectious disease for which no therapy exists. As a result, biomarkers must be examined to assess the degree of lung lesions and disease severity (Wang, 2020). COVID-19's pathological and physiological mechanisms, as well as diagnostic procedures, are still being investigated (Wang, 2020).

To reduce case fatality, clinical monitoring and effective treatment strategies were needed (Wang, 2020). It was necessary to look at other sensitive measures that could indicate lung lesion changes and disease severity (Wang, 2020).

CRP is a pentameric protein synthesized by the liver, whose level rises in response to inflammation (Sara *et al*.; 2022). CRP is an acute-phase reactant protein that is primarily induced by the IL-6 action on the gene responsible for the transcription of CRP during the acute phase of an inflammatory/infectious process (Sara *et al*.; 2022). The name CRP arose because it was first identified as a substance in the serum of patients with acute inflammation that reacted with the "c" carbohydrate antibody of the capsule of pneumococcus (Sara *et al*.; 2022).

CRP has both pro-inflammatory and anti-inflammatory properties (Sara *et al*.; 2022). It plays a role in the recognition and clearance of foreign pathogens and damaged cells by binding to phosphocholine, phospholipids, histone, chromatin, and fibronectin (Sara *et al*.; 2022). It can activate the classic complement pathway and also activate phagocytic cells via Fc receptors to expedite the removal of cellular debris and damaged or apoptotic cells and foreign pathogens (Sara *et al*.; 2022).

High levels of C-reactive protein (CRP) were noted in the vast majority of COVID-19 patients (3%–91%) and shown to be associated with disease severity (Lippi and Plebani, 2020, Young *et al*.; 2020). CRP levels give an idea for disease severity and prognosis. Additionally, it is considered to be a potential early marker for sepsis and mortality (Santoso *et al*.; 2020).

It is suggested by Young *et al*.; 2020 that CRP level, especially at admission, may be critical for grading disease severity. The patients who survived had a CRP level of ~40 mg/L, while those who died had 125 mg/L (60–160 mg/L) (Ruan *et al*.; 2020). Non-COVID etiologies (such as heart failure) should be considered for a patient with severe respiratory distress and normal CRP levels (Recep *et al*.; 2020).

In severe covid-19 patients, the inflammatory biomarker CRP was significantly elevated, which mean overproduction of inflammatory cytokines is one potential reason for C-reactive protein rises in COVID-19 (Victor *et al*.; 2020).

1.12.2. D. Dimer

D-dimer is a product of the blood clotting and break-down process that can be measured via analysis of a blood sample. D-dimer is released when a blood clot begins to break down (Ryu *et al*.; 2019).

Early identification of patients at risk for disease progression is a major concern among clinicians, for developing management strategies in order to prevent mortality outcomes (Seshadri *et al*.; 2021). Therefore, identification of better predictors of prognosis is of great clinical significance, and the need for prioritizing coagulation markers for prognostic abilities has been highlighted (Liao *et al*.; 2020, Zhou *et al*.; 2020).

Several researchers by Li *et al*.; 2020 and Coppelli *et al*.; 2020 have paid much attention to D-dimer, reporting its significant raise in severe cases and non-survivors, as compared to non-severe patients and survivors. It has proposed that, as a marker of coagulation, increased D-dimer reflect hypercoagulability and thrombotic burden, guiding clinicians for using anticoagulation in COVID-19 patients (Cummings *et al*.; 2020, Naymagon *et al*.; 2020).

Several studies by Di Micco *et al*.; 2020 and Long H. *et al*.; 2020 have been reported an increased D-dimer in positive relationship to disease severity, composite outcomes and high mortality events in COVID-19.

In accordance with previous evidence by Gao *et al*.; 2020 and Yu *et al*.; 2020, the results on the association of higher D-dimer with disease progression in COVID-19 support that severe patients are at higher risk of hypercoagulability. Also, a large body of evidence by Guan *et al*.;

2020 and Moreno-Pérez *et al*.; 2020 shows that, the non-surviving COVID-19 exhibit significantly higher D-dimer levels, reflective of hypercoagulability status. Several mechanisms explain higher D-dimer and hypercoagulability in COVID-19 (Tang *et al*.; 2020).

A body of evidence suggests a correlation between markers of inflammation and coagulopathy (Al-Samkari *et al*.; 2020), cytokine storm lead to thrombus formation through platelet activation (Hanif *et al*.; 2020, Connors *et al*.; 2020).

D-dimer was noted in 9%–43% of the patients investigated (Huang *et al*.; 2020). The level of D-dimer at admission was found to be associated with the risk of developing ARDS the requirement for intensive care and the risk of mortality (Huang *et al*.; 2020).

The high D-dimer level and the D-dimer levels significantly increased over time and associated with high mortality, and may be marker of infection/sepsis, cytokine storm and impending organ failure (Wu *et al*.; 2020).

In particular, D-dimer levels in intensive care patients were noted to be significantly higher than those in patients who were not admitted to intensive care units (Huang *et al*.; 2020). Prothrombin time was significantly increased in survivors and patients who died. Disseminated intravascular coagulation in patients with COVID-19 may develop over time due to poor prognosis (Tan *et al*.; 2020).

1.12.3. Ferritin

Ferritin is a major protein concerned with iron storage, its level in the blood serves as an indicator of the amount of iron stored in the body, and it can become elevated due to the presence of conditions featuring significant inflammation (Charles *et al*.; 2021).

Ferritin becomes elevated as an outcome of the activation of macrophages and hepatocytes in COVID-19 cases (Recep *et al*.; 2020). Unlike other viral infections, ferritin is elevated at a moderate level in cytokine storm syndrome (Recep *et al*.; 2020). It is thought to be used as a predictive marker of sepsis mortality (Lippi *et al*.; 2020, Santoso *et al*.; 2020). Evidence by Zhou *et al*.; 2020 was suggests that the amount of serum ferritin rises during the COVID-19 pandemic, indicating a higher risk of mortality.

Serum ferritin is used as a diagnostic measure for iron deficiency anemia since it is an indirect marker of total amount of iron stored in the body (Wang *et al*.; 2010). During viral infection, the amount of circulated ferritin rises, which can be used as marker of viral replication (Li *et al*.; 2020).

1.12.4. Procalcitonin

Procalcitonin is a glycoprotein calcitonin pro-hormone released by the thyroid Para-follicular cells. In case of a microbial infection, PCT levels are significantly raised as it is released by all parenchymal tissue under the influence of endotoxins and pro-inflammatory cytokines (Sibtain *et al*.; 2021).

Procalcitonin highly regarded and utilized as a biomarker of bacterial infection, contrasting opinion exists on the efficacy of PCT as a prognostic tool for COVID-19 (Bhandari *et al*.; 2020, Cao *et al*.; 2020). Moreover, cytokines released in COVID-19, particularly interferon

(INF)-γ, have a negative effect on PCT levels, adding to the strength of this prognostic tool (Cleland *et al*.; 2020).

Early studies by Ke *et al*.; 2020 in the wake of the pandemic have shown higher levels of PCT in severe COVID-19 cases. Lippi *et al*. 2020 have reported that the PCT levels are expected to quintuple in severe cases. Various other authors have also supported the view that any considerable increase from baseline PCT levels reflects the onset of a critical phase of the viral infection (Li *et al*.; 2020).

The level of Procalcitonin in COVID-19 usually remains within the normal range at admission (< 0.5 in 95% of the patients) (Guan *et al*.; 2020); however, it is likely that this level could be higher in patients who need admission to ICU (3%–35) (Lippi *et al*.; 2020). Few studies by Recep *et al*.; 2020 have found increased Procalcitonin levels.

This should primarily suggest an alternative diagnosis (bacterial pneumonia); however, any progressive PCT increase in patients who are hospitalized/admitted to ICU may be associated with this poor prognosis or a secondary bacterial infection (Zhou *et al*.; 2020, Wu *et al*.; 2020). Patients with elevated PCT were noted to have a 5-fold more severe COVID-19 infection risk (OR, 4.76; 95% CI، 2.74–8.29) (Lippi *et al*.; 2020).

1.12.5. Lactate dehydrogenase

Lactate dehydrogenase (LDH) is one of markers of importance, mainly because higher LDH levels have been related to worse outcomes in patients with other viral infections in the past (Tao *et al*.; 2018).

Studies by Lippi *et al*.; 2020 have shown high LDH levels, ranging from 27% to 76%, in patients. High LDH levels in patients with severe

COVID-19 are considered to be a potential marker that indicates lung injury and tissue damage (Recep *et al*.; 2020).

In one of the studies conducted by Recep *et al*.; 2020, it was observed that advanced age, hypertension and high LDH levels at admission were associated with the elevation in hospital mortality rates. LDH was higher in severe cases than in mild and moderate cases (Chen *et al*.; 2020).

Since LDH is existing in lung tissue, with severe Covid-19 patients may expect to discharge more LDH into bloodstream, for instance the disease is characterized by severe type of interstitial pneumonia that frequently progresses to acute respiratory distress syndrome (Henry *et al*.; 2020).

1.12.6.Antibodies (IgM and IgG)

Immunoassays are techniques for detecting and quantifying antigen/antibody interactions (Elham *et al*.; 2020). They may provide useful information on the dynamics of virus infections and previous exposures (Lee *et al*.; 2020). Antibodies, on the other side, are resistant to degradation more than viral RNA and fewer affected via transport, storing and selection (Younes *et al*.; 2020).

Antibodies, also known as immunoglobulins, have been formed through the immune system to protect the host from foreign invaders such as bacteria and viruses (Elham *et al*.; 2020).

Microbial infections typically cause IgM to be produced as the first line of protection, and later, as long-term immunity and immunological memory, IgG is developed (Elham *et al*.; 2020).

IgM and IgG have been detected in the blood of patient after (3-6) days and eight days, during SARS infection (Li *et al*.; 2020). As a result, the presence of both antibodies can aid in determining the date of infection.

IgM and IgG antibodies to SARS-CoV-2 can be detected 3–4 days after premorbid (Lee *et al*.; 2020). Antibody detection in acute- and convalescent-phase serum samples by indirect immunofluorescence assay and enzyme-linked Immunosorbent assay (ELISA) with cell culture extract are currently the most commonly methods used for serodiagnosis of SARS-CoV-2 infection in clinical microbiology laboratories (Peiris *et al*.; 2003).

While IgM antibodies can be produced as quickly as viral genetic material in the respiratory tract, the timing of immunoglobulin development (from 4 days to 10–14 days after onset of symptoms) restricts its use in acute phase diagnosis (Padoan *et al*.; 2020, Xiang *et al*.; 2020).

Specific SARS-CoV-2 antibodies are detection by serological tests in blood of patient are presently necessary for: (a) Follow up with patients; (b) enable serological surveillance on a local, state, and national scale; and (c) Recognize people who have already been exposed to the virus (Jennifer, 2020).

1.12.7.Interleukin- 6

IL-6 is known as a pleiotropic cytokine produced and secreted by a wide range of immune and non-immune cells such as DCs, mast cells, monocytes, macrophages, keratinocytes, meningeal cells, fibroblasts, vascular endothelial cells (ECs), as well as T and B lymphocytes following infections or tissue damage (Mitra *et al*.; 2020). IL-6 can be involved in modulating many host immune responses (Mitra *et al*.; 2020). Studies have also shown that IL-6, along with TNF-α and IL-1β, can affect many components and immune responses in viral infections, particularly the role of IL-6 in patients with COVID19 (Liu T. *et al*.; 2020).

On the other hand, increased expression of IL-6 in some viral infections, such as COVID-19, causes multiple damages to the lung tissue and leads to infection progression. Studies on patients infected with CoV (that is, SARS-CoV, MERS-CoV, and SARS-CoV-2) have shown that lymphopenia and cytokine storms are two significant immunopathology findings in these patients (Yang X. *et al*.; 2020, Gupta *et al*.; 2020).

The current study by Saeid *et al*.; 2021 suggests that high levels of various cytokines are correlated with the disease severity and immunopathogenesis of COVID-19 and these findings may contribute in a better understanding of the immune dysfunction in this infection. In addition, serum levels of cytokines and chemokines have been identified as prominent biomarkers for early identification of severe patients and predict the clinical progression of COVID-19 patients (Saeid *et al*.; 2021).

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), also known as the 2019 novel Coronavirus (2019-nCoV), has caused a recent outbreak of Coronavirus Disease (COVID-19) (Chen *et al*.; 2020, Wang *et al*.; 2020). Although most cases were mild to moderate, some patients developed severe symptoms characterized by respiratory dysfunction and/or multiple organ failure (Yang *et al*.; 2020).

In the current situation, identification of COVID-19 disease progression mainly relies on the clinical manifestation, while no effective biomarker has been proposed (Zulvikar *et al*.; 2020). It has been suggested that one of the possible mechanisms underlying rapid disease progression is a cytokine storm (Chen *et al*.; 2020, Wang *et al*.; 2020). Previous retrospective studies by Chen *et al*.; 2020 indicated that an elevated level of interleukin-6 (IL-6) was associated with a high case fatality of COVID-19 infection.

Cytokines are vital in regulating immunological and inflammatory responses (Zulvikar *et al*.; 2020). Among them, IL-6 is of major importance because of its pleiotropic effects (Chen *et al*.; 2020). The evidence presented by Zulvikar *et al*.; 2020 that circulating IL-6 levels are closely linked to the severity of COVID-19 infection. An increase in IL-6 levels has previously been observed in patients with respiratory dysfunction (Wang *et al*.; 2020), implying a possible shared mechanism of cytokine-mediated lung damage caused by COVID-9 infection.

Furthermore, it seems that the highly pathogenic SARS-CoV-2 is associated with rapid virus replication and a tendency to infect the lower respiratory tract, resulting in an elevated response of IL-6-induced severe respiratory distress (Zulvikar *et al*.; 2020). Thus, the results suggest that serial measurement of circulating IL6 levels may be important in identifying disease progression among COVID-19-infected patients (Zulvikar *et al*.; 2020).

It is reasonable that immediate initial evaluation of IL-6 level be performed upon hospital admission of COVID-19 patients, due to its potential benefits to assess worsening clinical features and disease progression in COVID-19 (Zulvikar *et al*.; 2020).

Several studies have correspondingly demonstrated a significant increase in IL-6 serum levels in patients suffering from COVID-19 (Chen *et al*.; 2020, Gong *et al*.; 2020, Zhou *et al*.; 2020). This cytokine as an inflammatory mediator also plays an essential role in innate and adaptive immune responses; though, it can also have protective and anti-inflammatory properties in some pathologic states (Mitra *et al*.; 2020). Furthermore, regarding the findings of recent studies, IL-6 and its receptors could be high potential diagnostic and therapeutic targets in COVID-19 patients (Chiappelli *et al*.; 2020).

1.12.8. Interleukin-4

Interleukin-4 (*IL-4*) is member of the T helper 2 (Th2) family of cytokines and this group also includes *IL-3*, *IL-5*, and *IL-9*, while the gene that encoding *IL-4* in humans is located on chromosome 5q31 within a cluster of Th2-related cytokine genes including *IL-3*, *IL-5*, and *IL-9* (Milena *et al*.; 2021). *IL-4* plays a critical role in allergic inflammation and parasite infection, *IL-4* has the capacity for switching immunoglobulin (Ig) class of IgE and IgG4, stimulate B cell proliferation, activation of eosinophil, basophils and mast cells, additionally *IL-4* involved in collagen production by fibroblasts, and they induce vascular cell adhesion molecule (VCAM)-1 expression on endothelial cells, furthermore *IL-4* acts a key role in promotion of Th2 differentiation (Milena *et al*.; 2021).

Inflammatory factors are often increased in severe and critical covid-19 patients, including interleukin (IL), colony-stimulating factor (CSF), chemokine, interferon (IFN), tumor necrosis factor (TNF), chemokine and growth factor (GF) (Qingqing *et al*.; 2021). Most of cytokines are produced by T lymphocytes, fibroblasts and mononuclear macrophages, and can in turn act on these cells, and these cytokines could promote each other and jointly mediate inflammation (Qingqing *et al*.; 2021).

The early symptoms of coronavirus disease 19 (COVID-19) patients were fever, dry cough and fatigue (Chan *et al*.; 2020), but severe patients may develop even multiple organ failure, which may be related to the levels of cytokines (Qingqing *et al*.; 2021). The production of cytokines is related to the individual immune function, so Qingqing *et al*.; 2021 assumed that the severity of COVID-19 can be predicted according to their levels.

Qingqing *et al*.; 2021 measured the serum levels of these cytokines in COVID-19 patients and non-COVID-19 patients by ELISA, and the results showed that the serum level of IL-4 in COVID-19 patients were significantly higher than those in non-COVID-19 patients, which indicated that there was a cytokine storm in patients with COVID-19.

1.13.The Effect of Age and Gender on Covid-19 Infections and Mortality

Covid-19 has spread over 300 countries since the first outbreak was detected during Dec 2019 (in China, Wuhan) (Ali *et al*.; 2020). There hasn't been a biomarker for the disease yet. The majority of the risk factors identified were based on general clinical findings, such as age, gender, and chronic diseases (Yang *et al*.; 2020).

Males have been reported more often than females among COVID-19 patients, particularly in deceased cases, also male cases outnumbered female cases in previous research by Ali *et al*.; 2020, males appeared to have extra severe disease or be in serious state of disease. Furthermore, men have a 2.4 times greater rate of dying than women (Pan *et al*.; 2020, Yang *et al*.; 2020).

There is no known reason for males having a higher COVID-19 prevalence than females. Nonetheless, it has been recently discovered that this sex disparity could be due to a number of complications which raise the infection risk or death in men indirectly (Ali *et al*.; 2020). Men are effected by cardiovascular risk factors (heart attack, hypertension, and diabetes, for example) and high-risk habits (social isolation, cigarette consumption, alcoholic use, and certain environmental exposure) (Sharma *et al*.; 2020). Female sex hormones can also play a role in immune response control (Ali *et al*.; 2020).

Another risk factor for COVID19 evolution was age, and the fifth decade could be a critical age (Ali *et al*.; 2020). Furthermore, the findings showed that age can be considered a risk factor for death (Ali *et al*.; 2020).

The majority of Chinese data in this sense agrees that the infection was mainly seen in older people (Ali *et al*.; 2020). 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).

A further Chinese research was applied on 52 Covid-19 patients who were chronically ill. They were on average 59.7 years old, and 61.5% died within 28 days (Yang *et al*.; 2020).

Chapter Two

Materials and Methods

2.Materials and methods

2.1.Patients

The patients were checked by RT-PCR, though nasopharyngeal swabs from a people that probably infected with SARS-CoV-2 (Covid -19) and 50 blood samples from a people in which that have positive result of RT-PCR (infected with SARS-CoV-2 (Covid -19) were kindly provided from the medical staff of Marjan Teaching Hospital from October/2021 to January/2022. Forty blood samples from a people that non-infected with SARS-CoV-2 were also provided and considered as control group which were confirmed that they were not infected with Covid-19 by RT-PCR**.**

2.2.Materials

2.2.1. Equipment

The equipment that used in the present study shown in table (2-1).

Table (2-1): The equipment which were used in the study

NO.	Laboratory Equipment	The industrial company
1	Collection tube	Biocomma limited (Spain)
2	Disposable syringe (5ml)	EASYMED
3	EDTA tube	HIGHTOP
4	Eppendrof tube	AFCO
5	Filter paper	Supertek
6	Gilson blue tips	AFCO
7	Jell tube	HIGHTOP
8	Micropipette from100-1000 micro letter Micropipette from 0.1-3 micro letter Micropipette from 10-100 micro letter Micropipette from 0.5-10 micro letter	Eppendrof Research Plus (Germany) BIOHIT NICHIRYO (Japan)
9	PCR tube	Bionear
10	PCR tube rack	Watson Bio Lab
11	Spin column	Biocomma limited (spin)
12	Swab	Biocomma limited (spin)
13	White Pipette tips	ExpellPLUS ™
14	Yellow Pipette tips	AFCO

2.2.2.Devices

The devices that used in the present study shown in table (2-2).

Table (2-2): The devices which were used in the study

NO.	Laboratory Devises	The industrial company
1	AFIAS-6 (Automated fluorescent immunoassay system)	Boditech Med Inc.
2	Autoclave	Hirayama
3	Eppendrof Centrifuge 5418	Eppendrof
4	Gel Electrophoresis devise	Advance
5	Gel Documentation	ATTA-E Graph (Japan)
6	Incubator	Memmert
7	Microplate reader	Molecular Devices, LLC
8	Microwave Oven	Shownic
9	Thermo cycler	Techne
10	Refrigerator	Vestel
11	Real-time cycler	Bioneer
12	Sensitive balance	Kern (Germany)
13	Vortex mixer	Bioneer
14	Water bath	Memmert

2.2.3.Chemical and Biological materials

The Chemical and Biological materials that used in the present study shown in table (2-3).

Table (2-3): Chemical and Biological materials .

NO.	Materials	The industrial company
1	**Agarose**	INTRON BIOTECHNOLOGY
2	**Ladder 100-1000bp**	Promega - USA
3	**Ladder 50bp**	Promega - USA
4	**Loading dye**	INtRON Biotechnology
5	**Nuclease-Free Water**	Promega - USA
6	**PCR Master Mix**	Promega - USA
7	**Primers (lyophilized)**	Bioner-Korea
8	**Red safe stain**	INtRON BIOTECHNOLOGY
9	**Restriction enzymes**	New England Biolabs
10	**Tris Borate EDTA Buffer 10X (TBE)**	Promega - USA
11	**Viral transport media**	Vincen

2.2.4. Kits and their contents

The kits that used in the present study shown in table (2-4).

Table (2-4): Kits and contents which were used in the study.

NO.	Kits	The industrial company	Contents
1	**Virus DNA/RNA purification kit (Spin column)**	Biocomma	▪ Buffer GLX ▪ Buffer PD ▪ Buffer PW ▪ RNase-free ddH_2O ▪ Spin column RC2 ▪ 2.0 Collection tube ▪ Proteinase K (20 mg/ml)
2	**Real Line SARS-CoV-2 PCR Detection Kit**	BIORON diagnostics	▪ Paraffin-sealed PCR-Mix ▪ RT-PCR-buffer ▪ Mineral Oil ▪ Positive Control ▪ Internal Control (RNA-IC) ▪ Strips caps ▪ Enzyme Taq/RT
3	**Blood/Cultured cells genomic DNA extraction Mini Kit.**	FAVORGEN BIOTECH CORP.	▪ RBC lysis Buffer. ▪ FATG Buffer. ▪ FABG Buffer. ▪ W1 Buffer. ▪ Wash Buffer (Concentration). ▪ Elution Buffer. ▪ Proteinase K. ▪ FABG Mini Column. ▪ Collection tube. ▪ User manual.

2.2.5.Molecular materials

2.2.5.1.Primers

The primers that used in the present study shown in table (2-5).

Table (2-5): List of Primers that used in the study

Gene	Sequences	bp	Ref.
***IL-4* gene**	F-ACTAGGCCTCACCTGATA	252bp	Peng *et al*., 2013
	R-GTTGTAATGCAGTCCTCC		
***ACE* gene**	F-CTGGAGACCACTCCCATCCTTTCT	490bp	Sevim *et al*., 2021
	R-GATGTGGCCATCACATTCGTCAGAT	190bp	

2.2.5.2.Restriction enzyme

The reaction mixture for BsmF1 (Bacillus stearothermophilus F 1) restriction enzyme that used in the present study was shown in table (2-6).

Table (2-6): The reaction mixture for BsmF1 restriction enzyme that used in the study.

Materials	Volume (µl)
PCR product	10
Enzyme	1
10x NEB Buffer	5
DH_2O	34
Total	50

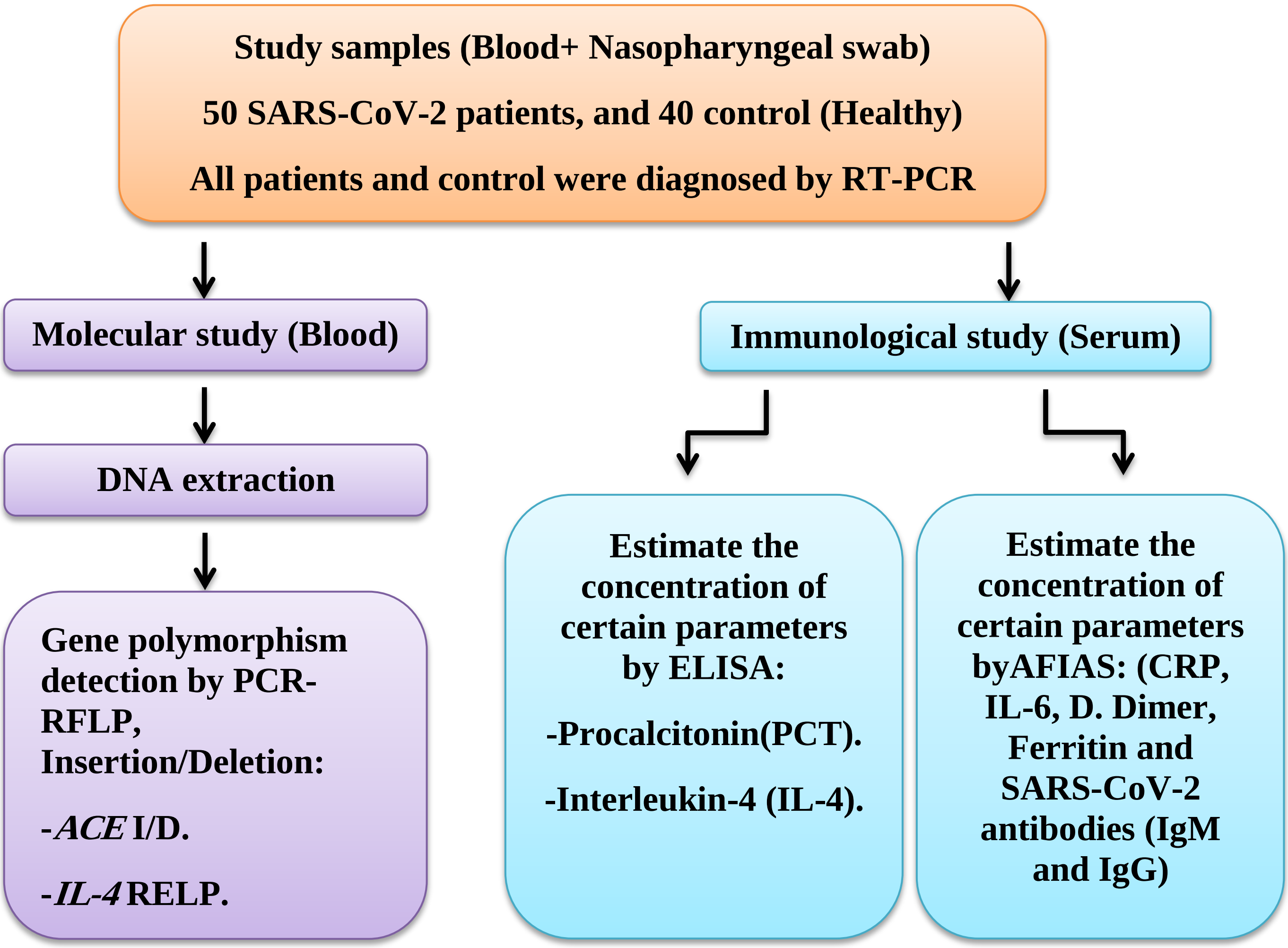
The incubation time and temperature for restriction enzyme that used in the present study was shown in table (2-7).

Table (2-7): The incubation time and temperature for restriction enzyme (BsmF1).

Restriction enzymes	Incubation time	Incubation temp.	The industrial company
BsmF1 5' G G G A C N_{10} ↓ 3' 3' C C C T G N_{14} ↑ 5'	**4 hours**	**37℃**	**Bio Labs**

2.3.Methods

2.3.1.Design of the study



2.3.2.Blood DNA extraction Mini Kit (Favorgen Biotech Corp).

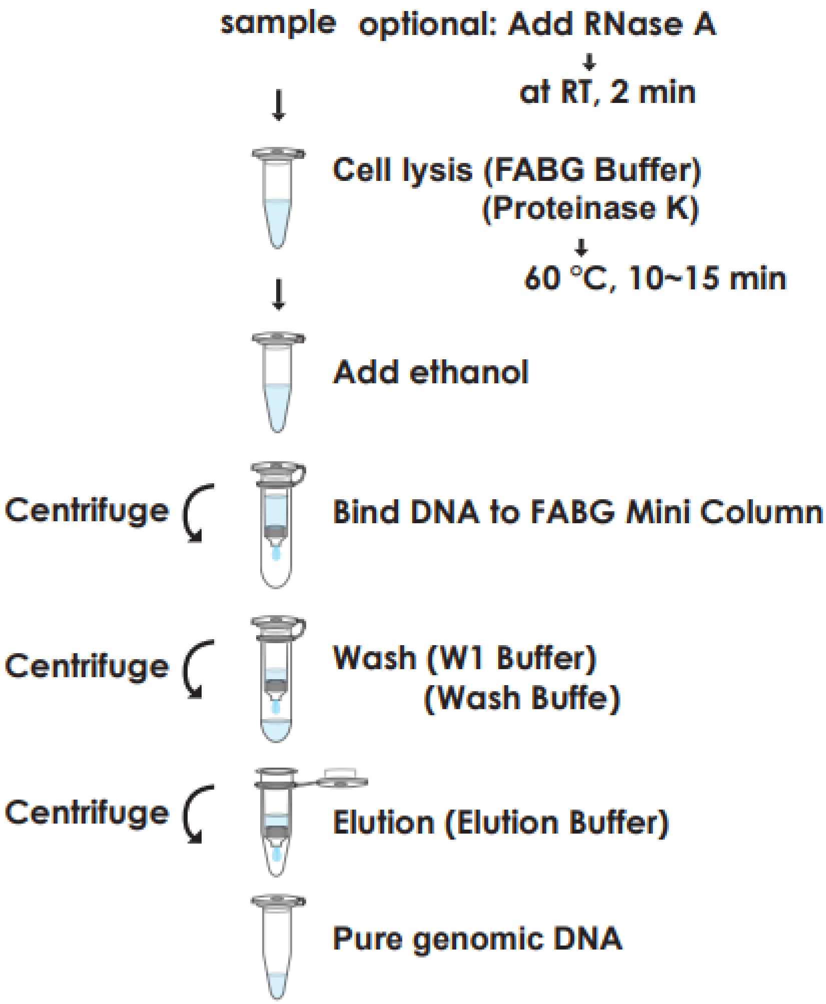
This kit is used for extraction of genomic DNA from fresh blood, frozen blood, cultured cells and fungus.

2.3.2.1.Procedure

1. **Sample preparation:** Two hundred microliter of blood was transferred up to a 1.5ml microcentrifuge tube, 30 µl of proteinase K was added to the sample and was mixed briefly and then was incubated for 15 min at 60 °C.
2. **Cell lysis:** Two hundred microliter of FABG buffer was added to the sample and was mixed by vortex and then was incubated in a 70 °C water bath for 15 min to lyse the sample and during incubation, the sample was inverted every 3 min.
3. **DNA binding:** Two hundred microliter of ethanol (96-100%) was added to the sample and was vortexed for 10 sec, the sample was pipetted to mixed well if there is any precipitate formed. FABG column was placed to a collection tube, the sample mixture was transferred carefully to FABG column and was centrifuged at speed 14000 rpm for 1 min, and the collection tube was discarded and the FABG column was placed to a new collection tube.
4. **Column washing:** Four hundred microliter of W1 buffer was added to the FABG column and was centrifuged for 30 sec at speed 14000 rpm and then the flow-through was discarded and the FABG was placed back to the collection tube, then 600 µl of wash buffer was added to the FABG column and was centrifuged for 30 sec at speed 14000 rpm and then the flow-through was discarded and the FABG was placed back to the collection tube and then was

centrifuged for an additional 3 min at speed 14000 rpm to dry the column.

5. **Elution:** The dry FABG column was placed to a new 1.5 ml microcentrifuge tube, then 100 µl of preheated Elution buffer was added to the membrane center of FABG column, and then the FABG column was incubated at 37 °C for 10 min in an incubator, then was centrifuged for 1 min at full speed 14000 rpm to elute the DNA and then the DNA fragment was stored at 4 °C or -20 °C. The brief procedure shown in figure (2-1).



Figure(2-1): Brief procedure for DNA extraction.

2.3.3.Virus DNA/RNA Purification Kit (spin column)

2.3.3.1.Preparation

Before first use, the specified volume of reagent was added to Buffer PD, Buffer PW. Tip and collection tubes which were free of nucleic acid and nuclease were prepared. The solution precipitated were observe before use, if there was precipitation in solution, was dissolved in 37 °C bath and cool to RT before use. (biocomma® Nucleic Acid Purification Kit, CMC Medical Devices & Drugs S.L. C/ Horacio, 18. 29006. Màlaga. Spain)

2.3.3.2.Protocol

1. Sample preparation : The nasopharyngeal swab was taken, it was putted directly in sterile viral transport medium for preservation for later use .
2. Lysis : 100-300 µl of processed sample was taken and 500 µl of Buffer GLX and 20 µl of Proteinase K were added and were mixed by vortex for 1 min and was placed at room temperature for 5 min to complete lysis.
3. Adsorption : A supernatant has been transferred into spin column RC2 which was put inside 2 mL collection tube, The flow-through has been removed, then a spin column was retained into 2 ml collection tube after centrifugation for 1 minute at 12,000 rpm.
4. Wash : **A.** At room temperature, 500 µl of Buffer PD was applied to the spin column and centrifuged for 1 minute at 12,000 rpm, and was discarded the flow through, the spin column RC2 was put back into the collection tube.

   B. At room temperature, 700 µl of Buffer PW was applied to the spin column and was centrifuged for 1 min at 12,000 rpm, and was discarded the flow through, the spin column RC2 was put back into the collection tube and was repeated this step.

C. The flow through was discarded after centrifuged for 2 min at 12,000 rpm and the spin column RC2 was put at room temperature for 5-10 mins.

5. Elution : The spin column was placed into a new 1.5 ml collection tube, 50-100 µl of RNase-free dH_2O was added and placed the column at room temperature for 2 mins then centrifuged at 12,000 rpm for 2 mins at room temperature to elute and collect the RNA.

2.3.4.Sterilization Methods

The 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 $inch^2$.

2.3.5.Tris Borate EDTA Buffer (TBE Buffer) preparation

Tris Borate EDTA Buffer was prepared by diluting 100 mL Tris Borate EDTA (10x) in 900 mL distilled water to make Tris Borate EDTA (1x), which was then used to prepare Agarose for the Gel electrophoresis.

2.3.6.Agarose preparation

For preparation of Agarose gel followed the steps :

1. One hundred ml of 1X TBE buffer was taken in flask.
2. Agarose powder (1.5, 2, 2.5 and 3) was add to the 1x TBE buffer to prepare agarose gel in concentrations 1.5%, 2%, 2.5% and 3% respectively.
3. The solution was heated to boiling using microwave oven until all agarose particles were dissolved.
4. The solution was left to cool down.

5. About 5 µl of red save was added to the agarose solution.

2.3.7.Real Line SARS-CoV-2

SARS-CoV-2 RNA detection by using qualitative assay kit by Real Time PCR with gene distinction procedure by using BIORON diagnostic material .

2.3.7.1.Intended use

The Real Line SARS-CoV-2 Detection Kit is a pathogen-detection-based in vitro Nucleic Acid Test (NAT). The Real Line SARS-CoV-2 Detection Kit used the RT-PCR approach to detect different genes of SARS-CoV-2 (COVID-19 virus, 2019-nCoV) and SARS-like coronaviruses in human biological samples.

2.3.7.2.Samples

RNA extraction from the nasopharyngeal swab , were detected using the Real Line SARS-CoV-2 Kit, which was based on a professional prescription.

2.3.7.3.Principles of the procedure

A method of analysis was dependent on reverse transcription to selected RNA fragment, followed by cDNA amplification and identification of PCR materials in Real Time. Each specimen was analyzed in a separate tube containing the RT-PCR Master Mix. The reaction was based on a multiplex analysis with a simultaneous detection of multiple targets in one tube. The Internal Control (IC) RNA was intended to check the quality of extracted RNA used for subsequent reverse transcription in real-time PCR analysis. The Positive Control plasmid (PC) DNA supplied with the kit was intended for the evaluation of the results of the test , as well as specific gene variables .

2.3.7.4.PCR Amplification with Reverse Transcription (RT-PCR)

1. The required number of tubes was marked with paraffin sealed RT-PCR-mix according to the number of samples to be analyzed, 1 tube for Positive Control PC and 1 tube for Negative Control NC.
2. The RT-PCR-buffer and Enzyme Taq/RT was vortexed thoroughly for 3-5 sec, then was spinet briefly for 1-3 sec.
3. The mixture of **RT-PCR-buffer** and **Enzyme Taq/RT** was prepared. To one mixture tube was added:
   15 x (N+1) µl of **RT-PCR-buffer**,
   0.5x (N+1) µl of **Enzyme Taq/RT**,
   Where: N – is the amount of the samples, including PC and NC. The tube was vortexed and then was spinet briefly for 3-5 sec
4. Fifteen microliters of Enzyme Taq/RT was added and RT-PCR-buffer was mixed into the each PCR tube and was avoided paraffin layer break.
5. One drop (~20 µl) of mineral oil was added into each tube, then were closed.
6. The tubes with samples were vortexed and PC and NC for 3-5 sec and was spinet down the drops by centrifuging for 1-3 sec.
7. Ten microliters of RNA sample was added into corresponding RT-PCR tube. The tubes were closed tightly. 10 µl of NC was added which passed the whole NA extraction procedures into corresponding tube. 10 µl of PC was added into PC tube. The tubes were closed tightly and was avoided paraffin layer break.

8. Tubes were vortexed for 3-5 sec and were spinet the tubes briefly for 3-5 sec. The tubes were closed tightly and was avoided paraffin layer break.
9. The tubes were placed into the Thermal Cycler. The Real Line Cycler was programed as shown in table (2-8).

Table (2-8): The condition of Bio Rad Cyclers

Step 1:	32 °C	20 min	1 cycle
Step 2:	95 °C	5 min	1 cycle
Step 3:	94 °C	10 sec	50 cycles*
	60 °C*	15 sec *	
*** measurement of fluorescence**			

10. Channels was chosen: **FAM / Green**
HEX / Yellow
ROX / Orange
Cy5 / Red
11. The places of all of test tubes were programmed with samples, positive and negative controls which according to real-time PCR system's instruction manual, then the program was run.

2.3.7.5. Data analysis

The analysis was carried out automatically when Real-Line Cyclers were used. The analysis was based on the presence or lack of a single in all other situations. The Real-time PCR Thermal Cyclers automatically detected and analyzed data. Analysis will be performed by Real-Time PCR application. The analysis of PCR results have been show in the table (2-9).

Table (2-9): RT-PCR results interpretation

Detection Channel				Interpretation
FAM/Green	HEX/Yellow	ROX/Orange	Cy5/Red	
S -gene SARS-CoV-2	IC	SARS-CoV-2 N -gene	SARS-CoV-2 E -gene	
Analyzed samples				
+	Not considered	+	+	**RNA of SARS-CoV-2 was detected✯**
+	Not considered	-	-	**RNA of SARS-like Coronaviruses was detected**, RNA of SARS-CoV-2 was not detected
-	+	-	-	RNA of SARS-like Coronaviruses was detected, RNA of SARS-CoV-2 was not detected
Positive Control sample				
+	Not considered	+	+	**Positive result**
Negative Control sample				
-	+	-	-	**Negative result**
✯ the simultaneous presence of SARS-CoV-2 coronavirus and other coronaviruses like SARS-CoV in the RNA sample was possible.				

2.3.8.Conventional Polymerase Chain Reaction (PCR)

2.3.8.1.Primers Preparation

All primers used in this study were prepared for amplification studied genes, and that by dissolved the primers (Forward and Reverse) in 300 µl of Nuclease Free Water according to the supplied company instructions (**Humanizing Genomics Macrogen**) to obtained working solution. The tubes were shaken, then the final solution was prepared by diluted 10 µl of primers (Forward and Reverse) in 90 µl of Nuclease Free Water (10 pmol/µl).

2.3.8.2.Polymerase Chain Reaction Mixture

The mixture of polymerase chain reaction was prepared to amplify the IL-4 and ACE genes by mixing components master mix with the forward and reverse primers, the DNA template and Nuclease free water as shown in Table (2-10).

Table (2-10): Polymerase Chain Reaction Mixture

NO.	Materials	Volume	Concentration
1	**DNA Template**	6 µl	**50 ng**
2	**Master Mix**	12.5 µl	1X
3	**Forward Primer**	1 µl	10 pmol/ml
4	**Reverse Primer**	1 µl	10 pmol/ml
5	**Nuclease-Free water**	4.5 µl	
Total	Final Volume 25 µl		

2.3.8.3.Amplification Conditions

Polymerase Chain Reaction or thermocycler was used to amplified the IL-4 and ACE genes, the amplification conditions each gene have been controlled as shown in a table (2-11).

Table (2-11): Amplification Conditions for Polymerase Chain Reaction

Gene	Initial denaturation °C(min)	One cycle conditions			Cycles number	Final Extension °C(min)
		Denaturation °C(min)	Annealing °C(min)	Extension °C(min)		
***IL-4* gene**	**94 (5min)**	**94 (1min)**	**57.8(1min)**	**72 (30 sec)**	**35**	**72 (5min)**
***ACE* gene**	**94 (5min)**	**94 (20 sec)**	**56.1 (1min)**	**72 (20 sec)**	**37**	**72 (5min)**

2.3.8.4.Loading of DNA and Electrophoresis

The gene amplification products from the polymerase chain reaction (PCR) were loaded using the Agarose gel. The agarose was then fully dissolved and cooled at room temperature. The agarose has poured in casting tray that combs were fixed on it and then was lifted to harden at room temperature.

The gene amplification products (Amplicons) and loading dye were loaded into the pits that resulted after lifting the combs. The DNA ladder (5μl) was putted in a first and last pits in first line of pits and only in a first pit in second line for the purpose of comparing the molecular size of the resulting bands then relay tank was filled with a buffer (1X TBE) and the electrophoresis of IL-4 and ACE genes amplification products were performed with a voltage of 100 V for a duration of 45 minutes. Later the gel visualized using UV trans-illuminator and documented by digital camera(Mukh, 2021).

2.3.9.Automated Fluorescent Immunoassay System COVID-19 Ab (AFIAS)

2.3.9.1.Principle

This test employs a sandwich immunodetection process, in which fluorescence-labeled conjugates in dried detection buffer (DB) bind to antibody in the sample, forming antibody-antigen complexes, and migrate onto the nitrocellulose matrix, where they are caught by immobilized anti-human IgG and anti-human IgM on the test strip. The further antibodies in a sample, the more antigen-antibody complexes form, resulting in a greater fluorescence signal on the detector antigen, which was processed to reveal anti-CoV IgG and IgM concentrations in the sample (Trivedi *et al*.; 2019).

2.3.9.2.Contents

Cartridge Box contains (Cartridge, Pipette tip (zipper bag), C- tip (zipper bag), Spare cartridge zipper bag, ID chip and Instruction for use).

2.3.9.3.Test Procedure

1. General mode was selected in the instrument for AFIAS tests.
2. One hundred microliters of sample was taken (serum) with a pipette and was dispensed it into the sample well on the cartridge.
3. The cartridge was insert into the cartridge holder.
4. A tip was inserted into the tip hole of cartridge.
5. The 'START' icon on the screen was taped.
6. Test results were displayed on the screen after l0 minutes.

2.3.9.4.Interpretation of test result

- The AFIAS measurement instrument measures the test outcome immediately and showed the results as Positive, Negative, or Indeterminate.
- Ancillary value was served in the form of a cut-off index (COI) as shown in tables (2-12).

Table (2-12): Cut-off index (COI) for IgG and IgM

Cut-off index (COI)	Result	Note
< 0.9	Negative for IgG and IgM	No need to retest
$0.9 \leq$ Titer < 1.1	In determinate	Need to retest
≥ 1.1	Positive for IgG and IgM	Need to confirmation test

2.3.10.Automated Fluorescent Immunoassay System C-Reactive protein (AFIAS CRP)

2.3.10.1.Principle

The test employs a sandwich immunodetection protocol, in which the indicator antibody with in buffer attaches with antigen in the sample, producing antigen-antibody complexes that migrate towards the nitrocellulose matrix and are caught by another immobilized-antibody upon on test strip. A further antigen inside a sample, so more antigen-antibody complexes form, resulting in a stronger fluorescence signal upon on sensor antibody, whereby the instrument processes for AFIAS analysis to check CRP concentration in the sample (Pepys and Hirschfield, 2003).

2.3.10.2.Contents

AFIAS CRP consists of (Cartridge, Pipette tip, ID chip, Instruction for use). Every cartridge packaged in an aluminum pouch own two components, a detector and cartridge part. Cartridge part contains a test strip, a membrane that has anti-human CRP on the test line, while rabbit IgG on the control line. Detector part contains anti-human CRP-fluorescence conjugate, anti-rabbit IgG fluorescence conjugate, bovine serum albumin (BSA) as stabilizer and sodium azide in phosphate buffer saline 1(PBS) as preservative.

2.3.10.3.Test procedure

The test procedure was the same of AFIAS Covid-19 Ab.

2.3.10.4.Interpretation of Test Result

- A tool for AFIAS examination calculate test result automatically also displays CRP concentration of the test sample in terms of mg/L.
- The cut-off : 10 mg/L.
- The working range of the AFIAS CRP was 0.5-200 mg/L.

2.3.11.Automated Fluorescent Immunoassay System D. Dimer (AFIAS D. Dimer)

2.3.11.1.Principle

The principle of AFIAS D. Dimer was the same of AFIAS Covid-19 Ab. (Performance of two relatively new quantitative D-dimer assays (Innovance D-dimer and AxSYM D-dimer) for the exclusion of deep vein thrombosis (Elf *et al*.; 2009).

2.3.11.2.Contents

AFIAS D-Dimer consists of (Cartridge, Pipette tip, ID chip and Instruction for use). Each cartridge packaged in an aluminum pouch has two components, a detector and cartridge part. Cartridge part contains a test strip, the membrane which own anti-human D-Dimer at the test line, while streptavidin at the control line. Detector part contains anti-human D-Dimer-fluorescence conjugate, biotin-BSA-fluorescence conjugate, bovine serum albumin (BSA) as stabilizer and sodium azide in phosphate buffer saline (PBS) as preservative.

2.3.11.3.The test procedure

The test procedure of AFIAS D. Dimer was the same of AFIAS Covid-19 Ab.

2.3.11.4.Interpretation of Test Result

- AFIAS test device calculated the result automatically also displayed D-Dimer concentration of the test sample it referred to ng/mL (FEU, Fibrinogen equivalent units).
- Cut-off : 500 ng/mL .
- Working range of AFIAS D-Dimer : 50-10,000 ng/mL .

2.3.12.Automated Fluorescent Immunoassay System Ferritin (AFIAS Ferritin)

2.3.12.1.Principle

The measurement was included a sandwich immunodetection method; the detector recombinant protein in buffer related to antibody in sample, forming recombinant protein-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antigen on test strip. The more antibody in sample forms the more recombinant protein-antibody complex and leads to stronger intensity of fluorescence signal on detector recombinant protein, which was processed by the instrument for AFIAS tests to show ferritin concentration in sample (Mary *et al*.; 2009).

2.3.12.2.Contents

AFIAS Ferritin consists of (Cartridge, Pipette tip, ID chip and Instruction for use). Each cartridge packaged in an aluminum pouch has two components, a detector and cartridge part. Cartridge part contains a test strip, a membrane that has anti-human ferritin on the test line, while keyhole limpet hemocyanin (KLH) at the control line. Detector part contains anti human ferritin-fluorescence conjugate, anti KLH-fluorescence conjugate, sucrose, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffer saline (PBS) as a preservative.

2.3.12.3.The test procedure

The test procedure of AFIAS Ferritin was the same of AFIAS Covid-19 Ab.

2.3.12.4.Interpretation of Test Result

- The instrument for AFIAS tests calculated the test result automatically and displayed ferritin concentration of the test sample in terms of ng/mL .
- Reference range – Women 20-250 ng/mL - Men 30-350 ng/mL .
- The working range of the AFIAS Ferritin was 10-1,000 ng/mL .

2.3.13. Automated Fluorescent Immunoassay System Interleukin-6 (AFIAS IL-6)

2.3.13.1.Principle

This test uses a sandwich immunodetection method ; the detector antibodies in buffer bind to antigens in the sample, forming antigen - antibody complexes and migrate onto nitrocellulose matrix to be captured by the other immobilized antibodies on test strip. More antigens in the sample will form the more antigen antibody complexes which lead to stronger fluorescence signal by detector antibodies, which is processed by instrument for AFIAS tests to show IL - 6 concentration in the sample (Istemi *et al*.; 2010).

2.3.13.2.The content

Cartridge Box contains (Cartridge, Pipette tip (zipper bag), C- tip (zipper bag), Spare cartridge zipper bag, ID chip and Instruction for use).

2.3.13.3.The test procedure

1. General mode was selected in the instrument for AFIAS tests.
2. 100 µl of sample was taken (serum) with a pipette and was dispensed it into the sample well on the cartridge.
3. The cartridge was insert into the cartridge holder.
4. A tip was inserted into the tip hole of cartridge.
5. The 'START' icon on the screen was taped.
6. Test results were displayed on the screen after l0 minutes.

2.3.13.4. Interpretation of Test Result

- The instrument for AFIAS test calculates the test result automatically and displays IL-6 concentration of the test sample in terms of pg/ml.
- Reference value: 7 pg/ml.
- Working range: 2-2500 pg/ml.

2.3.14. LDH

2.3.14.1. Intended Use

Quantitative measurement of lactate dehydrogenase activity in serum.

2.3.14.2.Principle

From plasma or serum, take 10µl dispense on a FUJI DRI-CHEM SLIDE LDH-PIII .After depositing, the specimen spreads uniformly on the spreading layer and diffuses into the underlying coloring layer. As the process proceeds, large molecular components such as proteins or dye components are filtrated, and only small molecular components are able to permeate and diffuse into the coloring layer. The LDH catalysis the reaction of lactic acid salt with Nicotinamide adenine dinucleotide (NAD +) while spreading uniformly in the spreading layer. The formed reduction type coenzyme (NADH) reduces nitrotetrazolium blue (NTB) by the catalytic reaction of diaphorase to form a diformazan dye (purple). The increase of absorbance by the generated dye is measured from 1 min to 2 min at 540 nm by reflective spectrophotometry

2.3.14.3.The contents

Slid and QC card.

2.3.14.4. The test procedure

- The new QC-card was been read when you switch to a new box of slides .
- Set slides on FUJI DRI-CHEM ANALYZER .
- The sample tube was Set in the specified sample rack.
- The “START” key Pressed to initiate testing .

2.3.15.Human Interleukin 4, IL-4 ELISA Kit (BT LAB).

2.3.15.1. Intended Use

This Sandwich kit is for the accurate quantitative detection of Human Interleukin 4 (also known as IL4) in serum, plasma, cell culture supernatants, Ascites, tissue homogenates or other biological fluids.

2.3.15.2. Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human IL4 antibody. IL4 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human IL4 Antibody is added and binds to IL4 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated IL4 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human IL4. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

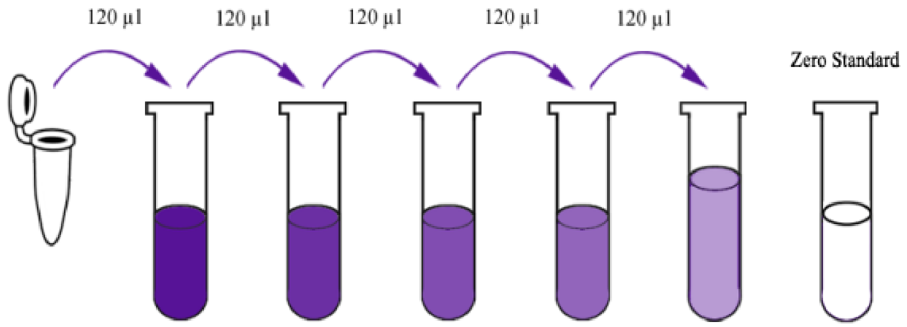
2.3.15.3.The contents

Standard solution (1280ng/L), pre-coated ELISA plate, standard diluent, streptavidin-HRP, stop solution, substrate solution A, substrate solution B, wash buffer Concentrate (25x), biotinylated Human IL4 antibody, user instruction and plate sealer.

2.3.15.4. Reagent Preparation

- All reagents were brought to room temperature before use.
- **Standard:** Reconstitute the 120ul of the standard (1280ng/L) with 120ul of standard diluent to generate a 640ng/L standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (640ng/L) 1:2 with standard diluent to produce 320ng/L, 160ng/L, 80ng/L and 40ng/L solutions. Standard diluent serves as the zero standard (0ng/L). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

640ng/L	Standard No.5	120ul Original standard + 120ul Standard diluent
320ng/L	Standard No.4	120ul Standard No.5 + 120ul Standard diluent
160ng/L	Standard No.3	120ul Standard No.4 + 120ul Standard diluent
80ng/L	Standard No.2	120ul Standard No.3 + 120ul Standard diluent
40ng/L	Standard No.1	120ul Standard No.2 + 120ul Standard diluent



Standard concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
1280ng/L	640ng/L	320ng/L	160ng/L	80ng/L	40ng/L

- **Wash Buffer:** Dilute 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

2.3.15.5. Assay Procedure

1. All reagents, standard solutions and samples were prepared as instructed. All reagents were brought to room temperature before use and the assay was performed at room temperature.
2. The number of strips required for the assay were determined. The strips were inserted in the frames for use. The unused strips were stored at 2-8°C.
3. 50ul of standard was added to standard well.
4. 40ul of sample was added to sample wells and then 10ul Human IL-4 antibody was added to sample wells, then 50ul streptavidin-HRP was added to sample wells and standard wells (Not blank control well), and then were mixed well and covered the plate with a sealer and incubated for 60 minutes at 37°C.

5. The sealer was removed and the plate was washed 5 times with wash buffer. The wells were soaked with 300ul wash buffer for 30 seconds to 1 minute for each wash. The plate was blotted onto paper towels or other absorbent material.
6. 50ul of substrate solution A was added to each well and then 50ul substrate solution B was added to each well. The plate was incubated and covered with a new sealer for 10 minutes at 37°C in the dark.
7. 50ul of Stop Solution was added to each well, the blue color was changed into yellow immediately.
8. The optical density (OD value) of each well were determined immediately used a microplate reader set to 450 nm within 10 minutes after the stop solution was added.

2.3.15.6.Calculation of Result

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis, as seen in figure (2-2).

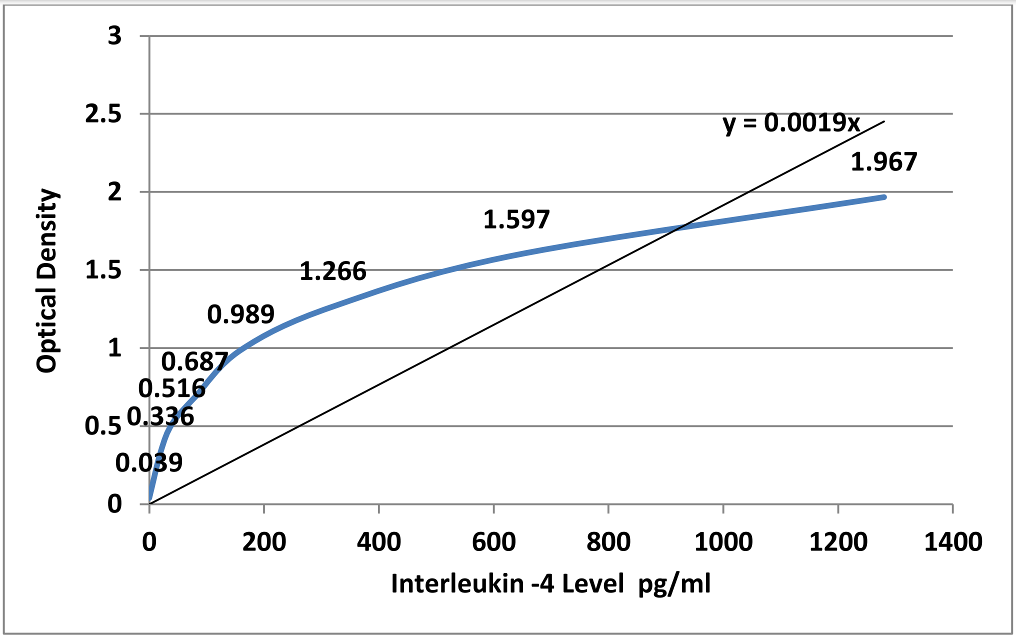


Figure (2-2): Curve the average of optical density of standard (450 nm) with the IL-4 concentration pg/ml.

2.3.16. Human Procalcitonin ELISA Kit (BT LAB).

2.3.16.1. Intended Use

This Sandwich kit is for the accurate quantitative detection of Human Procalcitonin (also known as PCT) in serum, plasma, cell culture supernatants, Ascites, tissue homogenates or other biological fluids.

2.3.16.2. Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human PCT antibody. PCT present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human PCT Antibody is added and binds to PCT in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated PCT antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human PCT. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

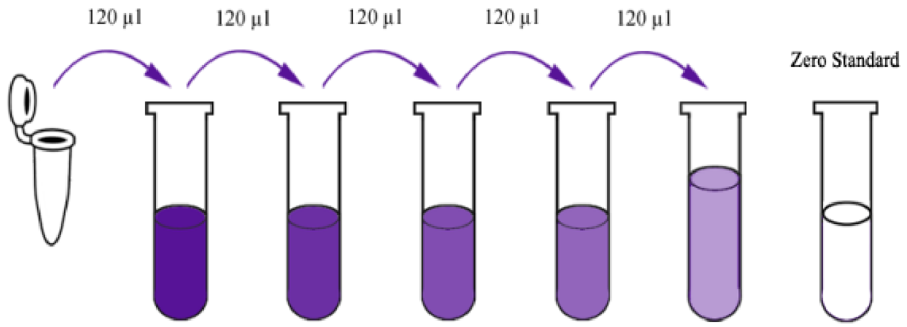
2.3.16.3.The contents

Standard solution (2400pg/ml), pre-coated ELISA plate, standard diluent, streptavidin-HRP, stop solution, substrate solution A, substrate solution B, wash buffer Concentrate (25x), biotinylated Human PCT antibody, user instruction and plate sealer.

2.3.16.4. Reagent Preparation

- All reagents were brought to room temperature before use.
- **Standard:** Reconstitute the 120ul of the standard (2400pg/ml) with 120ul of standard diluent to generate a 1200pg/ml standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (1200pg/ml) 1:2 with standard diluent to produce 600pg/ml, 300pg/ml, 150pg/ml and 75pg/ml solutions. Standard diluent serves as the zero standard (0ng/L). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

1200pg/ml	Standard No.5	120ul Original standard + 120ul Standard diluent
600pg/ml	Standard No.4	120ul Standard No.5 + 120ul Standard diluent
300pg/ml	Standard No.3	120ul Standard No.4 + 120ul Standard diluent
150pg/ml	Standard No.2	120ul Standard No.3 + 120ul Standard diluent
75pg/ml	Standard No.1	120ul Standard No.2 + 120ul Standard diluent



Standard concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
2400pg/ml	1200pg/ml	600pg/ml	300pg/ml	150pg/ml	75pg/ml

- **Wash Buffer:** Dilute 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

2.3.16.5. Assay Procedure

1. All reagents, standard solutions and samples were prepared as instructed. All reagents were brought to room temperature before use and the assay was performed at room temperature.
2. The number of strips required for the assay were determined. The strips were inserted in the frames for use. The unused strips were stored at 2-8°C.
3. 50ul of standard was added to standard well.
4. 40ul of sample was added to sample wells and then 10ul anti-PCT antibody was added to sample wells, then 50ul streptavidin-HRP was added to sample wells and standard wells (Not blank control well), and then were mixed well and covered the plate with a sealer and incubated for 60 minutes at 37°C.

5. The sealer was removed and the plate was washed 5 times with wash buffer. The wells were soaked with 300ul wash buffer for 30 seconds to 1 minute for each wash. The plate was blotted onto paper towels or other absorbent material.
6. 50ul of substrate solution A was added to each well and then 50ul substrate solution B was added to each well. The plate was incubated and covered with a new sealer for 10 minutes at 37°C in the dark.
7. 50ul of Stop Solution was added to each well, the blue color was changed into yellow immediately.
8. The optical density (OD value) of each well were determined immediately used a microplate reader set to 450 nm within 10 minutes after the stop solution was added.

2.3.16.6.Calculation of Result

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis, as seen in figure (2-3).

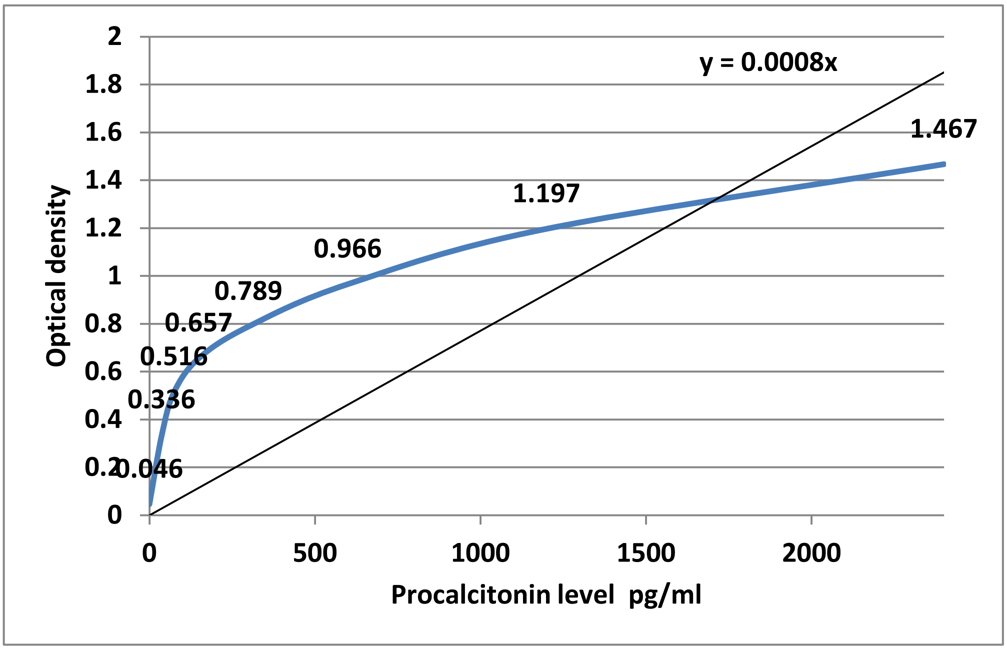


Figure (2-3): Curve the average of optical density of standard (450 nm) with the PCT concentration pg/ml.

2.3.17.Statistical analysis

The results of all samples (90, 50 patients of SARS-CoV-2, 40 of control) was conducted by using statistical program (IBM SPSS statistics version 24). The significant association was conducted using the chi-square test. The results less than < 0.05 consider significant, the impact of polymorphism on the risk for SARS-CoV-2 infection and a confidence interval 95% (CL , the add ratio (OR) and a confidence 95% CL values) were determinant. By direct counting the allele frequency was estimated. Hardy-Weinberg equilibrium (HWE) were used for calculated the allele frequency.

Chapter Three
Results and Discussion

3. Results and Discussion

3.1. Distribution of certain criteria of SARS-CoV-2 infected patients.

There were many patients received 2 dose of protective Corona vaccine before infected with virus, they revealed mild sign and symptoms rather than newly infected population, The vaccinated patients 34% in comparison with non-vaccinated 66% as in figure (3-1).

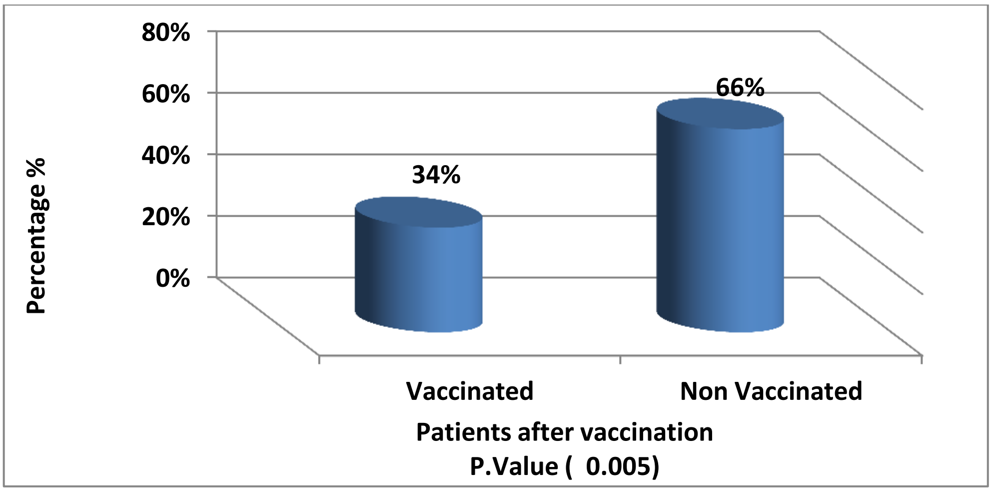


Figure (3-1): The percentage of vaccinated and Non vaccinated patients of COVID-19 in this study.

In the USA, a total of 10262 COVID-19 cases were reported in vaccinated people by April 30, 2021, of whom 2725 (26.6%) were asymptomatic, 995 (9.7%) were hospitalized, and 160 (1.6%) died (Günter, 2021). People who are vaccinated have a lower risk of severe disease but are still a relevant part of the pandemic (Günter, 2021).

The classical sign and symptoms of SARS-CoV-2 infection were different, among them fever and cough, the high percentage were showed in patients with mild fever, followed by moderate to high fever. While there was about 44% of infected patients having disease with no cough appear at the time of study, lower percentage (4%) of high cough. These results were listed in figures (3-2) and (3-3).

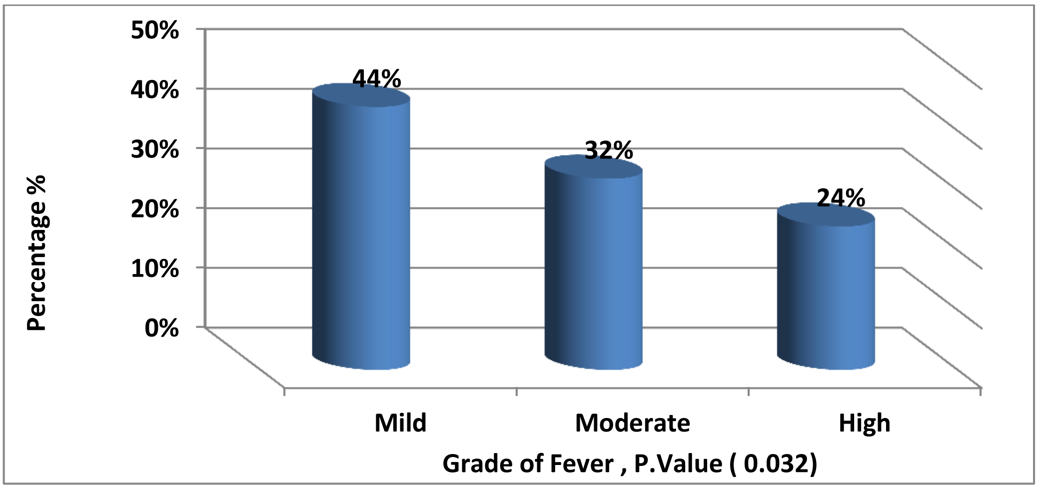


Figure (3-2): The frequency of severity of fever of infected patients with COVID-19.

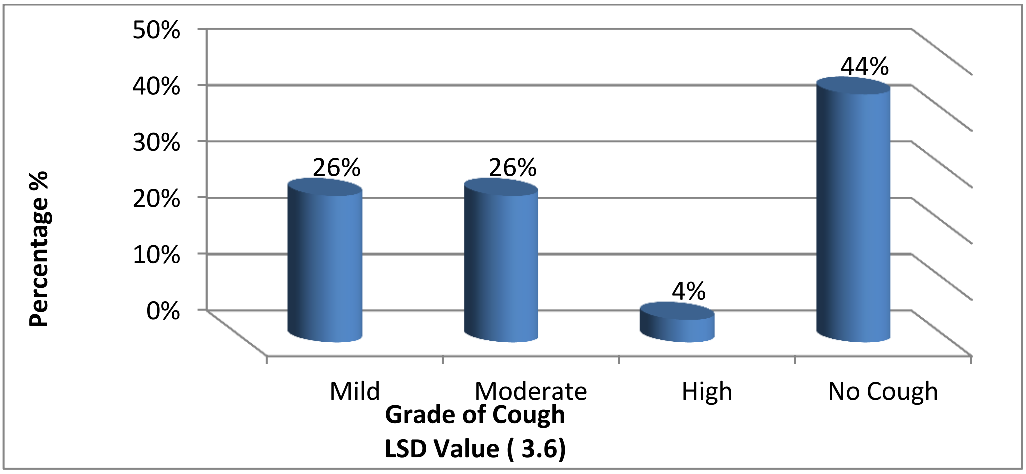


Figure (3-3): The frequency of severity of cough of infected patients with COVID-19.

Most people infected with the SARS-CoV-2 will have mild respiratory illness symptoms such as nasal congestion, runny nose, and a sore throat and other mild symptoms of COVID-19 include Low-grade fever (Donna, 2021). A cough is one of the common symptoms of COVID-19, but it is not always present, and can be infected with the coronavirus and not have a cough (Lisa *et al*.; 2022).

The comparison of all studied parameters with healthy control revealed that, decreased platelets, increased of (CRP , Ferritin , D.Dimer and LDH) as diagnostic markers of SARS-CoV-2 infected patients . The immunological parameters also have been increased in response to corona viral infection such as (IL-6 , IL-4 , PCT , IgG and IgM). This results might be refer to that the immune system work as protective role and having prognostic indicator for disease, activity and past infection (immunization). These result were showed in Table (3-1).

Table (3-1): Comparison of studied parameters in patients of COVID-19 and control .

Group Statistics	Variables	N	Mean ± SD	P. Value
WBC	Patients	50	7.7658±2.67	0.659
	Control	40	7.9835±1.98	
Eosinophil count	Patients	50	.2302±0.42	0.079
	Control	40	.3427 ± 0.13	
Platelets	Patients	50	171.68±48.97	0.000
	Control	40	269.49 ±47.80	
CRP	Patients	50	25.76 ±15.92	0.000
	Control	40	1.49 ±0.57	
Ferritin	Patients	50	276.58 ± 15.83	0.000
	Control	40	147.61 ±39.99	
D. Dimer	Patients	50	503.97 ±56.78	0.000
	Control	40	222.24 ±35.14	
LDH	Patients	50	236.48 ±70.50	0.000
	Control	40	134.37± 66.71	
IL-6	Patients	50	13.17 ±9.32	0.000
	Control	40	2.07 ±1.83	
IL-4	Patients	50	122.89 ±61.95	0.003
	Control	40	95.05± 10.40	
PCT	Patients	50	299.95± 63.84	0.003
	Control	40	226.40 ±32.04	
IgG	Patients	50	12.75 ± 2.14	0.000
	Control	40	0.93 ±0.09	
IgM	Patients	50	1.77 ±0.71	0.002
	Control	40	0.08± 0.22	

Total White Blood Cell (WBC) count and basophil count median values showed a no significant difference between the two groups (Abozer *et al*.; 2020). Added to lymphocytopenia, a reduced number of eosinophil, or eosinopenia, has been reported in more than half (52.9–78.8%) of patients who tested positive for COVID–19 (Zhang *et al*.; 2020, Li *et al*.; 2020). Patients with COVID-19 pneumonia exhibit coagulation abnormalities, most commonly elevated levels of fibrinogen and D-dimer, often with mild thrombocytopenia (Wool *et al*.; 2021).Elevated levels of IL-6, CRP, PCT, and D-dimer were detected in subjects with fatal covid-19 outcomes during treatment (Marija *et al*.; 2022). Significantly higher in-hospital mortality was observed in individuals whose IL-6 values were equal to or higher than 74.98 pg/mL, followed by CRP values higher than 81 mg/L, PCT values equal to or higher than 0.56 ng/mL, and D-dimer values equal to or higher than 760 ng/mL FEU (Marija *et al*.; 2022). Multiple organ damage was reported by Aleena *et al*.; 2022, through elevated circulating LDH concentrations as it is present in almost all body organs, therefore its high levels showed injury to the heart, liver, lymph nodes, spleen, lungs, kidneys, pancreas, liver and striated muscles. Evidence by Zhou *et al*.; 2020 suggested that the amount of serum ferritin rises during the COVID-19 pandemic, indicating a higher risk of mortality. The serum concentrations of IL-6 and IL-4 were elevated in COVID-19 patients, the levels were significantly higher in moderately and severely affected COVID-19 patients as compared with the control group, However, no significant differences were detected between controls and recovered from the COVID-19 group, suggesting that the severity of IL-6 and IL-4 were positively co-related to the severity of COVID-19 (Mohammed Yousif. *et al*.; 2021). IgM and IgG have been detected in the blood of patient after three to six days and eight days, during SARS infection (Li *et al*.; 2020).

3.2.Age groups distribution of studied parameters.

The frequency of patients age groups were listed in the figure (3-4), the young patients at age range (20-29 years) were (36%) more percentage than others, followed by adult age at (50-59 as well as > 60 years) at (18% and 20 %) respectively.

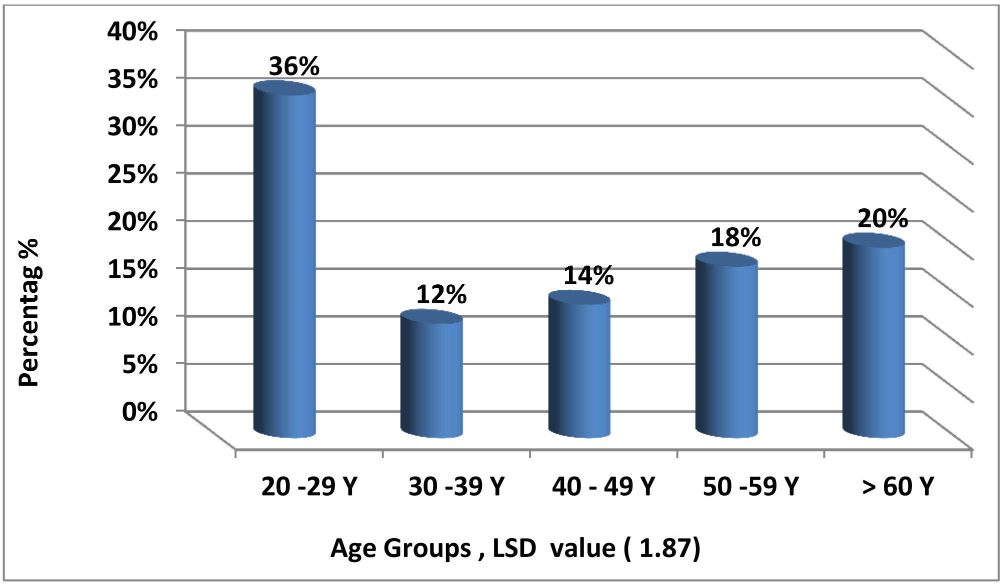


Figure (3-4): Age range frequency distribution.

Data for laboratory confirmed COVID-19 cases from South Korea, Australia, New Zealand, Japan and the Netherlands exhibited essentially identical profiles, with a bimodal distribution that showed highest rate of confirmed SARS-CoV-2 infections among individuals in the 20-29 years age cohort (21%-27% of total), and a second lower peak for the 50-59 or 60-69 age cohorts (16-18% of total) (Dudley J. *et al*.; 2022).

3.2.1.Age and hematological parameters.

The total white blood cells, eosinophil count and platelets count were measured to evaluation hematological element in response to

SARS-CoV-2 infection. There was no differences in WBC, and eosinophil count at both patients (at all ages) and control, while reduced the platelets count in (> 60 years) comparison with control, although it was within normal limit. This result was mentioned in table (3-2).

Table (3-2): Age range groups and hematological parameters .

Hematological Parameters		N	Mean ± SD	LSD Value
WBC	20 -29 Y	18	8.01 ±2.30	0.66
	30 -39 Y	6	7.42± 1.34	
	40 - 49 Y	7	6.53 ±0.97	
	50 -59 Y	9	8.68 ±3.55	
	> 60 Y	10	7.56 ±3.73	
	Control	40	7.98 ±1.98	
Eosinophil count	20 -29 Y	18	0.36 ±0.60	0.34
	30 -39 Y	6	0.16 ±0.14	
	40 - 49 Y	7	0.17 ±0.10	
	50 -59 Y	9	0.15 ±0.35	
	> 60 Y	10	0.12 ±0.27	
	Control	40	0.34 ±0.13	
Platelets	20 -29 Y	18	180.44± 49.03	24.3
	30 -39 Y	6	178.16 ±45.58	
	40 - 49 Y	7	181.42± 46.82	
	50 -59 Y	9	173.55± 65.04	
	> 60 Y	10	143.50± 31.64	
	Control	40	269.49± 47.80	

Hematological changes differed between survivors and non-survivors with COVID-19. Lymphopenia and eosinopenia could be predictors for poor prognosis of COVID-19 patients. Initial counts of eosinophil might guide in usage of glucocorticoids for COVID-19 treatment (Xunliang *et al*.; 2021). A low percentage of eosinophil might be considered as a biomarker of pneumonia of COVID-19, but not as a biomarker of pneumonia severity (Wujtewicz *et al*.; 2020).

3.2.2.Age range groups in relation to CRP, Ferritin, D. Dimer and LDH levels.

There were different parameters used as patients state monitor and diagnoses, among them are (CRP, Ferritin, D.Dimer and LDH enzyme), the mean ± SD of such parameters were listed in the table (3-3), it referred to that CRP were high at young age (30-39 years and adult age of > 60 years old) in 34.99 and 63.34 mg/l) respectively. While the ferritin level showed that increased level in comparison with control although it within normal. The D-Dimer level as well as LDH were increased in SARS-CoV-2 infection in comparison with control.

Table (3-3): Age distribution in relation to CRP , Ferritin , D. Dimer and LDH levels in patients and control.

Age and Diagnostic markers		N	Mean ± SD	LSD Value
CRP	20 -29 Y	18	8.89 ±2.81	4.6
	30 -39 Y	6	34.99± 7.92	
	40 - 49 Y	7	16.99± 4.60	
	50 -59 Y	9	18.41 ±7.59	
	> 60 Y	10	63.34 ±22.52	
	Control	40	1.49 ±0.57	
Ferritin	20 -29 Y	18	235.66± 51.86	46.5
	30 -39 Y	6	283.45 ±21 .70	
	40 - 49 Y	7	360.08± 44.12	
	50 -59 Y	9	268.11 ±44.32	
	> 60 Y	10	295.30 ± 41.83	
	Control	40	147.61 ±39.99	
D. Dimer	20 -29 Y	18	457.47 ±72.81	34.8
	30 -39 Y	6	535.70 ±43.53	
	40 - 49 Y	7	631.58 ±64.61	
	50 -59 Y	9	443.10 ±75.90	
	> 60 Y	10	534.11 ±91.61	
	Control	40	222.24 ±35.14	
LDH	20 -29 Y	18	204.27 ±54.66	26.8
	30 -39 Y	6	227.16± 17.15	
	40 - 49 Y	7	248.42± 82.96	
	50 -59 Y	9	150.00 ±59.96	
	> 60 Y	10	369.50 ±20.40	
	Control	40	134.37 ±26.71	

The age-specific rates at which individuals infected with SARS-CoV-2 develop severe and critical disease was essential for designing public policy, for infectious disease modeling, and for individual risk evaluation, particularly in young populations where they can be 2 orders of magnitude more frequent than deaths. The estimated probability of severe, critical, and fatal disease outcomes (ISR, ICR, and IFR, respectively) were shown for each age and location (Daniel and Gustavo 2022). Though the study group only included patients ≥18 years, they showed that ferritin was better at predicting all-cause mortality in progressively younger cohorts of patients. This might suggest that inflammation plays a larger role leading to mortality in younger vs. older adults (Jonathan *et al*.; 2020).

3.2.3. Age range in relation to Immunological markers

Certain cytokines (IL-6 and IL-4 level) were studied in SARS-CoV-2 patients, in which that the cytokines level were increased at all age groups especially in young age (30 - 39 years) for IL-6 and 20 - 29 years of IL-4, because it have higher level than other age range groups. Procalcitonin and anti –SARS-CoV-2 IgG have increased level at all age range groups, the higher level of IgG antibody were showed in adult age groups in comparison with other age range groups .While anti-SARS-CoV-2 IgM were increased at different age range groups such as (20 -29 years, 40 - 49 Years and 50 -59 years) as shown in table (3-4).

Table (3-4): Age distribution and Immunological markers (IL-6,IL-4, PCT, IgG and IgM)

Age and immune markers		N	Mean ± SD	LSD Value
IL-6	20 -29 Y	18	7.06 ±2.56	5.44
	30 -39 Y	6	25.04 ± 4.38	
	40 - 49 Y	7	14.61 ±6.51	
	50 -59 Y	9	18.92 ± 4.17	
	> 60 Y	10	10.86 ±6.43	
	Control	40	2.07 ±1.83	
IL-4	20 -29 Y	18	146.98± 26.99	23.7
	30 -39 Y	6	105.26 ±16.11	
	40 - 49 Y	7	100.45 ±23.17	
	50 -59 Y	9	107.01 ±27.34	
	> 60 Y	10	120.10 ±53.30	
	Control	40	95.05 ±10.40	
PCT	20 -29 Y	18	332.22 ± 77.34	34.8
	30 -39 Y	6	278.95 ±32.95	
	40 - 49 Y	7	377.85 ±27.70	
	50 -59 Y	9	248.61 ±75.13	
	> 60 Y	10	246.12 ±24.93	
	Control	40	226.40± 32.04	
IgG	20 -29 Y	18	11.79 ±2.22	3.54
	30 -39 Y	6	2.97 ±1.16	
	40 - 49 Y	7	18.42 ±3.15	
	50 -59 Y	9	15.06 ± 6.17	
	> 60 Y	10	14.31 ±3.68	
	Control	40	0.93 ±0.16	
IgM	20 -29 Y	18	1.38 ±0.69	0.67
	30 -39 Y	6	0.13 ±0.30	
	40 - 49 Y	7	2.63 ±1.88	
	50 -59 Y	9	4.53 ±1.00	
	> 60 Y	10	0.39 ±0.18	
	Control	40	0.08 ±0.028	

Various studies of COVID-19 patients have detected elevated IL-4 levels as part of the cytokine storm associated with severe respiratory symptoms (Víctor *et al*.; 2020). A study of 452 patients infected with

SARS-CoV-2 also reported that the elevation of IL-6 levels was more marked with more severe symptoms (Víctor *et al*.; 2020). Elderly patients with COVID-19 are more likely to progress to severe disease. Reasonably, age-related comorbidities are the leading reason for the increased mortality observed in this age group (Liu *et al*.; 2020). Also found that in non-hospitalized patients, higher antibody levels correlated with older age, male sex, higher BMI, and higher Carlson Comorbidity Index score (Maya *et al*.; 2021).

3.3. Frequency of Gender distribution

The male and female patients were chosen at equal percentage in and have 27.8% for both in comparison with gender matching control as in Figure (3-5).

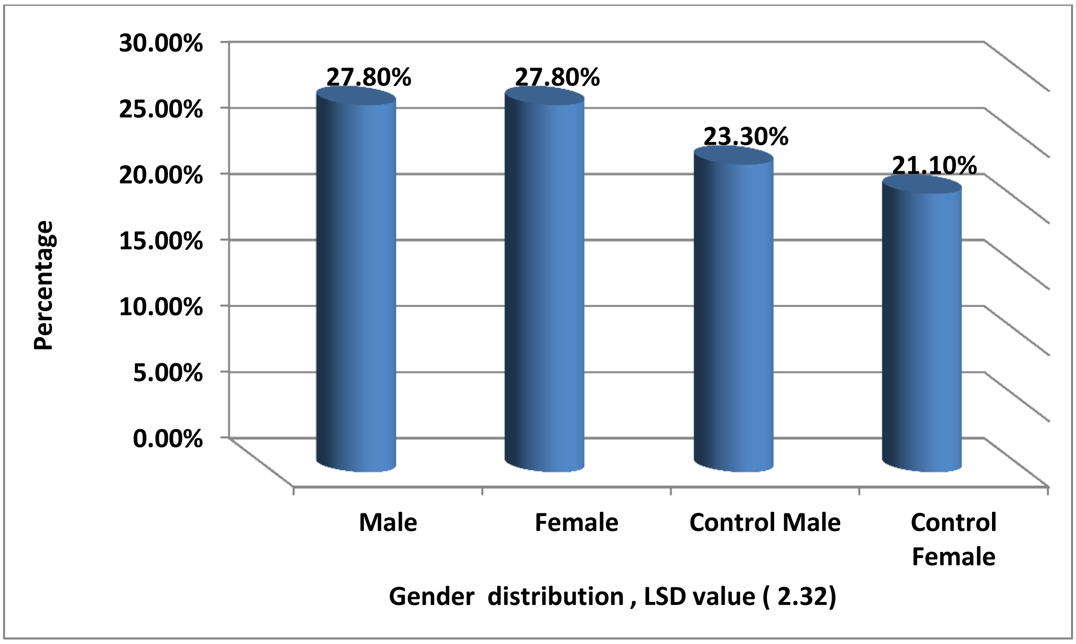


Figure (3-5): The frequency of Gender distribution.

In a study by Jian *et al*.; 2020, similar susceptibility to SARS-CoV-2 between males and females was observed in 1,019 patients who survived the disease (50.0% males), collected from a public data set and in a case series of 43 hospitalized patients (51.2% males).

3.3.1. Gender distribution and hematological parameters.

There were no differences in hematological parameters among both male and female of SARS-CoV-2 infected patients, as listed in the table (3-5).

Table (3-5): Gender distribution and hematological parameters

Hematological Parameters		N	Mean± SD	LSD Value
WBC	Male	25	7.91 ±2.32	1.86
	Female	25	7.61 ±3.03	
	Con Male	21	8.20 ±2.47	
	Con Female	19	7.73 ±1.27	
Eosinophil	Male	25	0.22 ±0.30	0.42
	Female	25	0.23± 0.51	
	Con Male	21	0.31± 0.17	
	Con Female	19	0.36± 0.07	
Platelets	Male	25	173.76± 53.47	15.40
	Female	25	169.60 ±45.04	
	Con Male	21	259.35 ±58.93	
	Con Female	19	280.69 ±28.99	

Lower counts of platelets, basophils, lymphocytes, and eosinophil were observed in COVID-19 cases compared with controls in both males and females (Felipe *et al*.; 2021). Male and female COVID-19 patients had a lower levels of eosinophil and basophils, as well as significantly higher levels of gamma-glutamyl transferases (GGT), C-reactive protein (CRP), and ferritin when compared with control individuals in the same age group (Felipe *et al*.; 2021). Most of the papers reviewed here did not indicate the presence of thrombocytopenia or platelet differences between patients with severe disease and those exhibiting mild disease (Huang *et al*.; 2020, Wang *et al*.; 2020, Liu *et al*.; 2020).

3.3.2. Gender distribution in relation to CRP, Ferritin, D.Dimer and LDH levels

The levels of CRP , Ferritin , D.Dimer and LDH were increased at both male and female, the Ferritin , D.Dimer are higher in male than female, while LDH is higher in female than male patients, this result were listed in table (3-6).

Table (3-6): Gender distribution in relation to CRP, Ferritin, D.Dimer and LDH levels.

Diagnostic Markers		N	Mean ± SD	P. Value
CRP	Male	25	25.86 ± 4.59	2.23
	Female	25	25.66 ± 5.41	
	Con Male	21	1.65 ±0.75	
	Con Female	19	1.31 ±0.16	
Ferritin	Male	25	346.35 ± 39.69	32.4
	Female	25	206.81 ± 82.94	
	Con Male	21	48.22 ±16.21	
	Con Female	19	257.45 ± 25.82	
D. Dimer	Male	25	509.18 ± 78.91	48.3
	Female	25	498.77± 38.26	
	Con Male	21	210.46± 45.79	
	Con Female	19	235.27± 2.38	
LDH	Male	25	172.12 ±17.23	32.4
	Female	25	300.84 ± 92.30	
	Con Male	21	131.00 ±17.62	
	Con Female	19	138.10 ±17.33	

It has been reported by Qin *et al.*; 2020, that male patients have a greater inflammatory reaction, with higher levels of LDH, ferritin, and CRP but a lower lymphocyte count than females, adjusted by age and comorbidity.

3.3.3. Gender distribution in relation to immunological markers (IL-6 , IL-4, PCT, IgM and IgG).

The male patients have higher level than female of (IL-6 , IL-4 , PCT and IgG level) , while lower level of IgM antibody . This result might be show that the higher immunological activity in male patients than female . This result was mentioned in table (3-7).

Table (3-7): Gender distribution in relation to immunological markers (Il-6, IL-4, PCT, IgM and IgG).

Immunological Markers		N	Mean ± SD	P. Value
IL-6	Male	25	13.51 ± 7.84	1.23
	Female	25	12.83 ±2 .06	
	Con. Male	21	2.17 ±1.53	
	Con. Female	19	1.97 ±0.41	
IL-4	Male	25	142.40± 29.98	23.4
	Female	25	103.38 ±25.44	
	Con . Male	21	92.78 ±11.23	
	Con. Female	19	97.56 ±9.04	
PCT	Male	25	366.95± 20.64	46.2
	Female	25	232.95± 53.13	
	Con. Male	21	225.29 ±38.25	
	Con. Female	19	227.63 ±24.39	
IgG	Male	25	13.34 ± 4.24	2.41
	Female	25	12.16 ±2.87	
	Con. Male	21	1.46 ±1.412	
	Con. Female	19	.35 ±0.94	
IgM	Male	25	1.74± 0.38	0.51
	Female	25	1.81± 0.08	
	Con. Male	21	0.13± 0.30	
	Con. Female	19	0.03± 0.06	

Male patients had higher neutrophil, CRP, and inflammatory cytokine levels such as (IL-6 and IL-4), and lower lymphocyte levels, which were related to the poor prognosis and severe clinical symptoms of male COVID-19 patients (Bin *et al*.; 2021). Procalcitonin (PCT) levels were higher in males 0.56 ng/mL vs. 0.12 ng/mL in female (Federico *et al*.; 2021). Klein SL *et al*. reported higher antibody levels in convalescent male patients, suggesting that males might need more antibodies to recover from COVID-19 (Bin *et al*.; 2021).

3.4. Vaccinated patients in relation to Immunological markers (IL-6 , IL-4 , PCT , IgM and IgG)

The table (3-8) showed that increased of IL-6 level in Non-vaccinated patients than vaccinated and control , IL-4 , PCT , IgM and IgG) were elevated in vaccinated patients more than non-Vaccinated. This result might be show that the vaccinated population have more immunity than non-Vaccinated, the cytokine and antibodies (IgM and IgG) make a role in protective measure against the SARS-Cov2 infection.

Table (3-8):Vaccinated patients in relation to immunological markers.

Immune Markers		N	Mean± SD	P, Value
IL-6	Vaccinated	17	7.73 ±1.70	.000
	Non Vaccinated	33	15.97 ± 3.05	
	Control	40	2.07 ±1.83	
IL-4	Vaccinated	17	148.14 ±18 .02	.000
	Non Vaccinated	33	109.88 ±23.80	
	Control	40	95.05 ±10.40	
PCT	Vaccinated	17	383.60 ±22.85	.000
	Non Vaccinated	33	256.85 ±26.74	
	Control	40	226.40 ±32.04	
IgG	Vaccinated	17	13.09 ± 2.37	.000
	Non Vaccinated	33	12.58 ± 2.20	
	Control	40	.93 ±0.96	
IgM	Vaccinated	17	3.01 ±1.81	.002
	Non Vaccinated	33	1.14 ±0.87	
	Control	40	0.08 ±0.22	

In a new *MMWR* examining more than 7,000 people across 9 states who were hospitalized with COVID-like illness, CDC found that those who were unvaccinated and had a recent infection were 5 times more likely to have COVID-19 than those who were recently fully vaccinated and did not have a prior infection (New CDC Study. 2021). The data demonstrate that vaccination can provide a higher, more robust, and more consistent level of immunity to protect people from hospitalization for COVID-19 than infection alone for at least 6 months (New CDC Study. 2021).

3.5. The immunological markers levels in association with severity of cough and fever.

The result of febrile patients were associated with different cytokine gradually variation according to fever grade , the IL-6 was elevated in association of fever increased the higher level was revealed at high fever patients in comparison with other grade of fever. IL-4 , PCT and Anti-SARS-CoV-2 IgM antibody were increased in patients with mild fever or in first few days of infection. While Anti SARS-CoV-2 –IgG antibody was elevated in patients at moderate fever grade as shown in table (3-9).The cough criteria might be started late in time of primary infection and might be prolonged at time more than other symptoms of disease due to several immunological points by noted that the result of IL-6 was increased in moderate cough degree of patients followed by patients with no cough in comparison with others , IL-4 and PCT were elevated at patients with no cough in comparison with others , The Anti-Sars-Cov2 Antibodies (IgM and IgG) were associated with mild to moderate cough degree , as listed in Table (3-10).

Table (3-9): The immunological markers levels in association with severity of fever in patients that infected with COVID-19.

Immune Markers	Fever grade	N	Mean ± SD	P. Value
IL-6	Mild	22	12.50 ±2.23	0.475
	Moderate	16	9.84 ±1.06	
	High	12	18.83 ± 4.35	
IL-4	Mild	22	140.64 ±29.75	0.194
	Moderate	16	111.77 ±24.33	
	High	12	105.17 ±23.51	
PCT	Mild	22	314.31 ±16.38	0.760
	Moderate	16	302.50 ±20.23	
	High	12	270.20 ± 43.13	
IgG	Mild	22	11.93 ± 2.66	0.424
	Moderate	16	15.89 ± 2.90	
	High	12	10.07 ±2.98	
IgM	Mild	22	2.45 ±1.34	0.524
	Moderate	16	1.30 ±0.38	
	High	12	1.17 ±0.82	

Table (3-10): The immunological markers levels in association with cough degree .

Cough grade		N	Mean ± SD	LSD Value
IL-6	Mild	13	10.18 ±2.64	3.81
	Moderate	13	16. 15± 4.34	
	High	2	4.98 ±1.85	
	No Cough	22	14. 82±2.1 9	
IL-4	Mild	13	106.39 ±24.34	4.02
	Moderate	13	118.58 ±25.70	
	High	2	102.36 ±14.51	
	No Cough	22	137.05 ±31.53	
PCT	Mild	13	293.55 ±21.8 7	33.0
	Moderate	13	260.48± 33.40	
	High	2	212.50± 7.07	
	No Cough	22	335.00± 16.5 2	
IgG	Mild	13	14.39 ± 3.07	3.72
	Moderate	13	13.65 ±2.93	
	High	2	19.60± 4.15	
	No Cough	22	10.63 ±1.2 6	
IgM	Mild	13	1.80 ±3.64	0.55
	Moderate	13	1.21 ±3.76	
	High	2	.01 ±.00	
	No Cough	22	2.25 ±0. 96	

Fever, body aches, and low appetite, which consistently correlated with higher antibody levels in both univariate and multivariable analyses, can be signs of a systemic inflammatory response, which is likely key for developing a strong anti-SARS-CoV-2 antibody response (Maya *et al.*; 2021). The severity of COVID-19 is known to be closely correlated to cytokines storms, when the immune system is unable to counteract the virus, cytokine storms in patients may lead to macrophage hyperactivity and further systemic abnormal reactions (Lang *et al.*, 2020; Liang *et al.*, 2020; Makaronidis *et al.*, 2020). Large-scale data demonstrated by Huating *et al.*; 2022, that the circulating levels of IL-6 and IL-4 are potential risk factors for severity and high mortality in COVID-19. On the other hand, numerous studies reported that PCT levels correlated significantly with the severity of disease, complications, and clinical outcome of COVID-19 (Del *et al*.; 2020) (Garrido *et al*.; 2021).

3.6. Qualitative Determination of SARS-CoV-2 virus by real Time PCR.

Qualitative Real Time-PCR was used to detect SARS-CoV-2 virus by detection of specific genes in Covid-19 patients, using a general method (Dan – Gene) to evaluate the presence of (ORF1ab/N), and a specific protocol (Bioron) to distinguish viral gene types such as (S, N, and E) as a diagnostic tool.

The amplification curve of S-gene began at cycle 32 above the ct (cycle threshold line), and the concentration of RFU (relative flourescent unit) increased to 700 units, as shown in figure (3-6). This result suggested that the patients were SARS-CoV-2 infected at S-gene level; the presence of S –gene may be linked to other SARS-CoV-2 genes, such as (N and E gene).

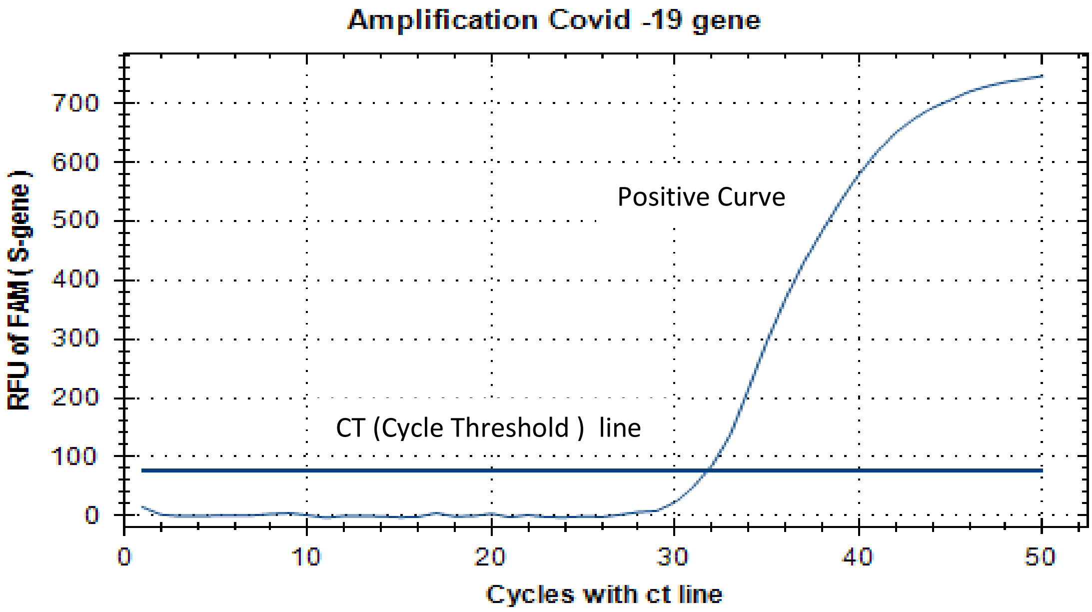


Figure (3-6): Amplification of Covid -19 S - gene by Real Time-PCR

While the amplification curve of E-gene began at cycle 32 above the ct (cycle threshold line) and the concentration of RFU (relative flourescent unit) up to 2000 Unit, as shown in the following figure (3-7). This result suggested that the patients were SARS-CoV-2 infected at E-gene level; the presence of E-gene may be related to other SARS-CoV-2 genes, such as (N and S gene).

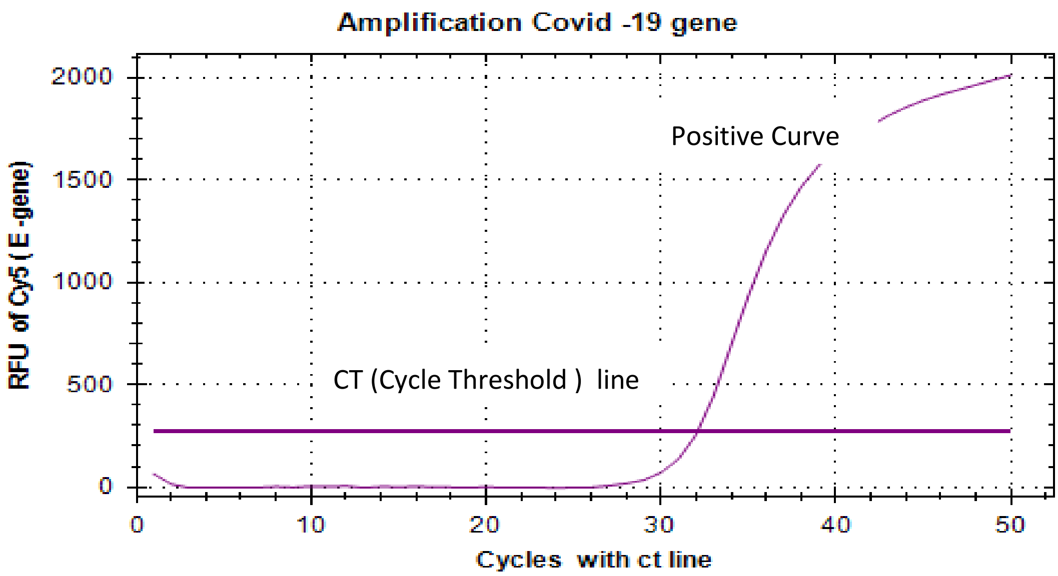


Figure (3-7): Amplification of Covid -19 E- gene by Real Time-PCR

The amplification curve of N- gene began at cycle 32 above the ct (cycle threshold line) and developed to 2100 RFU (relative flourescent unit). This result suggested that the patients were SARS-CoV-2 infected at N-gene level; the existence of N-gene could be linked to other SARS-CoV-2 genes such as (E and S gene) as shown in figure (3-8).

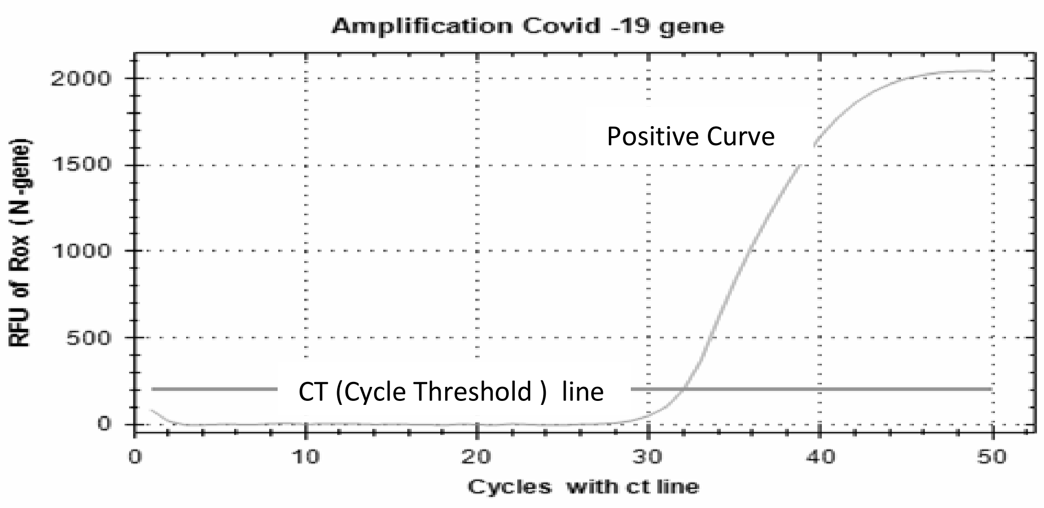


Figure (3-8): Amplification of Covid -19 N- gene by Real Time-PCR

The internal RNA control was used to determine the material quality, amplification, and density of viral nucleic acid at a qualitative and semiquantitative level of gene detction , as mentioned in figure (3-9).

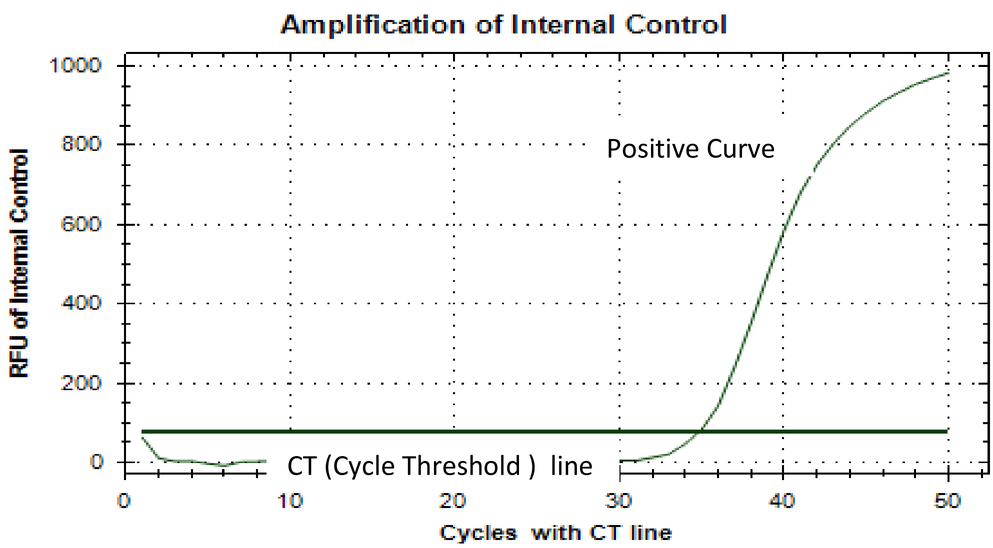


Figure (3-9): Amplification of Control RNA by Real Time-PCR

A diagnostic RT-PCR was established as a specific test after SARS-CoV-2 has been collected a specimen from lower respiratory tract (Konrad *et al*.; 2020). The ORF1ab sequence's RNA-dependent RNA polymerase (RdRp) gene, as well as the SARS-CoV-2 genome's E, N, and S genes, were used in RT-PCR tests (Corman *et al*.; 2020). The nucleic acid false-negative results were caused by an inaccurate sampling position, and inadequate sampling capability, an unusual sample distribution system (Xie *et al*.; 2020). To improve detection sensitivity, For identification, the majority of producers utilize two or more viral nucleic acid sequence regions (Reusken *et al*.; 2020), such as the SARS-CoV-2 genome's 0RF1ab sequence, E gene, N gene, and S gene (Chan *et al*.; 2020).

3.7. Genetic polymorphisms of some related-genes associated with COVID-19.

The genomic DNA in figure (3-10) was extracted from the blood samples as a first step to amplify the target region of *ACE* gene and *IL-4* gene.

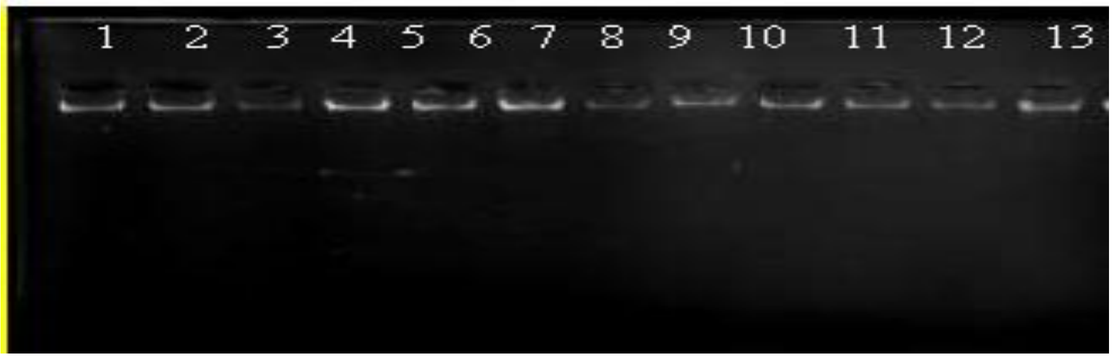


Figure (3-10): Electrophoresis pattern of gnomic DNA extracted from blood samples of patients and healthy control groups.

Lane 1 refers to genomic DNA from blood samples (1-8 patients & 9-13 control) ; Electrophoresis conditions, 1% agarose, 75 V, 20 mA for 1h (10 µl in each well), stained with Red Safe.

3.7.1. *ACE* gene I/D Polymorphism

This study found I/D Allele Frequencies for Patients, which genotypes found DD, ID, and II genotypes of *ACE* gene found 42%, 38% and 20% respectively. The D allele frequency was 59%, and I allele frequency was 41% for patients. The percentage of genotypes in control group were 32%, 25% and 43% for D/D, I/D and I/I, respectively, while the allele frequency was 59 and 41 for I and D allele , respectively. Table (3-11) depicts the distribution of *ACE* gene I/D polymorphisms in the COVID-19 patients and controls. The distinction in the presence of the D allele between patient and control used to be statistically sizable (59% vs. 41%, respectively, p < 0:05).

Table (3-11): Genotype and allele distribution *ACE* (I/D) gene polymorphism in patient and control, shown the Odd Ratio value.

ACE I/D	Patients (N=50)		Control n=40	OR(95%CI)	P-value
genotypes	**I/I , n (%)**	10 (20%)	17 (43%)	**Reference group**	
	D/D, n(%)	21 (42%)	13 (32%)	1.5 (0.63 -3.5)	0.35
	I/D , n(%)	19 (38%)	10 (25%)	2.71 (1.05-6.9)	0.03
Allele Frequency	**I, n (%)**	41 (0.41%)	59 (0.59%)	0.48 (0.27-0.84)	0.01
	D, n (%)	59 (0.59%)	41 (0.41%)	2.07 (1.17-3.6)	0.01

OR for allele D/D =1.5 correlated with disease because it value more than one.
OR for allele I/D=2.71 high correlated with disease because it value more than one.
Risk Allele frequency (D) high in patients (59) more than control 41,
with high OR=2.07 compared with wild allele I=0.48.

Note: OR values of allele more than one(>1) , This allele considered risk allele =causes disease. =1 not effective, <1 defense against disease.

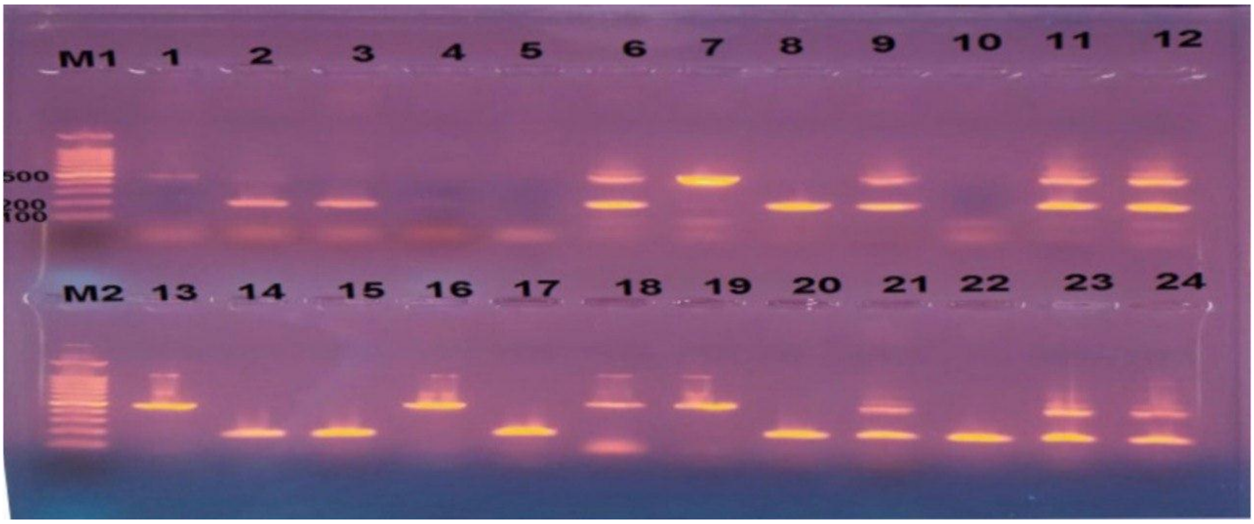


Figure (3-11): *ACE* (Insertion/ Deletion) gene polymorphism.

M leader 100 , lane(2,3,8,14,15,17,20and22) deletion genotype (Homozygote for Deletion) with size 190bp, lane(6,9,11,12,21,23and24) insertion /deletion genotype (Heterozygote), lane (1,7,13,16,18and19) insertion genotype (Homozygote for Insertion) with size 490bp, agarose concentration: (3%) Recognition showing I/D SNPs found in the patient with PCR .

Figure (3-11) gave the result of study that showed in the Allele 490bp object also these delivered about 2 companies at 190bp and 490bp identified for the heterozygous allele quality SNPs of genes found difficulty to dialogue considering that found begin, which versions of genes, delivered about with the aid of fantastic polymorphism, are concept to deliver about contrasts in contamination powerlessness and ailment seriousness (Mathew *et al*.; 2001). This has pushed the analysts to moreover discover the connection SNPs variations genes in patients. Expert SNPs variations regarding the SARS end result appears showed impacted by way hallo type D/I polymorphism (Saab *et al*.; 2007, Sun *et al*.; 2011). Found conveyance of genes first-rate SNPs variations genes which impact on the effects of 112 SARS more targeted in this examination. Our discoveries exhibit predominance from D/I genotype, D-allele carriage was everyday amongst COVID-19 sufferers contrasted with the populace. From factor when the sickness seriousness was once examined relying upon ACE first-rate variations genes the sufferers for D/I halo type a greater excessive medical direction, more from with D/D and I/I genotypes (Pati *et al*.; 2020),(Zheng *et al*.; 2020). Found contrasts of genes fantastic SNPs variations generally examined. This report, found recurrence of D alleles is more in American populations (89%) compare of Indian (69%) (Delanghe *et al*.; 2020). More of European nations, found Italy, France and Spain, recurrence from D alleles showing reachable at 87%. Then again, found proven which recurrence from II allele Asia countries is greater more of Europe populations (Delgado *et al.;* 2020).

3.7.2. Genotyping of *IL-4* (rs11209032) polymorphism using PCR-RFLP.

For (*IL4*) genotyping, the genomic DNA was amplified using specific primers and accomplished by the Thermo-cycler apparatus under the optimal conditions as mentioned in the table (2-11). The results revealed that the presence a single band (252 bp) of the target sequence of *IL4* gene in agarose gel as shown in figure (3-12).

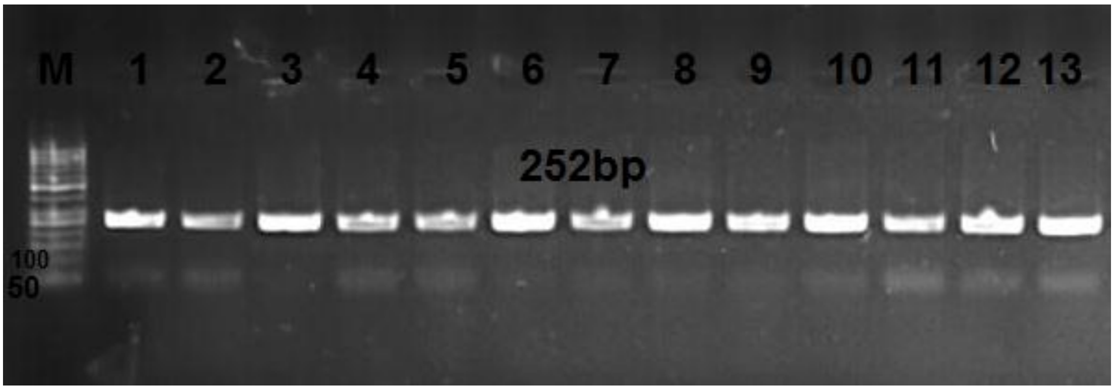


Figure (3-12): Electrophoresis pattern of PCR products of *IL4* genes.

M: refers to DNA size marker line 1 (50bp) DNA marker, line 1-8 IL4 genotype for patients, line 9-13 IL4 genotype for control. Electrophoresis condition: 2% agarose concentration , 75V,20mA for 120 min, stained with Red Safe.

The 252-bp PCR product of IL4-590 C>T was digested with BsmF1restriction endonuclease and the C allele was replaced by the T allele with a loss of digestion site. The homozygous C/C genotype had into 192bp and 60bp fragments; the heterozygotes mutant C/T genotype was completely digested into 252,192 bp, 60 bp and homozygotes mutant T/T genotype 252 bp fragments; all 3 fragments (252 bp, 192 bp, 60bp) corresponded to the heterozygous C>T genotype Figure (3-13).

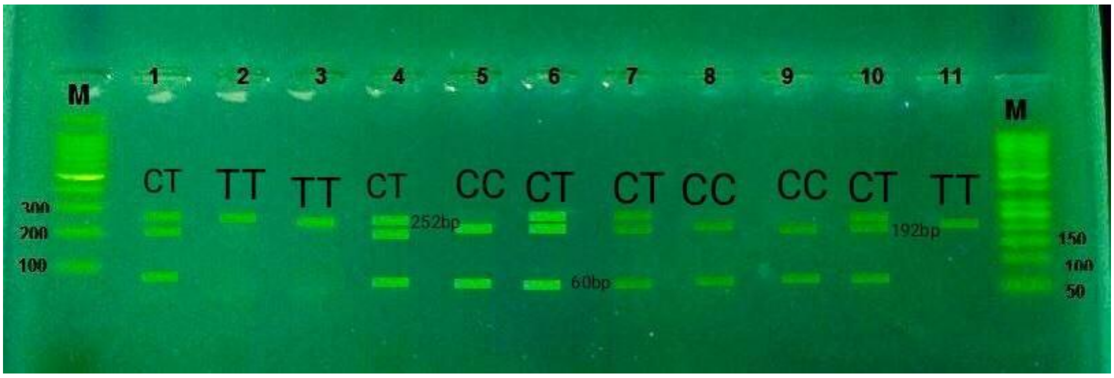


Figure (3-13): Electrophoresis pattern of *IL4* genotyping using RFLP technique (digested with BsmF1 restriction enzyme 2% agarose, 70V, 20mA, for 60 min), M; refers to DNA size marker(100bp) lane 1and line 13 (50bp) DNA marker, (5,8,9)– C/C homozygous genotype; (1,4,6,7and 10) – C>T heterozygous genotype ; (2,3,11) – T/T homozygous genotype PCR product**.**

The *IL-4* genotype showed critical variety between alleles in patients and control, the CC was showed up in 52.5% control while it was 24% in patients, TT genotype was more successive in patients (56%) than control (35%). CT genotype was more continuous in control (12.5%) than patients (20%), as shown in table (3-12) and figure (3-13).

Table (3-12): Genotype and allele distribution *IL-4* gene polymorphism in patient and control, shown the Odd Ratio value.

Genotype	SARS-CoV-2Patients (N=50)		Control n=40	OR(95%CI)	P-value
Genotypes	**CC, n(%)**	12 (24%)	21 (52.5%)	**Reference group**	
	CT, n(%)	10 (20%)	5 (12.5%)	1.75 (0.54-5.6)	0.3
	TT, n(%)	28 (56%)	14 (35%)	2.36 (1.003-5.56)	0.04
Allele Frequency	**C , n(%)**	34 (0.34%)	59 (0.59%)	0.35 (0.20-0.63)	0.0005
	T, n(%)	**66** (0.66%)	41 (0.41%)	2.79 (1.57-4.96)	0.0005

OR for heterozygous allele CT =1.75 correlated with disease because it value more than one.
OR for homozygous allele TT=2.36 high correlated with disease because it value more than one.
Risk Allele frequency (T) high in patients (66) more than control 41 with high OR=2.79 compared with wild allele C=0.35.

In present study, firstly reported that the role of genetic polymorphisms of IL-4 in COVID-19 in Iraq population, that found IL-4-590 C/T polymorphisms were associated with the COVID-19 risk, and the T allele of IL-4 promoter polymorphisms had significantly increased the susceptibility of COVID- 19 in Iraq population. This finding suggested that the IL-4-590C/T polymorphisms might be used as a genetic marker for the onset and development of COVID- 19 in Iraq population.

This study showed that serious COVID- 19 illness in Iraq patients was related with a typical haplotype of *IL-4* which contains a typical SNP (589T) known to increment transcriptional action of *IL-4*, as shown by utilization of a luciferase examine (Li *et al*.; 2016). Already, this variation in the advertiser local of the *IL-4* quality had been related with raised degrees of serum IgE and with the determination of asthma in geologically separate populaces (Korzycka *et al*.; 2015). This study shown that a typical *IL-4* haplotype was educational and recommend that a practically dynamic SNP inside the haplotype could be liable for the illness affiliation (Yadav *et al*.; 2012).

IL-4 is one of anti-inflammatory cytokine, produced by activated CD4+ lymphocytes, mast cells, and basophils and exerts an important role in the immune system on different cell types (Shang *et al*.; 2016). In humans the *IL-4* gene has been mapped to chromosome 14q32 (Micheal *et al*.; 2013).

3.8. Correlation between immunological parameters of SARS-CoV-2 infected patients

By using logistic regression module of immunological parameters were enrolled in the present study, the results of correlation were shown as the following :-

3.8.1. Correlation of Interleukin-6 with other immunological parameters .

The correlation of IL-6 level was refer to that, increased IL-6 lead to decreased of PCT at the first few days of infection, because the IL-6 more elevated during such time than other time of infection, PCT might be or not elevated at this time of infection. The figure (3-14) was describe such result.

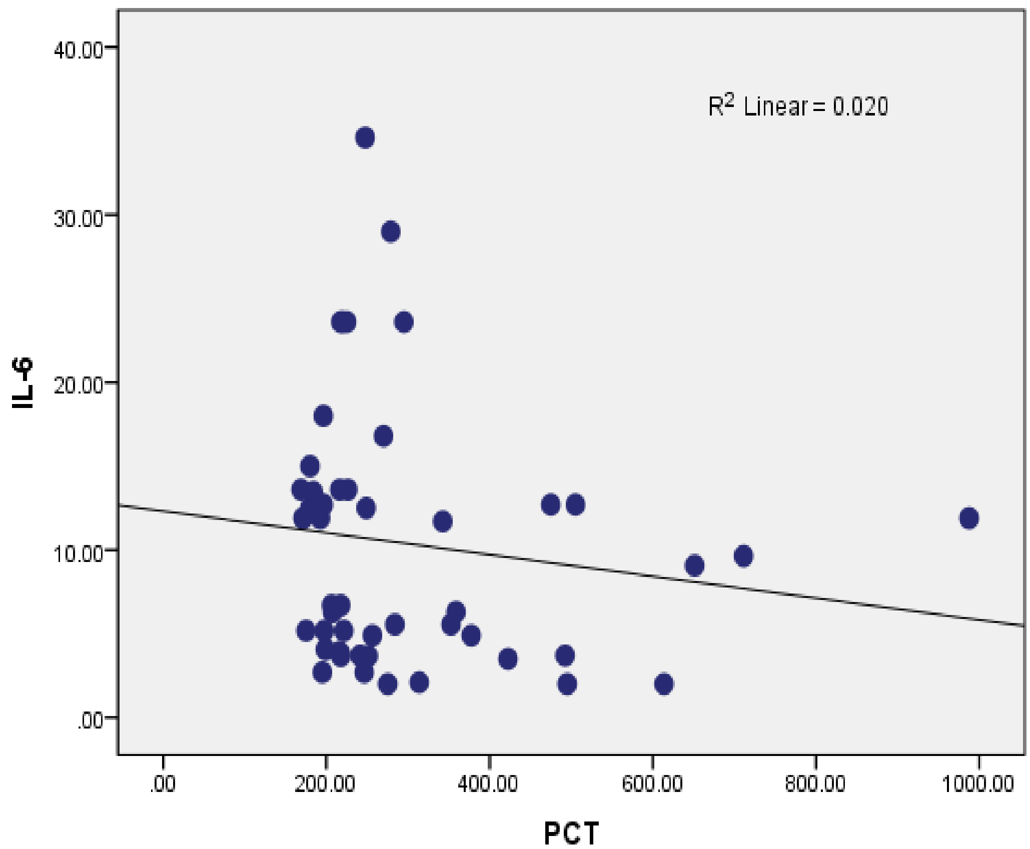


Figure (3-14): Correlation of IL-6 with PCT levels.

Using cytokines in combination with PCT may help to identify bacterial super infection in COVID-19 (Mazaheri *et al.*; 2022). In our COVID-19 cohort, CRP correlated positively with both IL-6 and IL-1β but there was no significant correlation between PCT and IL-6. It could be that the severity of COVID-19 in these patients with high IL-6 was due to virus driven inflammation rather than bacterial super infection (Mazaheri *et al.*; 2022). The immune system of SARS-CoV-2 patients may take a longer time to normalize , that of IL-6, IL-1β, TNF-α, CXCL-10, and reduced antiviral cytokines could be used as biomarkers of SARS-CoV-2 infection (Anuradha *et al.*, 2021).

As like as PCT, the correlation of IL-6 with IL-4 was show that reduced IL-4 in relation to IL-6 production , The IL-4 level might be refer to hypersensitivity interaction or allergic condition past infection with SARS-CoV-2 infection , Increased IL-6 in counter of IL-4 at the first few days of infection might be refer to that the immune response require more acute phase reactant than other or allergic reactant in respiratory environment. This result mentioned in figure (3-15). COVID-19 patients showed higher levels of IL-6, IL-10 and IL-13, but lower levels of IL-4 (Francisco *et al*.; 2021).

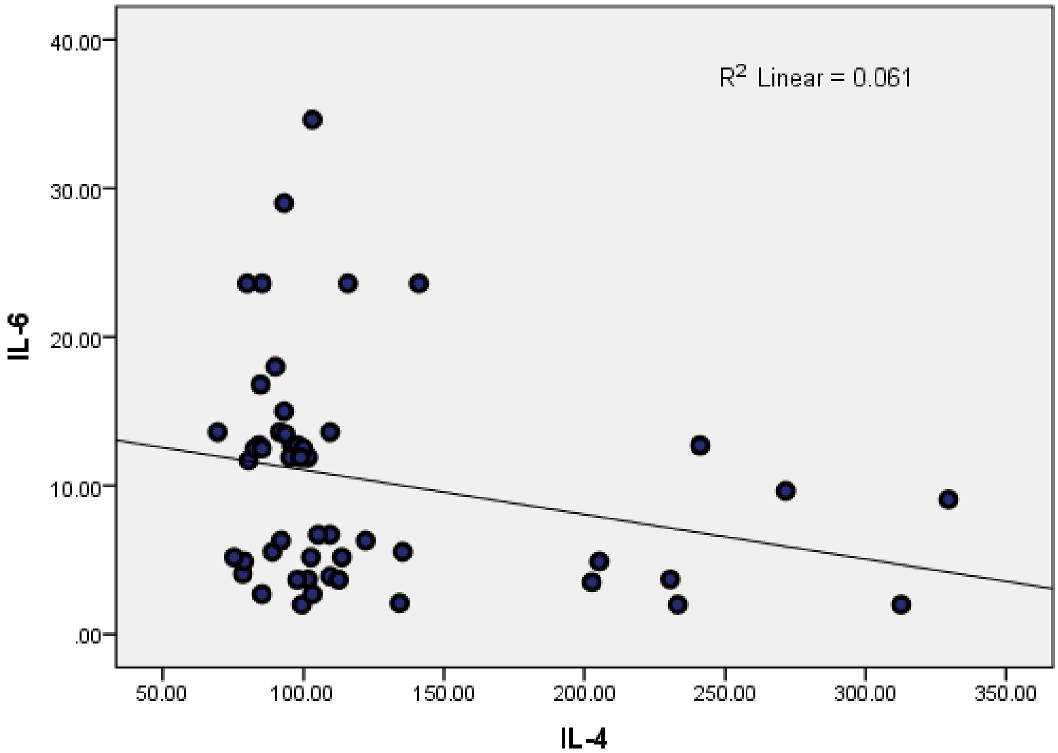


Figure (3-15): Correlation of IL-6 with IL- 4 in Sars-Cov2 infected patients.

The figure (3-16) showed that the IL-6 elevation might be accompanied with low Anti-SARS–CoV-2–IgG production, in which that the IgG antibody was increased after more than first week of infection in comparison with IL-6 increased during few hours or days from onset of infection.

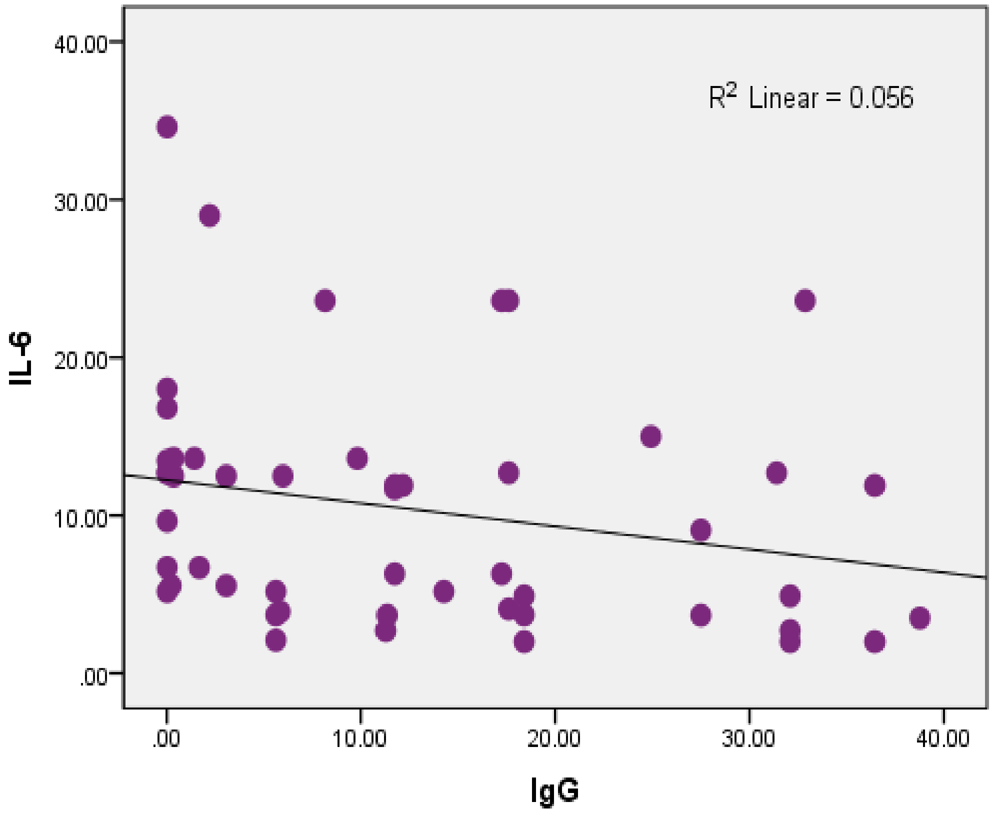


Figure (3-16): Correlation of IL- 6 in comparison with Anti-SARS-CoV-2 Ab.

Inhibitors of IL-6 or antagonists of IL-6R can be used as immunomodulatory agents for patients with SARS-CoV-2 induced disease (Lasky *et al*.; 2021). IL-6 is unable to cause immune damage on cells of target, thereby reducing the rate of inflammatory responses (Xu *et al*.; 2020) . Serum IL-6 is proposed to be considered in management and treatment of SARS-CoV-2 induced disease (Del vale *et al*.; 2020). Elevated levels of IL-6, CRP, PCT, D-dimer, and lower serum albumin levels were detected in subjects with fatal disease outcomes during treatment. These findings suggest a good prediction of in-hospital mortality in patients with COVID-19 who require admission to the ICU, especially when jointly using all four cutoff values (White *et al*.; 2021).

The result of increased in anti-SARS-CoV-2-IgM in comparison with low IL-6 level, this result might be show that the cytokine might be reduced after specific antibody produced against the SARS-CoV-2 infection. The Anti IgM antibody the first antibody were produced after first week of infection and gradually reduced during time, through replacement by IgG antibody type . As shown in figure (3-17).

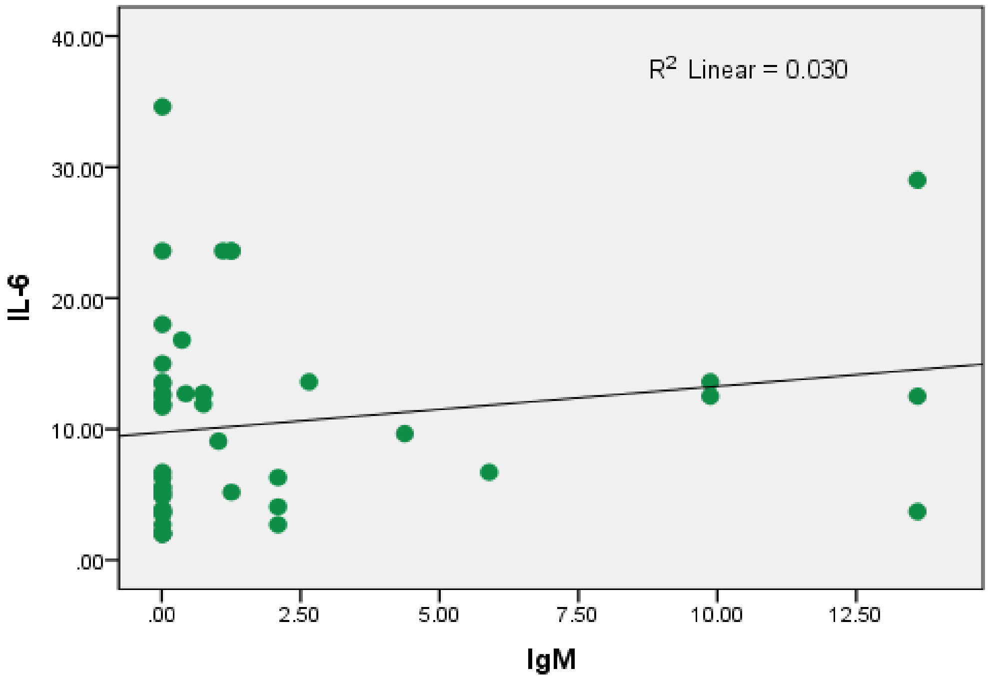


Figure (3-17): Correlation of IL- 6 with Anti-SARS-CoV-2 –IgM antibody.

Early responses of elevated cytokines such as IL-6 reflect the active immune responses, leading to high titers of IgG antibodies against COVID- 19 (Jing *et al*.; 2022). The COVID-19 related interstitial pneumonia triggers early IgG levels (higher than IgM) that gradually decrease over 12 months. Occasionally, it is possible to observe increase of IgM levels in presence of low concentrations of IgG and negative PCR for SARS-CoV-2 RNA. Baseline levels of IL-6 could be proposed as predictor of radiological mid/long-term squeal after COVID-19 related interstitial pneumonia (Domenico *et al*.; 2022).

3.8.2.Correlation of Interleukin-4 with other immunological parameters

The result of figure (3-18) showed that there is direct relationship between IL-4 and PCT, might be refer to that increased in IL-4 lead to increase in PCT level, this result might be showed that the secondary complication of SARS-CoV-2 infection was progress either to hypersensitivity interaction or secondary bacterial infection, This fact need to more studies and excrement to recoded as SARS-CoV-2 past infection complication.

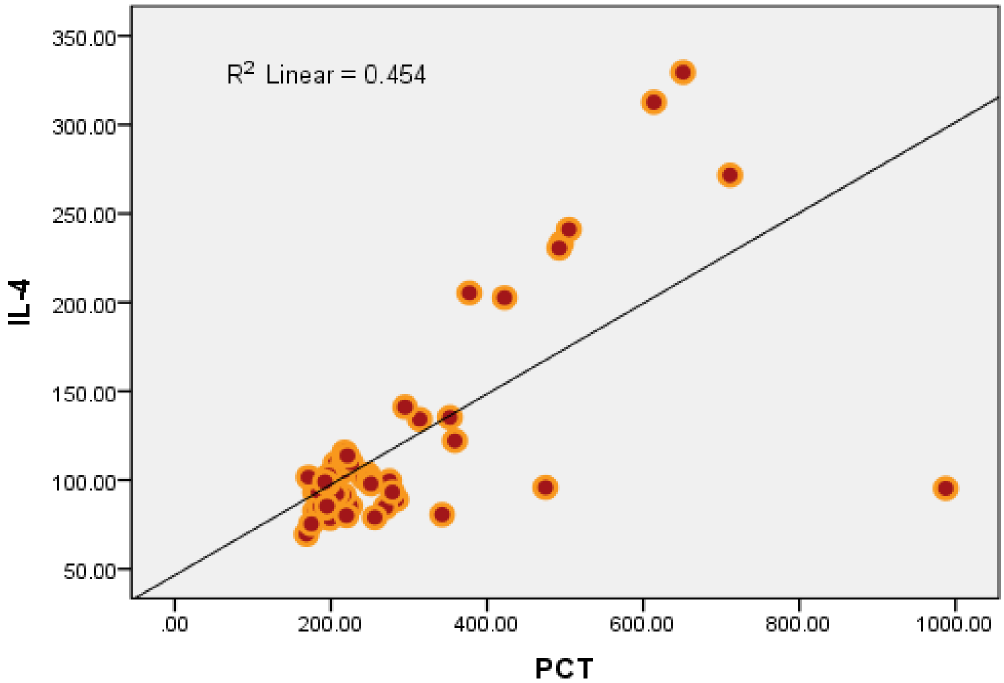


Figure (3-18): Correlation of IL-4 in relation to PCT in SARS-CoV-2 infected patients.

Increased level of Anti –SARS-CoV-2 –IgG antibody was lead to reduced IL-4 level, this result might be refer to that the active immune state in response to infection lead to enhancement of IgG production than IL-4. IgG prolonged for several months rather than IL-4 was reduced after few days or weeks of infection, this result mentioned in figure (3-19).

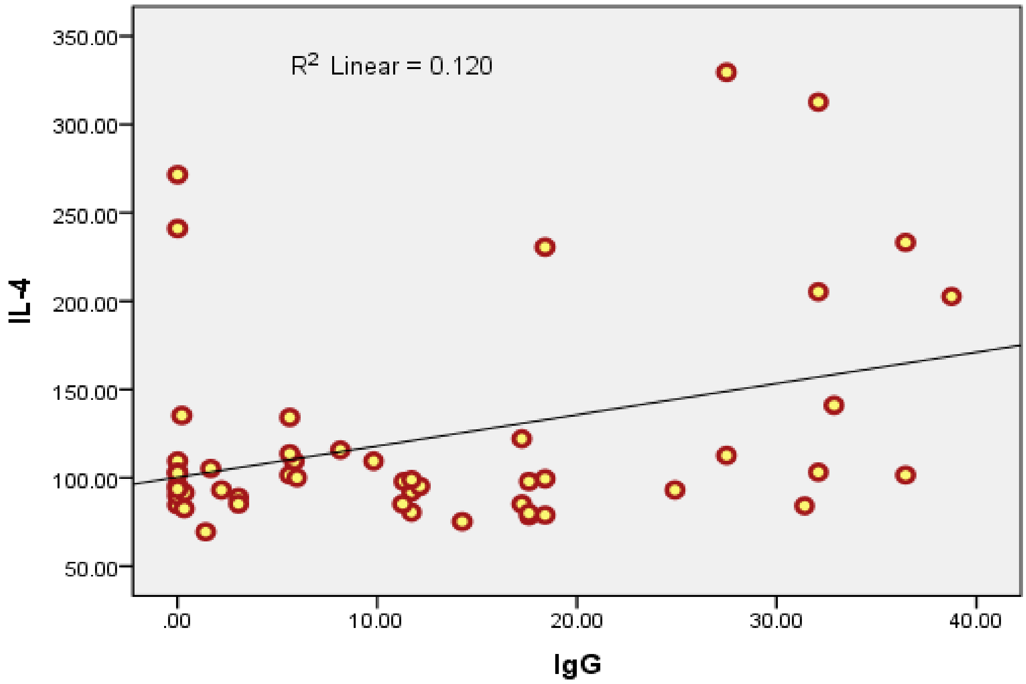


Figure (3-19): Correlation of IL-4 with IgG antibody.

This figure (3-20) showed that the low level of SARS-CoV-2 – IgM antibody was related with high level of IL-4, this result refer to that cytokine level was increased in first few days of infection before antibody production time. In addition to IL-6, CRP is a significant marker of COVID-19 inflammation.

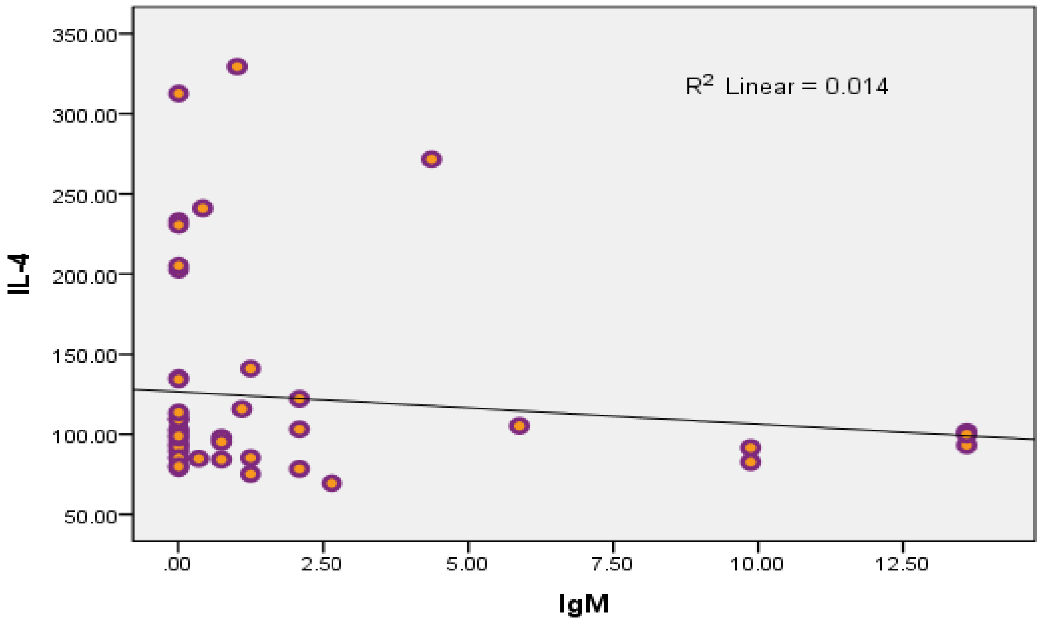


Figure (3-20): Correlation of IL- 4 with anti-SARS-CoV-2-IgM antibody.

Higher levels of serum CRP are associated with higher mortality in people with severe COVID-19 disease (Zhang *et al*.; 2021). Our findings show the severe COVID-19 patients' antibody levels were stronger than those of moderate patients, and a cytokine storm is associated with COVID-19 severity. There was a difference in immunoglobulin type between anti-S protein antibodies and anti-N protein antibodies in COVID-19 patients. And clarified the value of the profile in critical prevention (Yaolin *et al*.; 2021).

3.8.3.Correlation of PCT with Anti SARS-CoV-2 Antibody (IgM and IgG) .

The result of figures showed that the Anti-SARS-CoV-2-IgG antibody elevated in comparison with PCT level. While no relationship between PCT and Anti-SARS-CoV-2–IgM antibody. The PCT was produced during secondary infection, so that reduced PCT level might be refer to that reduce the chance of prognosis of secondary infection past SARS-CoV-2 infection. This results were shown in figures (3-21),(3-22) below. This study previously investigated the natural course of PCT and CRP and their value to identify secondary infections in critically ill COVID-19.

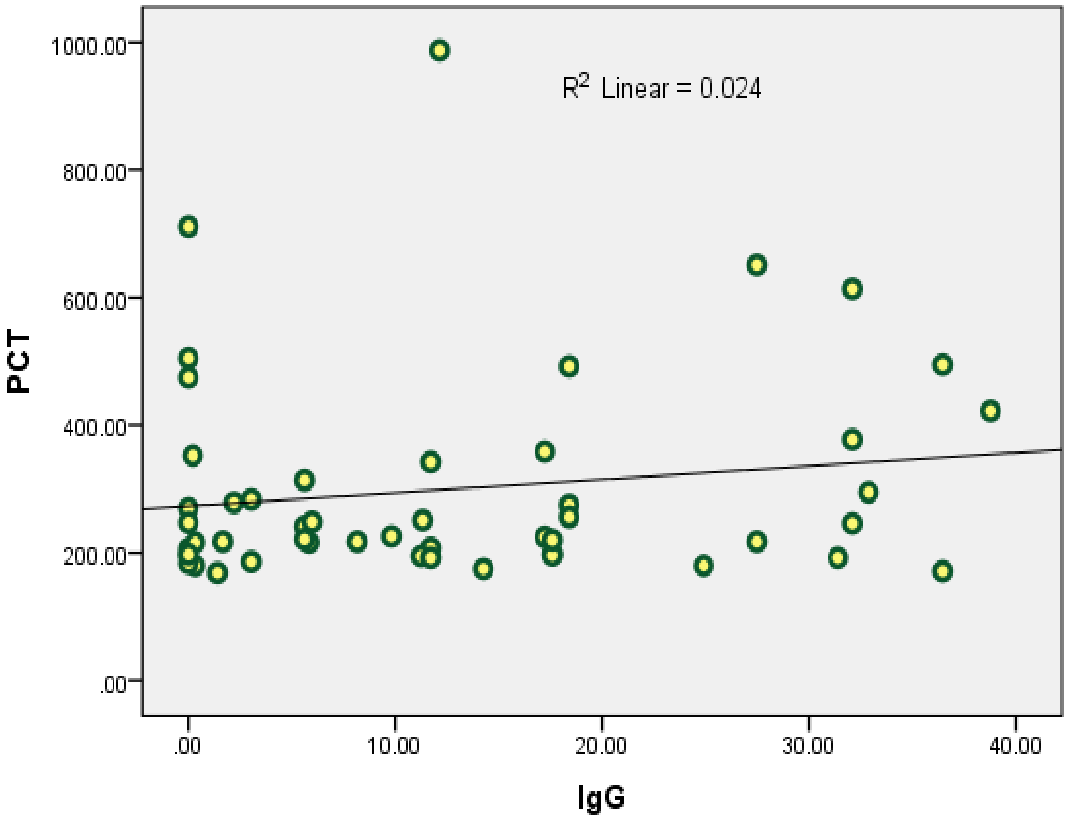


Figure (3-21): Correlation of PCT with Anti-SARS-CoV-2 –IgG Antibody.

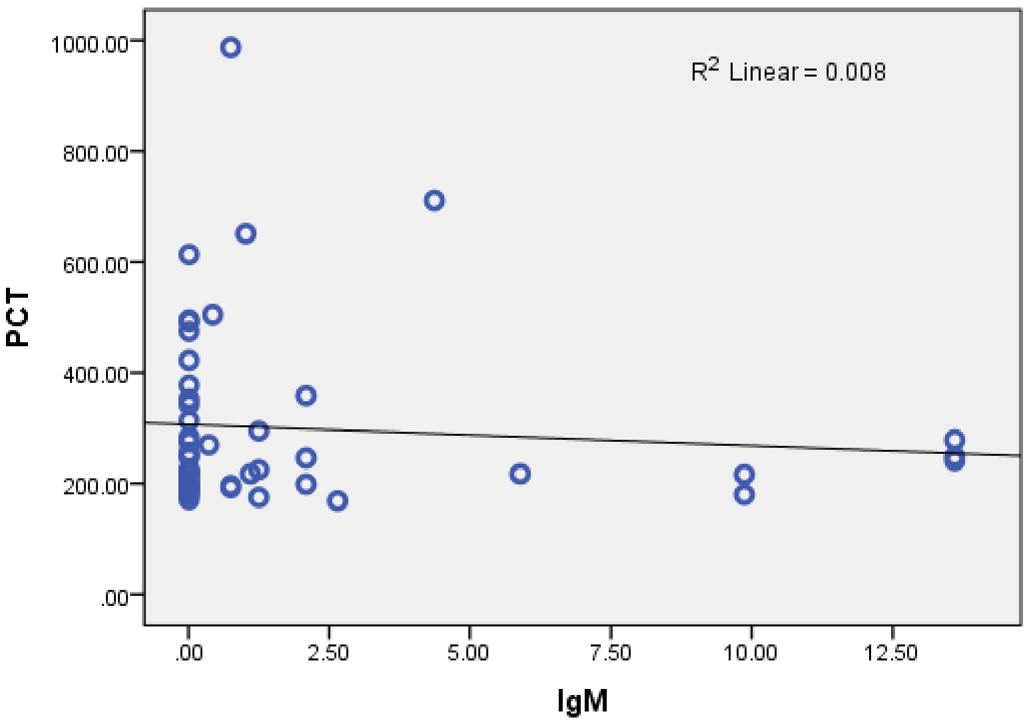


Figure (3-22): Correlation of PCT with Anti-SARS-CoV-2 –IgM Antibody.

The study showed that COVID-19 patients have elevated concentrations at ICU admission, that gradually decline, while a later increase in these biomarkers indicate a secondary bacterial infection (Berkel *et al*.; 2020). Elevated levels of IL-6, CRP, PCT, D-dimer, and lower serum albumin levels were detected in subjects with fatal disease outcomes during treatment. These findings suggest a good prediction of in-hospital mortality in patients with COVID-19 who require admission to the ICU, especially when jointly using all four cutoff values (White *et al*.; 2021).

3.8.4.Correlation of Anti-SARS-CoV-2 Antibody IgM with IgG .

The more elevation in Anti-SARS-CoV-2–IgG in comparison with Anti-SARS-CoV-2–IgM, might be refer to that the immunity status of patients infected with SARS-CoV-2 virus in which gradually active during time of exposure to infection by elevation of IgG instead of IgM, The IgG prolonged for several months with IgM might be reduced after few weeks of infection. This result was monitored in figure (3-23).

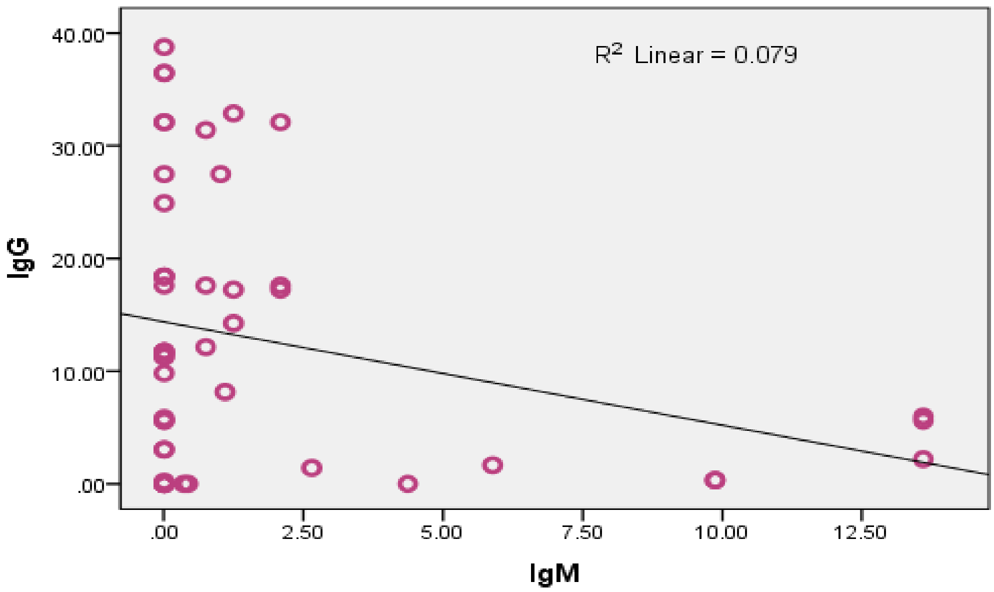


Figure (3-23): Correlation of Anti-SARS-CoV-2 –IgM and IgG in infected patients.

Microbial infections typically cause IgM to be produced as the first line of protection, and later, as long-term immunity and immunological memory, IgG is developed (Saghazadeh *et al*.; 2020). Based on serological studies of naturally infected populations, IgM antibodies are the first to be expressed and are mainly present in the circulation, promoting antigenic modulation (Galipeau *et al*.; 2020). IgG antibodies begin to appear later in the immune response because they undergo affinity maturation through somatic mutations, resulting in high affinity for the target antigen and an enhanced ability to neutralize the pathogen (Galipeau *et al*.; 2020).

3.9. Sensitivity and Specificity of all studied parameters with Anti-SARS-CoV-2 Antibodies .

The Roc curve analysis to determination of sensitivity and specificity between the all studied parameters , such as (CRP , D. Dimer , IL-6, IL-4, PCT, IgM and IgG against SARS-CoV-2, the result show that the CRP , D. Dimer , IL-6 , IL-4 , IgM and IgG level have more reliable and specific to monitor of the immune response in infected population, PCT have nonspecific indicator in which that used in SARS-CoV-2 diagnostic, might be used as prognostic marker to distinguish between bacterial and viral secondary complications. The Figure (3-24) and table (3-13) show the analysis curve and table of aria under the curve with statistical significant value monitor to each parameter .

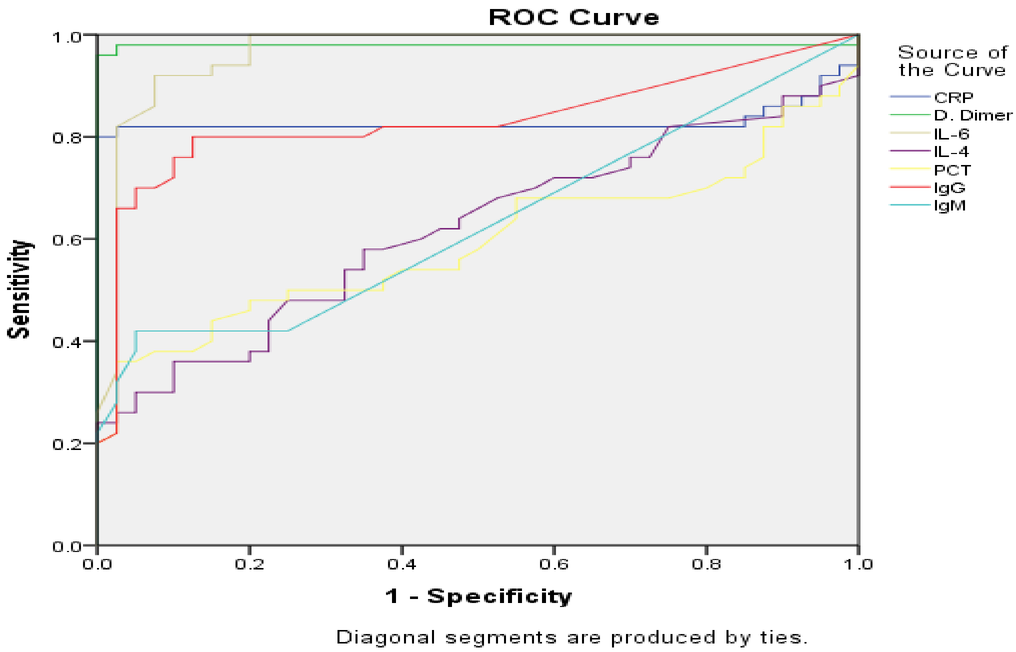


Figure (3-24): Roc curve of studied parameters.

Table (3-13): Analysis table of ROC curve with statistical significant value.

Area Under the Curve					
				Asymptotic 95% Confidence Interval	
Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Lower Bound	Upper Bound
CRP	.829	0.052	0.000	.728	.930
D. Dimer	.979	0.020	0.000	.941	1.000
IL-6	.966	0.020	0.000	.927	1.000
IL-4	.619	0.059	0.050	.504	.735
PCT	.593	0.061	0.130	.474	.712
IgG	.832	0.046	0.000	.742	.921
IgM	.632	0.058	0.033	.517	.746
The test result variable(s): IL-6, IL-4, PCT, IgG, IgM 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					

Conclusion and Recommendation

Conclusions and Recommendations

1.Conclusions

1. Gene differentiation of SARS-CoV-2 with allelic discrimination by real time PCR, The presence of S gene mostly in first few days of infection while E gene present in the end time of infection over 1 - 2 week of onset of infection as well as N gene but in less degree.
2. Genotype D/I in the ACE (I/D) polymorphism related of some infectious high effect on COVID-19 severity.
3. The patients that receive 2 dose of protective Corona vaccine before infected with SARS-CoV-2 revealed mild symptoms rather than newly infected population.
4. Decreased of platelets, increased of (CRP, Ferritin, D.Dimer and LDH) as diagnostic markers of SARS-CoV-2 infected patients, and increased of immunological parameters in response to SARS-CoV-2 infection such as (IL-6 , IL-4, PCT, IgG and IgM).
5. There was no differences in WBC, and eosinophil count at both patients (at all ages) and control, while reduced the platelets count in (> 60 years) comparison with control while there were no differences in hematological parameters among both male and female of SARS-CoV-2 infected patients.
6. Elevated IL-6 was revealed at patients with high fever and moderate cough also patients without cough, while IL-4, PCT and IgM were increased in patients with mild fever and patients without cough, while IgG was elevated in patients with moderate fever and mild to moderate cough.
7. IL-6 has negative correlation with PCT, IL-4, IgG and IgM, while IL-4 has positive correlation with PCT and negative correlation with IgG and IgM.

8. PCT level elevated in comparison with SARS-CoV-2 IgG antibody, while no relationship between PCT and SARS-CoV-2 IgM antibody.
9. The immunity status of patients infected with SARS-CoV-2 virus was gradually activation during time of exposure to infection by elevation of IgG instead of IgM.

2.Recommendations

1. Study other polymorphism related with **SARS-CoV-2** disease in Iraq population.
2. Investigation of other biomarkers for predication, diagnosis and monitoring of **SARS-CoV-2** hypersensitivity with complication.
3. Further studies recommended to exclude the prognostic criteria of Cov-19 patients in regarding to IL-4 and PCT as clinical distinguish parameters used to differentiate secondary bacterial infections from pulmonary hypersensitivity at ICU (Intensive Care Unit) patients.

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الخلاصة

فيروسات كورونا هي مجموعة من فيروسات الحمض النووي الرايبوزي والتي تسبب الأمراض للإنسان والفقاريات. يمكنها ايصال المرض إلى الجهاز التنفسي والجهاز الهضمي والكبد والجهاز العصبي المركزي للإنسان وهي فيروسات مغلفة تمتلك جينوم الحمض النووي الرايبوزي ذات الإحساس الإيجابي أحادية السلسلة. يمكن لهذا الفيروس أن يدخل جسم الإنسان عن طريق مستقبلات الإنزيم المحول للأنجيوتنسين 2 الموجودة في الأعضاء المختلفة والتي تكون مكملة لشكل السنبلة مما يسمح بحدوث ارتباط ناجح ويسهل دخول الفيروس إلى الخلايا الهدف.

تم جمع إجمالي 90 عينة دم 50 منها من الأشخاص الذين أصيبوا بفيروس سارس-2 و 40 عينه منها اصحاء غير مصابين بفيروس سارس-2 تتراوح اعمارهم (20-60 سنة) خلال تشرين الاول/2020 الى كانون الثاني/2021 تم فحص جميع المرضى عن طريق RT-PCR بمستشفى مرجان التعليمي . تم جمع عينات الدم من جميع مرضى كوفيد-19 والغير المريضين ، ثم تم استخراج الحمض النووي وتحليله من أجل تحديد جين الإنزيم المحول للأنجيوتنسين (I / D) ، والأنماط الجينية للانترلوكين 4 (PCR/RFLP) وترددات الأليلات بواسطة تفاعل البوليميريز المتسلسل و عن طريق الترحيل الكهربائي المستخدم للفحص.

وجدت النتيجة أن ترددات اليل (I/D) للمرضى، والتي وجدت تواترًا للأنماط الجينية DD و ID و II من جين ACE وجدت (21)42% و (19)38% و (10)20% على التوالي. كان تردد اليل D %59 مرة واحدة ، وكذلك تردد اليلI %41. عرض الترددات من DD و ID و II أيضًا الأنماط الجينية لجين ACE في الغير مريضين تظهر(13)32% ، (10)25%، (17)43% وجدوا تردد اليل D حوالي 41% ويمكن أن يكون تردد اليل I %59 ، كما تظهر نتائج IL-4 النمط الجيني تنوع حرج بين الأليلات في المرضى وغير المرضى ، ظهر CC في (21)52.5% مجموعة الاصحاء بينما كان (12)24% في المرضى ، وكان النمط الوراثي TT أكثر تتابعًا في المرضى (2) 56% من غير المرضى (14)35%. كان التركيب الجيني CT أكثر استمرارية في غير مرضى (5)12.5% من المرضى (10)20%. تمت دراسة بعض المتغيرات المناعية على مصل 50 مريضاً و 40 غير مريض باستخدام AFIAS... والمقايسة المناعية المرتبطة بالإنزيم (ELISA). وجدت النتيجة مستويات عالية من CRP و Ferritin و D-Dimer و LDH و PCT و IL-4 و IL-6 و IgG و IgM

299.95pg/mL, 236.48IU/L, 503.97ng/mL, 276.58ng/mL ,25.76mg/L) ومستويات منخفضة من كريات الدم 12.75, 13.17pg/mL, 122.89pg/mL, و1.77) البيضاء وعدد الحمضات والصفائح الدموية 7.7658Cells/cumm) على التوالي مقارنة بغير المرضى 0.2302Cells/cumm, و 171.68Cells/cumm) 226.40pg/mL ، 134.37IU/L ، 222.24ng/mL ، 147.61ng/mL ، 1.49mg/L) ، 7.9835Cells/cumm ، 0.08 ، 0.93 ، 2.07pg/mL ، 95.05pg/mL ، 0.3427Cells/cumm و 269.49Cells/cumm) على التوالي. تم إجراء التحليل الإحصائي باستخدام SPSS و ANOVA و Chi-Square.

التمايز الجيني لـفيروس سارس-2 مع التمييز الأليلي عن طريق تفاعل البوليميريز المتسلسل في الوقت الحقيقي ، ووجود الجين S غالبًا في الأيام القليلة الأولى من الإصابة بينما يكون الجين E موجودًا في نهاية وقت الإصابة على مدى 1- 2 أسبوع من ظهور العدوى وكذلك الجين N ولكن بدرجة أقل. يكون الجين N أكثر حفظًا واستقرارًا ، مع 90% من تماثل الأحماض الأمينية وطفرات أقل بمرور الوقت. النمط الوراثي D / I في تعدد الأشكال لجين ACE (I/D) المرتبط ببعض تأثيرات المعدية على معدل وفيات مرض كوفيد- 19. أظهر المرضى الذين تلقوا جرعتين من لقاح كورونا الوقائي قبل الإصابة بفيروس سارس- 2 أعراضًا خفيفة بدلاً من السكان المصابين حديثًا ، كما زاد مستوى IL-6 في المرضى غير الملقحين مقارنة بالتطعيم والسيطرة ، بينما (IL-4، PCT، IgM و IgG) في المرضى الذين تم تطعيمهم أكثر من غير الملقحين ، مما يشير إلى أن السكان الذين تم تطعيمهم لديهم مناعة أكثر من غير الملقحين. ظهرت نسبة عالية من مرضى كوفيد- 19 يعانون من حمى خفيفة وبدون سعال في وقت اجراء الدراسة. انخفاض عدد الصفائح الدموية وزيادة (CRP و Ferritin و D.Dimer و LDH) كمعايير تشخيصية للمرضى المصابين بفيروس سارس- 2 ، مع زيادة المعايير المناعية استجابة لعدوى سارس- 2 مثل (IL-6، IL-4 و PCT و IgG و IgM) نتيجة للدور الوقائي للجهاز المناعي وله مؤشر تنبؤي للمرض والنشاط والعدوى السابقة (التحصين). المرضى الصغار في الفئة العمرية (20 - 29 سنة) هم أكثر نسبة اصابه من البالغين (50 - 59 وكذلك > 60 سنة) بينما لا يوجد اختلاف في عدوى سارس- 2 بين المرضى الذكور والإناث. لا توجد فروق في عدد كريات الدم البيضاء وعدد الحمضات في كل من المرضى (في جميع الأعمار) الغير المرضى ، بينما انخفض عدد الصفائح الدموية في (> 60 سنة) مقارنة مع غير المرضى بينما لا توجد فروق في المعايير الدموية بين كل من الذكور والإناث. زيادة CRP و

ferritin و D. Dimer و LDH في سن مبكرة (30 - 39 سنة وعمر البالغين أكبر من 60 عامًا) وفي كل من الذكور والإناث من مرضى فيروس سارس- 2. زيادة مستوى IL-6 و IL-4 و PCT و IgG و IgM في مرضى كوفيد- 19 في جميع الفئات العمرية ، بينما يكون لدى المرضى الذكور مستوى أعلى من الإناث من (IL-6 و IL-4 و PCT و IgG المستوى) ، وانخفاض مستوى الأجسام المضادة IgM نتيجة لارتفاع النشاط المناعي في المرضى الذكور أكثر من الإناث. تم الكشف عن ارتفاع IL-6 في المرضى الذين يعانون من ارتفاع في درجة الحرارة والسعال المعتدل وكذلك المرضى الذين لا يعانون من السعال ، بينما تم زيادة IL-4 و PCT و IgM في المرضى الذين يعانون من حمى خفيفة والمرضى الذين لا يعانون من السعال ، بينما ارتفع IgG في المرضى الذين يعانون من حمى معتدلة وخفيفة و سعال معتدل. IL-6 له علاقة عكسيه مع PCT و IL-4 و IgG و IgM ، بينما IL-4 له علاقة طرديه مع PCT وعلاقة عكسيه مع IgG و IgM. ارتفع PCT مقارنة بالأجسام المضادة IgG لفيروس سارس- 2 ، بينما لا توجد علاقة بين PCT والجسم المضاد IgM . تم تنشيط حالة المناعة للمرضى المصابين بفيروس سارس- 2 تدريجياً خلال وقت التعرض للعدوى عن طريق ارتفاع IgG بدلاً من IgM.

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دراسة مناعية وجزيئية لتعدد اشكال النيوكليوتيدات المفردة لبعض الجينات المرتبطة بالمرضى المصابين بكوفيد-١٩ في محافظة بابل

تم تقديمها الى
مجلس كلية العلوم للبنات – جامعة بابل
كجزء من متطلبات الحصول على درجة الماجستير في علوم الحياة

من قبل

مصطفى رحيم طعمه المعموري

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