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Study of Some Immunological and Molecular Parameters in Patient Infected with COVID-19

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By

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بسمِ اللهِ الرحمنِ الرحيم

قَالُواْ سبحَانكَ لَا علمَ لَناَ إِلَّا مَا

عَلَّمتَنَا إِنَكَ أَنتَ العَلِيمُ الحَكِيمُ

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We certify that this thesis entitles **(Study of Some Immunological and Molecular Parameters in Patient Infected with COVID-19)** was prepared under our supervision at the department of Biology, College of Science for Women, University of Babylon as a partial requirement for the degree of Master in Biology.

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Dedication

To God Almighty.....

To the teacher of mankind, Muhammad bin Abdullah....

To the honorable members of the discussion committee....

To the respected search administrators....

To whom I had the honor of bearing his name, my dear father, our tent....

To the one who supported me in her prayers and supplications, my mother is the light of my eyes....

To those who have always supported me, my brothers and sisters.....

To the companion of the path and life, my dear wife, the mother of my children....

To everyone who taught me a letter, my dear professors....

To those who have been loyal to me, all my friends....

I dedicate this research to you

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Summary:

The present study was conducted in Babylon Governorate during the period from October 2021 to January 2022 on patients with Sars-Cov2 infection. As an patients found in the epidemiological wards of Babylon Governorate hospitals. The Study has done by collection of blood samples from 54 infected patients with CoVID-19 and from 41 healthy subjects as a control group for different age groups (Male and femal).

The results of the study revealed a significant increase in the levels of C-reactive protein, D.Dimer, Random blood sugar, Interferon- ɣ, Haemoglobin A1C, AntiSars-Cov2-IgG and IgM antibodies with no difference in the levels of Interleukin-6. The age group of adults at (50-59) years has a high level of C-reactive protein compared with other age groups as well as control groups. All age groups have higher levels of Random blood sugar compared to controls, except for the adult age group (60 years), which had the highest level of of random blood sugar due to stress, metabolism and hormonal changes. Adult age group (50 to 59 years old) had a higher level of D-Dimer rather than other age groups although it increased at all age groups, it was indicating thrombotic complications in affected patients. The young age group at (30-39 years, followed by 40-49 years old) has a high level of cortisol hormone compared to other age groups and control .

The young age group (20-29 years old) have a higher level of HBA1C compared with other age groups as well as the control groups.

High level of anti SARS-CoV2-IgG antibody compared to other age groups and control , at young age group (50-59 years, followed by 60 years).While anti SARS-CoV2-IgM antibody at the young age group (40-49 years) compared to other age groups.

High percentage of haemoglobin A1C in males more than in females, and the overall result of patient groups has increased compared to the control group, this result might be due to a lower level of haemoglobin in females than males.

The results showed a correlation between the ratios of haemoglobin A1C and cortisol level, Increased blood sugar and haemoglobin A1C increase the consumption of cortisol hormone and lead to a decrease in its level.

Increase in the level of interferon- γ more than cortisol in patients with diabetes during Sars-Cov2 infection. interleukin-6 increased more than cortisol in Sars-Cov2 diabetic patients. Increased interleukin-6 may activate the immune system by induction of acute phase protein production, and it was increased either at the initial onset of infection or during late infection with a poor or complex prognosis.

Increasing the level interleukin-6 over interferon-γ as a result of Sars-Cov2 infection, and there is a direct relationship between them, the interleukin-6 level was rapidly elevated instead of interferon-γ. This result may be due to interleukin-6 being more sensitive and higher production during this infection.

The results of multiple alignment of partial sequencing of the gene *IFITM3* showed 4 mutations located at sites 155G>A, 158G>T, 161G>A, and 168A>G. These mutations showed a high frequency of SNPs. After revealing the validity of these mutations, the results showed that the mutations at site 155 were rs7479267 G>A and on site158 was rs71452590 G > T, at position 161 was rs7478728 G > A and at 168 was rs6598045A>G.

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List of Abbreviations

No.	Abbreviations	Terms
1	+ssRNA	single-stranded positive-sense RNA
2	Abs	Absorption
3	ACE2	Angiotensin-converting enzyme 2
4	ACTH	Adrenocorticotropic hormone
5	ADP	Adenosine Diphosphate
6	AFIAS	Automated Fluorescent Immunoassay System
7	AIDS	Acquired immune deficiency syndrome
8	APPs	acute-phase proteins
9	ARDS	Acute respiratory distress syndrome
10	ARDS	acute respiratory distress syndrome
11	AT2	Angiotensin II receptor type 2
12	ATP	Adenosine triphosphate
13	BSA	bovine serum albumin
14	cAMP	Cyclic adenosine monophosphate
15	CD-4	Cluster of differentiation -4
16	CD-8	Cluster of differentiation-8
17	CK	creatine kinase
18	COI	cut-off index
19	COVID-19	coronavirus disease -2019
20	CoVs	Corona virus
21	CRF	case-fatality rate
22	CRP	C-reactive protein
23	CT	computed tomography
24	CTLs	cytotoxic T lymphocytes
25	CVD	Cardiovascular disease
26	DB	detection buffer

27	DCs	dendritic cells
28	DHEA	Dehydroepiandrosterone
29	DM	Diabetes mellitus
30	DMVs	double-membrane vesicles
31	DNA	Deoxyribonucleic acid
32	DNV	Dengue virus
33	E	Envelope
34	EBOV	Ebola virus
35	EDTA	Ethylene diamine tetra acetic acid
36	ELISA	Enzyme Linked Immunosorbent Assay
37	FDP	fibrin degradation product
38	GA	Golgi apparatus
39	G-CSF	granulocyte-colony stimulating factor
40	GLUT2	glucose transporter 2
41	GM-CSF	Granulocyte macrophage colony-stimulating factor
42	HbA1C	Haemoglobin A1C
43	HCoVs	human coronaviruses
44	HE	Hemagglutinin esterase
45	HIV	Human immunodeficiency virus
46	HIV-1	human immunodeficiency infection type 1
47	HLH	hemophagocytic lymphohistiocytosis
48	HPA	Hypothalamic pituitary adrenal
49	IAAP	Islet amyloid polypeptides
50	IBv	infectious bronchitis virus
51	ICTV	International Committee of Taxonomy of Viruses
52	ICU	intensive care unit
53	IFITM	interferon-induced transmembrane
54	IFN-l	Interferon One
55	IFN-ß	Interferon Beta
56	IFN-α	Interferon Alpha

57	IFN-γ	Interferon Gama
58	Ig A	Immunoglobulin A
59	IgM and IgG	immunoglobulin M– G
60	IL-6	Interleukin 6
61	IL-8	Interleukin 8
62	ILCs	innate lymphoid cells
63	IP-10	interferon inducible protein-10
64	IR	insulin resistance
65	ISG	IFN-stimulated gene
66	LDH	lactic dehydrogenase
67	LDL	Low density lipoprotein
68	LFIAs	lateral flow immunoassays
69	LSD	Least significant difference
70	M	Membrane
71	MAF	Minor allele frequency
72	MCP-1	monocyte chemoattractant protein-1
73	MERS- CoV	Middle East respiratory syndrome coronavirus
74	MHC	Major histocompatibility complex
75	MIP-1α	Macrophage inflammatory protein-1 Alpha
76	Mpro	major protease
77	mRNAs	messenger RNAs
78	N	Nucleocapsid
79	NAAT	nucleic acid amplification test
80	NCBI	National center for biotechnology information
81	NK	natural killer
82	NKG2A	natural killer group 2A
83	NO	Nitric oxide
84	Nsps	Non structural proteins
85	OD	optical density

86	OR	Odd ratio values
87	ORFs	Open reading frames
88	PBS	phosphate buffer saline
89	PCR	polymerase chain reaction
90	pp1a/pp1ab	polyprotein 1a/1ab
91	PRRs	pattern recognition receptors
92	RBD	recombinant receptor-binding domain
93	RBS	Random blood sugar
94	RNA	Ribonucleic acid
95	RTC	replication-transcription complex
96	RT-PCR	Real-time polymerase chain reaction
97	S	Spike
98	SARS	Severe acute respiratory syndrome
99	SARS-CoV	severe acute respiratory syndrome coronavirus
100	SGPT	glutamic-pyruvic transaminase
101	sgRNAs	subgenomic RNAs
102	SNPs	Single nucleotide polymorphisms
103	SNS	Sympathetic nervous system
104	SPSS	Statistical package for the social sciences
105	T1DM	Type 1 Diabetes Mellitus
106	T2DM	Type 2 Diabetes Mellitus
107	TCR	T cell receptor
108	T_H1	T helper 1
109	Tim-3	T-cell immunoglobulin mucin-
110	TNF-α	Tumor necrosis factor Alpha
111	US FDA	United states Food and drug administration
112	WHO	World Health Organization

Chapter One
Introduction

1.1. Introduction:

In December 2019, an outbreak of coronavirus infection with the unknown source (COVID-19) first emerged in Wuhan, China, resulting in more than 80,000 confirmed cases in this country and being transported to an increasing number of countries (Zhu *et al*., 2020). World Health Organization (WHO) reports global public health emergency over the COVID-19 outbreak on January 30, 2020. Therefore, the coronavirus infection nearby to severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) has become a global emergency and a crucial health issue for humans (Wu *et al*., 2020). COVID-19 spread to the world in a short time and was declared as public health emergency of international concern by World Health Organization. The disease has been found to be associated with various systemic and pulmonary sequelae pulmonary fibrosis being one of them (Mrigpuri *et al*., 2021).

The COVID-19 causes distinct clinical manifestations among affected individuals, ranging from asymptomatic cases to cases with mild, moderate, severe, or critical symptoms, where they might progress to an acute respiratory distress syndrome (SARS) or even to dysfunction of multiple organs, complications that lead to death (Kabeerdoss & Danda, 2020 ; Huang *et al*., 2020).

Multiple evidences support that the humoral response, mainly antibodies against the S protein, blocks virus attachment to susceptible ACE2+ cells (Zhou *et al*., 2020). However, there are still many questions regarding the significance of antibodies against the different viral proteins, and the cross reactivity of antibodies against other highly prevalent alpha- and beta-coronavirus, although it seems that cross reactivity occurs mostly within the beta-coronaviridae (Huang *et al*., 2020

; Guo *et al*., 2020), particularly between SARS-CoV and SARS-CoV-2 that share 90% of the amino acid sequence in S1 (Walls *et al*., 2020). Antibodies IgM and IgG can be detected early during the 1st week of symptom onset, whereas IgG can be detected at around 14 days after the initiation of symptoms however, given the short time elapsed since the beginning of the COVID-19 pandemic, it is not known how long the protecting levels of these blocking antibodies will remain active (Huang *et al*., 2020 ; Guo *et al*., 2020 ; Theel *et al*., 2020).

The risk of developing a more aggressive disease condition is influenced by age and the presence of comorbidities, such as diabetes, hypertension, obesity, and cardiovascular diseases (García *et al*., 2020 ; Ejaz *et al*., 2020). Markers, such as the increase in C-reactive protein (CRP), D-dimer, and prothrombin time, are important to predict the outcome of the disease (Xavier *et al*., 2020 ; Elshazli *et al*., 2020). Also, immunological disorders were observed, such as leukopenia (Violetis *et al*., 2020 ; Jenkins, Mccaw, & Goepfert 2020), as well as a significant increase in serum cytokines levels, such as IL-6, TNF-α, and IL-8 (Jenkins, Mccaw, & Goepfert 2020 ; Leisman *et al* 2020). Among the altered cytokines, interferons present conflicting results (Brodin, 2021). As well as a significant increase in serum cytokines levels, such as IL-6, TNF-α, and IL-8 (Jenkins, Mccaw, & Goepfert. 2020 ; Leisman *et al* 2020). Among the altered cytokines, interferons present conflicting results (Brodin, 2021). Interferons are divided into type I (IFN-α and IFN-ß), type II (IFN-γ), and type III (IFN-l) and have a fundamental role in the innate immune system, being part of the first line of defense against viral infections (Huang *et al*., 2019).

1.2. The aim of study:

The present study was planned to evaluation of the risk developing conditions such as diabetic to be give more aggressive status in which that associated with SARS–CoV2 infection (Covid -19), as well as the goal of this study is to analyze the main aspects of the immune response against SARS-CoV2.

To complete this aim, the following objectives were done:-

1.Estimation of Blood glucose level and HbA1C during acute state of Covid -19 infection according to age, sex and other demographic criteria.

2.Monitoring the specific anti Sars-Cov2 antibodies (IgM and IgG) by qualitative (rapid test), and confirmed by quantitative measurement by advance procedure.

3.Determining tye level of interleukin-6 in patients with diabetes as a complication of COVID-19 infection were estimation by ELISA technique.

4.The cortisol hormone were estimation by ELISA technique.

5.The specific *IFTIM3* gene expression was determination by PCR technique.

Chapter two

Literature Review

2.1. Coronavirus (CoVs):

The name "coronavirus" refers to the enveloping positive sense RNA viruses having spike-like projections on their surface that give them a crown-like appearance under an electron microscope. Coronaviruses have a diameter of 60 nm to 140 nm (Richman *et al*., 2016).

The severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003 and the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 produced human epidemics. Four corona viruses, HKU1, NL63, 229E, and OC43, have been in circulation in humans and often cause moderate respiratory disease. There are many notable distinctions and parallels between the current virus and earlier viruses. The case fatality rates for MERS-CoV and SARS-CoV are both significantly higher (40 percent and 10 percent, respectively). Despite having a DNA that is 79% identical to the first SARS-CoV, the current SARS-CoV-2 looks to be significantly more contagious (De Wit *et al*., 2016). The angiotensin-converting enzyme 2 (ACE2) receptor allows both MERS-CoV and SARS-CoV to infect cells (Wan *et al*., 2020).

The SARS-Cov-2 binds to ACE2 on alveolar epithelial cells and primarily infects lower airways initially. Both viruses are effective inflammatory cytokine inducers. The proposed mechanism for organ injury is the "cytokine storm" or "cytokine cascade." The virus triggers the release of inflammatory cytokines and chemokines into the pulmonary vascular endothelial cells as well as the activation of immune cells. Despite being frequently linked to acute respiratory infections in humans, coronavirus is a complex pathogen due to its capacity to infect a wide range of hosts and disorders (Fung *et al*., 2019).

Wild animals are a frequent source of zoonotic infections because of their frequent contact with humans. Both the Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV) and the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) are zoonotic pathogens that can lead to serious respiratory conditions in people (Luk *et al*., 2019; Ramadan and Shaib, 2019). Another pathogen that affects humans is the novel coronavirus known as 2019-nCoV. In 2019, three bronchoalveolar-lavage samples from Chinese adult patients with unexplained severe pneumonia were used for viral metagenomics, which led to the discovery of this new virus (Zhu *et al*., 2020).

According to illness development patients can be loosely split into two groups: asymptomatic or moderate cases, who typically recover, and severe cases, who suffer multi-organ failure, especially respiratory failure, and require admission to an intensive care unit (ICU), about 15% of whom (Wang *et al*., 2020). For COVID-19 to be resolved, an effective immune response to SARS-CoV-2 may be regarded as essential. However, several studies have found a substantial correlation between the severity of the disease and the concentrations of pro-inflammatory cytokines and immune cell subsets (Wang *et al*., 2020).

2.1.1. The Coronavirus in Historical Perspective:

The first human coronaviruses, HCoV-229E and HCoV-OC43, were identified in 1966 and 1967, respectively (Hamre & Procknow, 1966). The following time frame was crucial for the discovery of research milestones that significantly added to our understanding of coronaviruses: polyprotein processing (1986) (Denison & Perlman, 1986), the first full-length coronavirus genome sequence (1987) (Boursnell *et al*., 1987), and

the first recombinant coronaviruses created by targeted recombination (1992). (Koetzner *et al*., 1992).

There were 8,000 reported cases of SARS as a result of the zoonotic spread of the severe acute respiratory syndrome coronavirus (SARS-CoV) and the ensuing SARS epidemic in 2002–2003, 10% of which were fatal (Zhong *et al*., 2003). More than 2,500 human cases of MERS have been reported since 2012 as a result of the development of a second highly deadly coronavirus of zoonotic origin, MERS-CoV, which is linked to severe clinical symptoms and a 36% case fatality rate (Zaki *et al*., 2012).

The pathogenic SARS-CoV-2 recently spread quickly among humans after possibly originating in bats or from an undetermined intermediate host (Zhu *et al*., 2020). More than 200 nations have reported more over 40 million CoVID-19 cases as of October 2020, leading to more than 1 million fatalities (CoVID-19 Dashboard). Clinical manifestations range from asymptomatic or mild infections to acute lung inflammation and pneumonia, mostly in the elderly and patients with comorbidities. The SARS-CoV-2 targets both upper and lower respiratory tract tissues, and efficient human-to-human transmission occurs even before the onset of symptoms (Huang *et al*., 2020).

2.1.2. Classification and Taxonomy of CoVs:

The term "Corona viruses" (COVs) comes from a Greek word that means "crown," alluding to the look of COV viruses under an electron microscope that resembles a crown or corona (Woo *et al*., 2010).

The coronaviruses are enclosed, icosahedral symmetric particles with a diameter of 80–220 nm and a non-segmented, single-strand, positive-sense RNA genome of around 26–32 kb (Weiss & Navas, 2005).

The order Nidovirales, suborder Cornidovirineae, and family Coronaviridae each contain a sizable group of viruses known together as coronaviruses (CoVs). The two subfamilies of the coronaviridae are called letovirinae and orthocoronavirinae. Alphaletovirus is a member of the Letovirinae family, and the Orthocoronaviridae family is further divided into the genera Alphacoronavirus (CoV), Betacoronavirus (CoV), Gammacoronavirus (CoV), and Deltacoronavirus (CoV) based on phylogenetic study and genome structure (ICTV 2018) Figure (2-1) displays the Coronaviridae according to its most recent categorization (Lai *et al*., 2007).

The coronaviruses infect a variety of wild and domestic species; α- and βCoVs primarily infect birds, whereas γ- and δ CoVs infect mammals. In hospitalized patients with symptoms of the common cold, a human coronavirus (HCoV) known as B814 was initially discovered in 1960. (Tyrrell & Bynoe, 1965). Currently, 229E and NL63, which are members of the CoVs, and HKU1, OC43, SARS, MERS, and SARS-CoV-2, which are members of the CoVs, are the seven different HCoVs that infect people. SARS (SARS-CoV), MERS (MERS-CoV), and SARS-CoV-2 are examples of human respiratory and gastrointestinal diseases caused by alpha- and beta-coronaviruses, although gamma- and delta-coronaviruses can also infect birds in addition to mammalian and human hosts (Forni *et al*., 2017; Zhou *et al*., 2020).

The first human coronavirus (HCOV) was discovered in the nasal secretions of cold patients. Currently, 6 distinct COV strains have been identified as human pathogens (Kin, 2015). The two primary HCOVs (from lineages Alpha and Beta) that account for 15% to 29% of all colds are 229E and OC43. Between 2002 and 2003, SARS-COV caused an outbreak of a serious respiratory disease in China, and since 2012,

MERS-COV has been responsible for an outbreak of a serious respiratory disease in the Middle East (Raj *et al*., 2013).

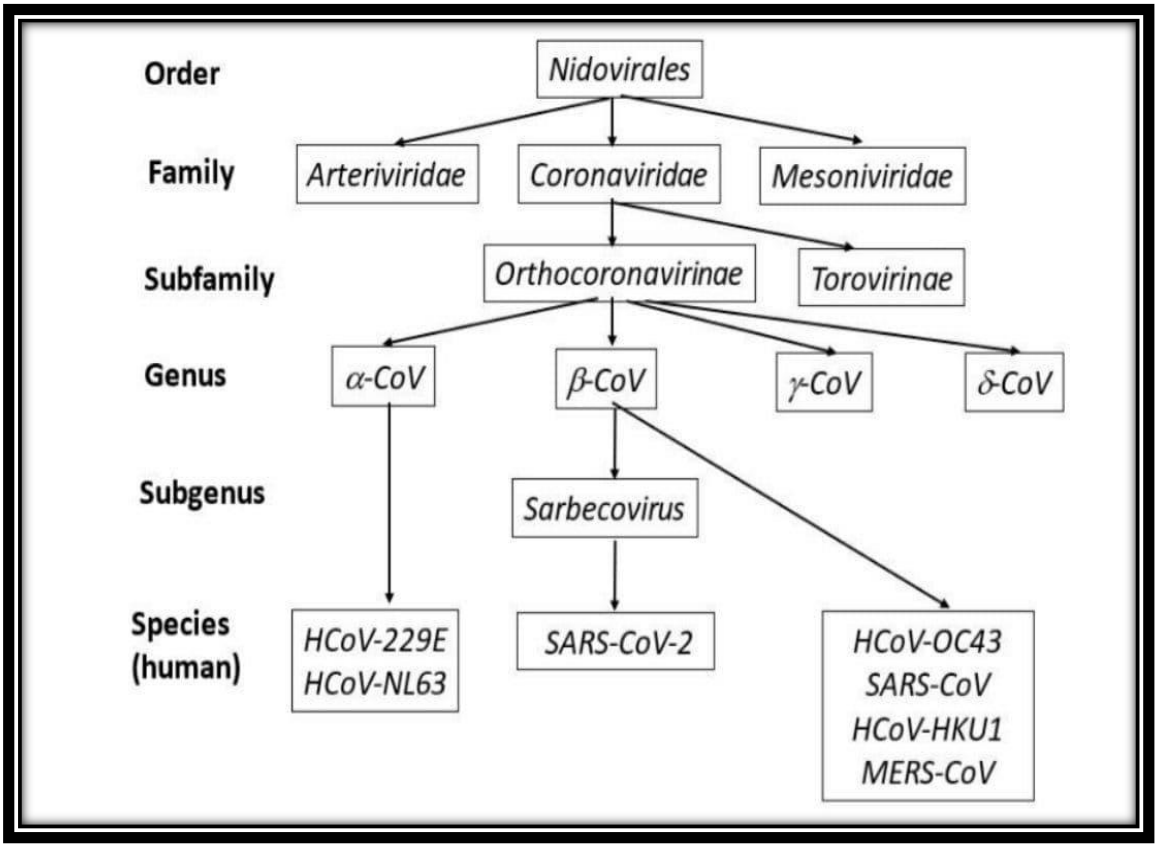


Fig (2-1): Classification of Human Coronaviruses (Malik, 2020)

Classification of Coronaviridae according to the International Committee of Taxonomy of Viruses (ICTV), with special emphasis on reservoir host, zoonotic importance, and major epidemics.

2.1.3. Coronavirus structural characteristics:

The coronaviruses are large, approximately spherical particles with distinctive surface projections (Goldsmith *et al*., 2004). With an average diameter of 80 to 120 nm, their size is extremely varied. 50 to 200 nm in diameter are regarded as extreme sizes (Masters, 2006). 40,000 kDa is generally the overall molecular mass. They are wrapped in a covering that contains many protein molecules (Lalchhandama, 2020). The lipid bilayer

envelope, membrane proteins, and nucleocapsid shield the virus when it is outside the host cell (Neuman *et al*., 2011). Four primary structural proteins the spike (S) glycoprotein, small envelope (E) glycoprotein, membrane (M) glycoprotein, and nucleocapsid (N) protein as well as a number of auxiliary proteins make up the SARS-CoV-2 (CoV19) virus's structure (Fig. 2-2). (Jiang *et al*., 2020).

The membrane (M), envelope (E), and spike (S) structural proteins are embedded in a lipid bilayer that makes up the viral envelope (Lai & Cavanagh, 1997). The structural proteins E and M work with the lipid bilayer to shape and maintain the size of the viral envelope, Interaction with the host cells requires the S proteins (Fehr & Perlman, 2015).

The M protein of the human coronavirus NL63, however, differs from other coronaviruses in that it possesses a binding site for the host cell (Naskalska *et al*., 2019). The envelope measures 85 nm in diameter. A unique pair of electron-dense shells that are comparatively opaque to the electron beam used to scan the viral particle appear to make up the virus's envelope in electron micrographs (Neuman *et al*., 2006).

The M protein, a type III membrane protein, is the major structural protein of the envelope and gives it its overall form. It has 218 to 263 amino acid residues and is 7.8 nm thick (Godet *et al*., 1992). Three domains make up this protein: a short N-terminal ectodomain, a triple-spanning transmembrane domain, and a C-terminal endodomain. The C-terminal domain creates a matrix-like lattice that increases the envelope's additional thickness. The amino-terminal domain of a protein may contain either N- or O-linked glycans, depending on the species. The assembly, budding, envelope formation, and pathogenic stages of the virus lifecycle depend on the M protein (Schoeman & Fielding, 2019).

The E proteins are small structural proteins that vary greatly between species. They range in size from 8.4 to 12 kDa and include 76 to 109 amino acids (Masters, 2006). A coronavirus particle contains only about 20 copies of the E protein component (Godet *et al*., 1992).

The corona- or halo-like appearance is caused by the spikes, which are the coronaviruses' most distinctive feature. A coronavirus particle typically contains 74 surface spikes (Alsaadi & Jones, 2019). Each spike measures 20 nm in length and is made up of a trimer of the S protein, which is itself made up of the S1 and S2 subunits. The two subunits are kept together by a noncovalent bond while they are exposed on the viral surface until they bind to the host cell membrane (Lalchhandama, 2020).

The transmembrane protein known as the spike, or S glycoprotein, has a molecular weight of around 150 kDa and is located in the virus's outer layer. By attracting the angiotensin-converting enzyme 2 (ACE2) produced in cells of the lower respiratory tract, S protein creates homotrimers that protrude from the viral surface and aids in the attachment of envelope viruses to host cells. The host cell's furin-like protease splits this glycoprotein into the S1 and S2 subunits. S1 proteins are the most important components in terms of infection, but they are also the most variable because they are responsible for host cell specificity. Part S1 is responsible for determining the host virus range and cellular tropism with the receptor binding domain make-up, while S2 functions to mediate virus fusion in transmitting host cells (Walls *et al*., 2020).

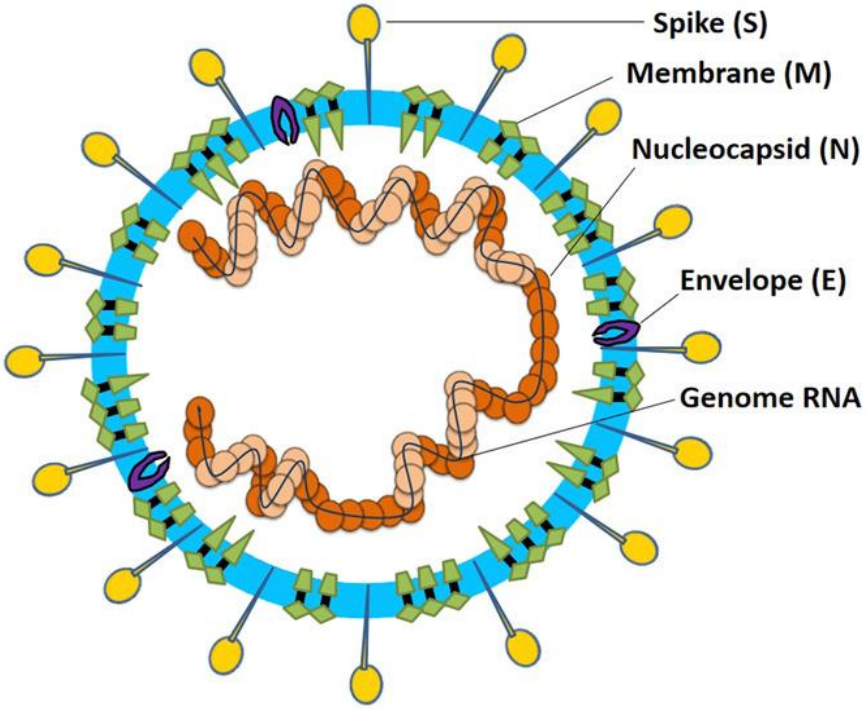


Fig. (2-2) Structure of severe acute respiratory syndrome coronavirus (Malik, Kumar & Soni, 2020)

2.1.4. Coronavirus Genome Structure:

The single-stranded positive-sense RNA (+ssRNA) genome of CoVs is around 30 kb in size and has a 5′-cap structure and 3′-poly-A tail. In order to create the replication-transcription complex (RTC) in double-membrane vesicles (DMVs), polyprotein 1a/1ab (pp1a/pp1ab), which encodes nonstructural proteins (nsps), is directly translated from genomic RNA (Snijder *et al.*, 2006). RTC then produces a nested group of subgenomic RNAs (sgRNAs) through discontinuous transcription (Hussain *et al.*, 2005). The 5′-leader and 3′-terminal regions of these subgenomic messenger RNAs (mRNAs) are shared. Between open reading frames, transcription regulatory sequences cause transcription to end and subsequently acquire a leader RNA (ORFs). These minus-strand sgRNAs act as the building blocks for subgenomic mRNAs (Perlman & Netland, 2009).

A normal CoV's genome and subgenomes have at least six ORFs, Except for Gammacoronavirus, which lacks nsp1, the first ORFs (ORF1a/b), which make up roughly two-thirds of the length of the entire genome, encode 16 nsps (nsp1–16). Two polypeptides, pp1a and pp1ab, are produced as a result of a 1 frameshift between ORF1a and ORF1b. These polypeptides are converted into 16 non-structural proteins (nsps) by virally encoded chymotrypsin-like (3CLpro) or major protease (Mpro) and one or two papain-like proteases (Masters, 2006). At least four major structural proteins are encoded by additional ORFs on the third of the genome, close to the 3′-terminus, including the spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins. Different CoVs also encode unique structural and accessory proteins such HE protein, 3a/b protein, and 4a/b protein in addition to the four basic structural proteins. The sgRNAs of CoVs are used to translate all of the structural and auxiliary proteins (Sawicki *et al*., 2007). Sequence research reveals that the 2019-nCoV is a member of the group of betacoronaviruses that also contains the SARS-CoV, MERS-CoV, Bat-SARS-like (SL)-ZC45, and Bat-SL ZXC21 and has the characteristic genome structure of a CoV. According to the phylogenetic tree of CoVs, 2019-nCoV is more distantly connected to SARS-CoV and more closely related to bat-SL-CoV ZC45 and bat-SL-CoV ZXC21 (Li *et al*., 2020).

2.1.5. COVID-19 Disease Pathogenesis:

The SARS-CoV-2 adheres to the surface of the otic canal, conjunctival mucosal membranes, and oral epithelial membranes. It is thought that the ACE 2 protein, which is abundantly expressed on a variety of human cells, including type II alveolar cells (AT2), oral, esophageal, ileal, cardiac, proximal tubule cells of the kidneys, as well as

urothelial cells of the bladder, mediates the internalization of the SARS-CoV2 (Zou *et al*., 2020).

A cellular enzyme known as furin cleaves the spike (S) protein of SARS-CoV2 at the S1/S2 location. For the virus to enter the lung cells, this cleavage is necessary (Hoffmann *et al*., 2020). The TMPRSS2 primes the activated S protein before it subsequently connects to ACE 2 receptors to enter the host cells. The SARS-CoV-2 genetic makeup is analogous to that of the original SARS-CoV, and these viruses have a lot of structural similarities in their (S) proteins. They both enter the host cell through the same receptor, but SARS-CoV-2 has a tenfold higher affinity for ACE 2 receptors (Wrapp *et al*., 2020).

The experimental findings imply that the essential function of ACE 2/angiotensin (1-7) in signaling pathways causing tissue injury and inflammation (Rodrigues *et al*., 2017). Angiotensin II is degraded by ACE 2 in order to produce angiotensins 1 through 7, which act as ACE II's antagonists (Tikellis *et al*., 2012).

Inflammatory cytokines like interleukins (IL-2, IL-6, IL-7, and IL-10), G-CSF, IP-10, MCP-1, MIP-1α, and TNF have a positive correlation with COVID-19 severity. Significantly lower lymphocyte counts are seen in patients with advanced illness (Huang *et al*., 2020). Patients with severe COVID-19 show a striking decrease in lymphocytic T cells (CD4+ and CD8+) and natural killer (NK) cells, according to flow cytometric studies. Additionally, functional depletion of T cells is linked to an increase in the expression of the natural killer group 2A (NKG2A), PD-1, and T-cell immunoglobulin mucin-3 (Tim-3) in the early stages of the disease (Diao *et al*., 2020).

The SARS-CoV-2 begins to replicate, migrate to the airways, and enter lung alveolar epithelial cells. A robust immune response could be

brought on by the SARS-CoV-2 virus' rapid multiplication in the lungs. Acute respiratory distress syndrome and respiratory failure are brought on by cytokine storm syndrome, which is thought to be the primary cause of death in COVID-19 patients (Huang *et al*., 2020).

The lungs are the primary site of histopathological abnormalities in COVID-19 patients, and investigations revealed that individuals with severe COVID-19 had bilateral widespread alveolar destruction, hyaline membrane development, desquamation of pneumocytes, and fibrin deposits in their lungs (Zeng *et al*., 2020).

2.1.6. Transmission of coronaviruses:

The SARS-CoV mostly spread through close contact between people, either by inhaling airborne droplets or by coming into contact with contaminated surfaces of gadgets (Otter *et al*., 2016). The primary method of MERS-CoV transmission from person to person, but this method is rarely regularly or sustainably used (Memish *et al*., 2020). Since MERS-CoV has been isolated from patient samples of their faeces, vomitus, urine, serum, and cerebrospinal fluid, theoretically, MERS-CoV might potentially transmit by contact with these substances (Memish *et al*., 2020).

Human-to-human transmission that took place in intimate interactions and was primarily transmitted by droplets and direct contact is the main route of transmission for SARS-CoV-2 (Li *et al*., 2020). SARS-CoV-2 is more contagious than SARS-CoV and MERS-CoV, although COVID-19 cannot yet have an accurate reproduction number (R0) because to the large number of asymptomatic infections that cannot be correctly accounted for at this time (Li *et al*., 2020).

The risk of pharyngeal virus shedding was extremely high at the onset of infection since the SARS-CoV-2 viral load in upper respiratory tract samples was already at its maximum during the first week of symptoms (Wolfel *et al*., 2020).

2.1.7. Diagnosis of COVID-19:

The individual differences in the clinical presentation of new SARS-CoV-2 (or COVID-19) include asymptomatic to severe respiratory distress syndrome and multi-organ failure. The epidemiological history, clinical signs, and confirmation by a variety of laboratory detection techniques, such as computed tomography (CT) scan, nucleic acid amplification test (NAAT), and serological tests, are the main components of the usual clinical diagnosis of COVID-19 (Corman *et al*., 2020). The chest's computed tomography is more sensitive and shows multifocal, bilateral, peripheral/subpleural ground glass opacities that typically impact the posterior parts of the lower lobes, with or without concomitant consolidations (Caruso *et al*., 2020).

The SARS-CoV-2 genetic material can be identified directly by diagnosing CoVID-19 or indirectly by assessing the humoral immune response to SARS-CoV-2. Real-time polymerase chain reaction (RT-PCR) is the most used technique for detecting genetic material from SARS-CoV-2 (Sethuraman *et al*., 2020). Supporting lab tests are Greater severity and a worse prognosis are associated with complete blood count lymphopenia, eosinopenia, and neutrophil/lymphocyte ratios below 3.13. A worse prognosis and a higher chance of cardiac injury are both associated with thrombocytopenia (Vabret *et al*., 2020). A number of factors contribute to lymphopenia, including the cytotoxic effects of the virus, the induction of apoptosis, IL1-mediated pyroptosis, and the

inhibition of bone marrow by inflammatory cytokines (Azkur *et al*., 2020). High levels of C-reactive protein (CRP), ferritin, D-dimer, procalcitonin, lactic dehydrogenase (LDH), prothrombin time, activated partial thromboplastin time, amyloid serum protein A, creatine kinase (CK), glutamic-pyruvic transaminase (SGPT), urea, and creatinine are associated with a worse prognosis, thromboe (Vabret *et al*., 2020).

2.2. Risk factors associated with COVID-19:

The infectious diseases, factors that can affect a person's likelihood of contracting COVID-19 infection include their employment status, level of education, income, and housing situation (Butler-Jones & Wong, 2016). These factors may affect their capacity to seek medical attention, follow through on treatment, or use physical barriers to prevent the spread of the disease. So both clinical and social aspects should be considered in successful risk prediction systems for community transmission (Khalatbari-Soltani *et al*., 2020). Particularly among populations with lower socioeconomic level, the latter characteristics have not received enough research (Khalatbari-Soltani *et al*., 2020).

Diabetes is one of the main comorbidities linked to the severity of infection in the COVID-19 pandemic (Yang *et al*., 2020). Age, gender, and underlying medical disorders like hypertension, cardiovascular disease, and chronic lung disease are additional bidi linkages. People with diabetes may experience late diabetes complications such diabetic renal disease and ischemic heart disease, which can make them frailer and exacerbate the severity of COVID-19 disease, which can result in kidney or heart failure. But in SARS patients, plasma glucose levels and diabetes were independent predictors of mortality and morbidity (Yang *et al*., 2006).

2.3. Diabetes Overview:

Diabetes is a chronic condition brought on by either insufficient insulin production by the pancreas or inefficient insulin utilization by the body. A hormone called insulin controls blood sugar. Uncontrolled diabetes frequently results in hyperglycemia, or elevated blood sugar, which over time seriously harms numerous bodily systems, including the neurons and blood vessels (Danaei *et al*., 2011). Type 1 diabetes, also referred to as juvenile or childhood-onset insulin-dependent diabetes, is characterized by inadequate insulin production and requires daily administration of insulin. Symptoms include excessive urination (polyuria), excessive thirst (polydipsia), constant hunger, weight loss, changes in vision, and fatigue. These signs could appear out of nowhere (Sarwar *et al*., 2010). Type1 diabetes is caused by the damage of β-cells the only cells in the body that produce insulin and the resulting progressive insulin deficiency. Diabetics suffer continuous hyperglycemia because their bodies cannot adapt to rises in blood sugar without insulin. For unknown reasons, cells are killed by a person's own immune system in between 70 -90% of cases. caused by the gradual loss of insulin due to the degeneration of cells, the only cells in the body that make insulin.Diabetics suffer continuous hyperglycemia because their bodies cannot adapt to rises in blood sugar without insulin. For unknown reasons, cells are killed by a person's own immune system in between 70-90% of cases. Family members of type 1 diabetics are more likely to become sick themselves because the condition is largely inherited (DiMeglio *et al*., 2018).

Pancreatic beta cells are destroyed in type 1 diabetes, and people with the condition tend to have higher levels of CD8+ T-cells and B-cells that are specifically directed against islet antigens than people without the

condition, which suggests that the adaptive immune system may be involved in beta cell degeneration. Additionally, type 1 diabetics frequently have impaired regulatory T cell function, which may worsen the autoimmune disease. Insulins, or the islet of Langerhans, become inflamed when beta cells are destroyed (DiMeglio *et al*., 2018). While insulin insufficiency is invariably the cause of hyperglycemia in type 2 diabetes. Reduced insulin-mediated glucose uptake from muscle, increased glucose synthesis from the liver, and increased free fatty acid mobilization from adipose tissue are all symptoms of insulin deficit (Lebovitz *et al*., 1998). The body's inefficient use of insulin causes type 2 diabetes, formerly known as non-insulin-dependent or adult onset. Type 2 diabetes affects more than 95% of those who have the disease. The symptoms of type 2 diabetes may resemble those of type 1 diabetes, but they are typically less severe. This kind of diabetes is primarily brought on by extra body weight and physical inactivity. As a result, the condition may not be discovered until after difficulties have already developed. This type of diabetes was previously only seen in adults, but it is now increasingly common in children as well (Sarwar *et al*., 2010).

Insulin insufficiency is always the cause of hyperglycemia in type 2 diabetes. Reduced insulin-mediated glucose uptake from muscle, heightened glucose synthesis from the liver, and enhanced free fatty acid mobilization from adipose tissue are all symptoms of insulin deficit. Postprandial hyperglycemia is the initial outcome, which is then followed by fasting hyperglycemia. Insulin resistance, whether inherited or acquired, can contribute to the onset of type 2 diabetes by raising the need for insulin and, in those whose cells have a low secretory reserve, insulin insufficiency (Lebovitz *et al*., 1998).

Over 90% of cases of diabetes mellitus, according to the World Health Organization (WHO), are T2DM, which is characterized by insufficient insulin secretion by pancreatic islet cells, tissue insulin resistance (IR), and an insufficient compensatory insulin secretory response (Stumvoll *et al*., 2005).

2.3.1. Diabetes and COVID-19:

The Type 2 diabetes is connected with lowgrade chronic inflammation induced by the excessive visceral adipose tissue. The peripheral insulin sensitivity and homeostatic glucose regulation are both impacted by this inflammatory disease. Chronic inflammation and hyperglycemia might cause an inappropriate and inadequate immune response (Iacobellis, 2020). Diabetes patients with COVID-19 are more likely to experience uncontrolled inflammatory reactions and severe hypercoagulability, which could lead to a worse result (Guo *et al*., 2020).

In hospitalized COVID-19 patients, hyperglycemia is a significant predictive indicator of prognosis and outcome. Previous research demonstrated that COVID-19 hyperglycemic patients had a greater cumulative incidence of severe illness than normoglycemic controls (Sardu *et al*., 2020). Possible explanations for this higher mortality include immune system alterations brought on by hyperglycemia and an increase in inflammatory cytokines (Schuetz *et al*., 2011). Higher binding affinity and efficient virus entry, decreased viral clearance, diminished T-cell function, increased susceptibility to cytokine storm disorder, and the presence of CVD are all mechanisms that probably make DM patients more vulnerable to COVID-19. The primary cellular locations for coronavirus entrance and inflammation are lung cells, especially pneumocytes (Hamming *et al*., 2004). Additionally, type-2 diabetes patients were shown to have greater blood levels of the cellular protease

furin (Fernandez *et al*., 2018). The results of these investigations provide credence to the idea that COVID-19 individuals with diabetes are more likely to experience unfavorable outcomes. In addition, a recent study found that patients with diabetes had slower SARS-CoV-2 clearance (Chen *et al*., 2020). ACE inhibitors and angiotensin II type-I receptor blockers are frequently used to treat diabetes and hypertension, which are frequent comorbidities linked to COVID-19. The expression of ACE2 is reportedly increased in diabetics taking these drugs, and the greater levels of ACE2 may therefore promote severe illness in COVID-19 patients (Wan *et al*., 2020).

The cell surface proteins are broken apart by the Furin. Furin targets the SARS-CoV-2 Sprotein S1/S2 cleavage site during infection.The cleavage is crucial because it enables the virus to fuse with the host cell membranes by exposing the fusion sequences on the COVID-19 spike protein (Braun *et al*., 2019).

2.4. Cortisol hormone overview:

A steroid hormone called Cortisol is created from cholesterol. It is produced in the zona fasciculata layer of the adrenal cortex. Adrenocorticotropic hormone (ACTH), which is secreted from the anterior pituitary, increases LDL receptors and the activity of cholesterol desmolase, which converts cholesterol to pregnenolone and is the rate-limiting stage in cortisol synthesis (Angelousi *et al*., 2020). Cortisol causes the pancreas to secrete more glucagon and less insulin, which causes an increase in lipolysis in adipose tissues. In addition to increasing liver glycogenolysis, liver gluconeogenesis, liver ketogenesis, and lipolysis while decreasing lipogenesis, glucagon is a peptide hormone released by the pancreatic alpha cells. Cortisol also increases the action of

glucagon, epinephrine, and other catecholamines (Ramamoorthy & Cidlowski, 2016).

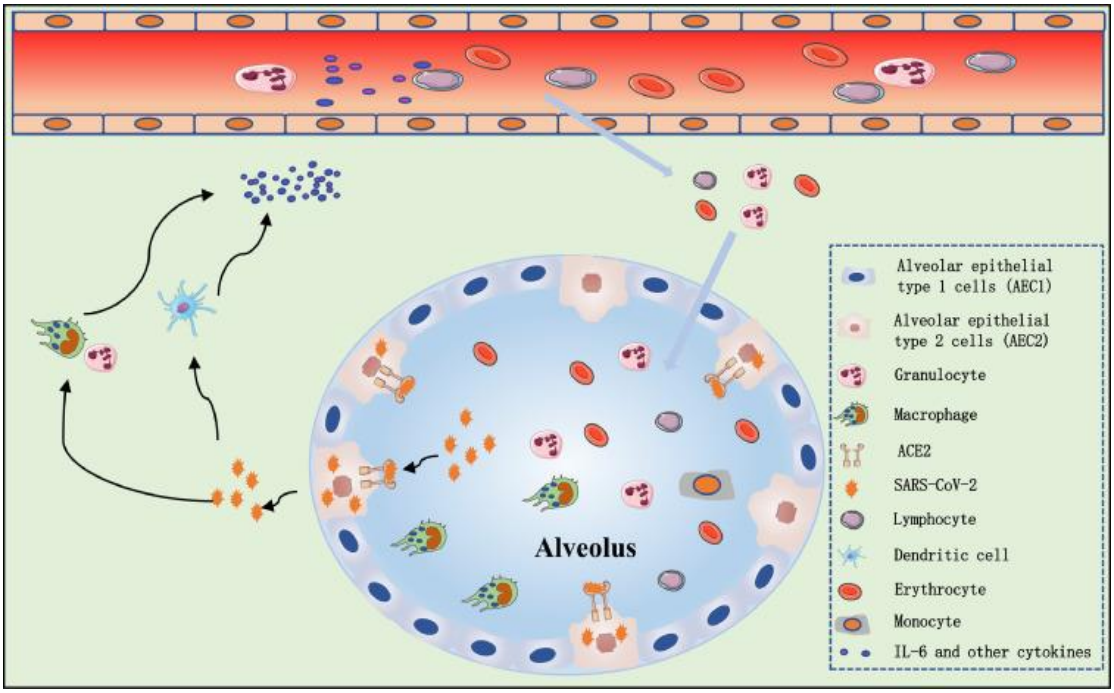
2.4.1. Cortisol hormone and CoVID-19:

In terms of cortisol dynamics, postmortem studies on individuals who died from SARS-CoV-1 have discovered necrosis and degeneration in the adrenal cortical cells, indicating a direct cytopathic action of the virus. The presence of ACE receptors in the adrenal cortical cells suggests that cortisol dynamics may also be changed in SARS-CoV-2 patients (Raamsman *et al*., 2000). Additionally, a thrombotic event at the adrenal has been linked to an acute adrenal insufficiency (Boscarino *et al*., 2008). Some of the SARS-amino CoV-1's acid sequences act as molecular mimics of the adrenocorticotropic hormone (ACTH). This may prevent the stress-related increase in cortisol because the antibodies made to fight the virus particles will unintentionally lower the level of circulating ACTH (Boscarino *et al*., 2008). It is possible to develop COVID-19-related critical illness-related corticosteroid deficiency.Due to insufficient glucocorticoid-mediated anti-inflammatory activity in response to the severity of stress brought on by a critical disease, an uncommon syndrome known as relative adrenal insufficiency develops (Hurst *et al*., 2005). Patients with adrenal insufficiency may have a higher chance of contracting SARS-CoV-2 infection due to their weakened natural defenses, which include dysfunctional neutrophil and natural killer cells (Kuo, 2013).

2.5. IL-6 and CoVID-19:

The Interleukin-6 (IL-6) is an essential part of the cytokine system and plays a central role in acute inflammation (Scheller & Rose-John, 2006). Four alpha helices make up the short polypeptide IL-6, which has a molecular weight of 19–28 kD, 184 amino acid residues, isoelectric

point of 5.0, glycosylation sites, and two disulfide linkages. It is usually seen in monomer form. The IL-6 gene, which has 5 exons and 4 introns, is found on chromosome 7p (Scheller *et al*., 2014**)**. Almost all immune and stromal cells, including B lymphocytes, T lymphocytes, macrophages, monocytes, dendritic cells, mast cells, and other non-lymphocyte cells such fibroblasts, endothelial cells, keratinocytes, glomerular Mesangial cells, and tumor cells, can produce IL6(Jones & Jenkins, 2018). The IL-6 contributes to host safety by inducing acute phase responses, haematopoiesis and immune reactions, It is developed quickly and transiently in response to infections and tissue injuries, High blood IL-6 levels have a strong correlation with COVID-19 severity and poor clinical outcomes, including as (ICU) hospitalization, ARDS, and mortality (Coomes & Haghbayan, 2020). IL-6 has been shown in numerous studies to be a key indicator of severe COVID-19 (Henry *et al*., 2020). An essential cytokine, IL-6 is associated with a number of inflammatory disorders. High levels of IL-6 in SARS-CoV-2-infected individuals were associated with patient symptoms such pulmonary inflammation and severe lung damage (Lu *et al*., 2020). When the SARS-CoV-2 binds to alveolar epithelial cells, it activates the innate immune system and adaptive immune system, causing a high number of cytokines to be released, including IL-6. Additionally, because of the pro-inflammatory effects of these substances, vascular permeability increased, allowing a lot of fluid and blood cells to enter the alveoli and cause dyspnea and potentially respiratory failure (Leiva-Juarez *et al*., 2018) (Figure2-3). According to the first gross examination report of a COVID-19 death autopsy, both lungs seem bronzed, and there was a significant amount of gray-white viscous liquid over flow following the incision. (Liu *et al*., 2020).



Figure(2-3) Mechanism of cytokine release syndrome in severe COVID-19 patients. Alveolar epithelial cells, namely Alveolar epithelial type 2 cells (AEC2), are infected by the SARS-CoV-2 through the ACE2 receptor. Virus is released as a result of epithelial cell death and increased cell permeability (Kaur, Allahbadia & Singh. 2020).

When the innate immune system is activated by the SARS-CoV-2 virus, macrophages and other innate immunity cells release a high number of cytokines and chemokines, including IL-6, in addition to capturing the virus. Antigen-presenting cells can also cause adaptive immunity to become active (mainly dendritic cells). Not only do T and B cells perform an antiviral role, but they also actively or passively encourage the release of inflammatory cytokines. Additionally, when inflammatory factors are stimulated, asignificant amount of inflammatory exudates and erythrocytes enter the alveoli,causing dyspnea and respiratory failure.

2.6. IFN-γ and CoVID-19:

The IFN-γ is a cytokine that is principally produced by cells of the immune system, including innate like lymphocyte populations, such as natural killer (NK) cells and innate lymphoid cells (ILCs), and adaptive immune cells, such as T helper 1 (T_H1) cells and $CD8^+$ cytotoxic T lymphocytes (CTLs). It signals through the IFN-γ receptor (IFN-γ R; comprising the IFN-γ R1 and IFN-γ R2 subunits), which can be expressed on most, if not all, cell types as well as IFN-γ was initially identified as 'macrophage activating factor', and macrophages are a major physiological target for IFN-γ action (Hu & Ivashkiv, 2009 ; Stark & Darnell, 2012).

The IFN- γ production in innate cells, such as lymphocytes, can be stimulated by cytokines (primarily IL-12 and IL-18), or after the activation of broadly reactive antigen receptors or pattern recognition receptors (PRRs) in response to tissue damage or microbial infection. An early burst of IFN-γ production occurs during infections prior to the emergence of an antigen-specific adaptive immune response. The T cell receptor (TCR) mediated identification of microbial (but also self or mutant self) peptides in the context of MHC class II or MHC class I molecules, respectively, is normally necessary for significant levels of sustained IFN-γ production by TH1 cells or CTLs (Hu & Ivashkiv, 2009 ; Villarino *et al*., 2017).

The Numerous studies have been done to determine how interferon (IFN) signaling affects COVID-19 disease progression. Both beneficial and harmful effects have been noted. When transmembrane receptors and downstream signaling kinases are active, IFN signaling can be divided into three main categories. Type I signaling involves (IFN-α, -β, -ε, -κ,

and -ω IFNs) (Secombes *& Zou*, 2017). The protective effects of IFN signaling in SARS-CoV-2 infections are illustrated by research indicating that severe COVID-19 is related with reduced IFN signaling (Hadjadj *et al*., 2020; Ziegler *et al*., 2021). the presence of autoantibodies that prevent some IFNs from working (Bastard et al., 2021). High viral load and severe COVID-19 are correlated with autoantibodies against type I IFNs in the nasal mucosa (Kim *et al*., 2021). High levels of type III IFNs and, to a lesser extent, type I IFNs are linked to lower illness risk or severity in the upper respiratory tract (Sposito *et al*., 2021). Increased type III IFN levels in bronchial aspirates are associated with lower virus loads and quicker clearance (Villarino *et al*., 2017). IFN-γ was observed to improve SARS-CoV-2 clearance and clinical status in a patient with rheumatoid arthritis and Sjogren syndrome who was on rituximab but whose condition had gotten worse despite receiving convalescent plasma, as in this series (Lukaszewicz *et al*., 2021). Exogenous IFN-γ and plasma exchange were used to treat a patient who had endogenous anti-IFN-γ antibodies for COVID-19, but curiously, he did not exhibit severe COVID-19 symptoms (Kacar *et al*., 2021).

2.7. CRP and COVID-19:

The C-reactive protein is a pentameric acute phase protein and acute pneumococcal pneumonia. CRP is expressed more frequently in inflammatory disorders such rheumatoid arthritis and several cardiovascular conditions, and it may play a role in the etiology of these conditions. The majority of cells that produce C-reactive protein are hepatocytes, although there are also adipocytes, lymphocytes, endothelial cells, macrophages, and smooth muscle cells. Any bacterial infection can cause CRP levels to increase up to 1000 times, and they can suddenly drop as soon as the infection is treated. Nitric oxide (NO) release and

cytokine production are pro-inflammatory effects that are countered by CRP by activating the complement system, apoptosis, and phagocytosis (Sproston & Ashworth, 2018).

The essential component in the severity of COVID-19 is the elevated levels of cytokines that have been found in COVID-19 patients, suggesting a cytokine storm and aggravating the immunological response to viral infection (Hu *et al*., 2021). The most important cytokine that is associated with COVID-19 patients' CRP levels is IL-6. CRP as an indirect biomarker of IL-6 is sufficient and reliable to detect the severity of COVID-19 instead of detecting all cytokines in the body since the level of IL-6 is inversely correlated with the amount of CRP in blood (Li *et al*., 2020 ; Mueller *et al*., 2020).

In COVID-19 patients, CRP levels can accurately predict the severity of the disease, harmful effects, prognosis, and mortality. High CRP levels in COVID-19 patients at the time of hospital admission suggest that CRP can be used as a standalone biomarker for earlier disease severity identification (Chen *et al*., 2020; Ahnach *et al*., 2020). The prognosis of COVID-19 is closely correlated with high CRP levels in patients, and this relationship must be used in clinical practice to determine the severity of COVID-19 disease (Poggiali *et al*., 2020; Stringer *et al*., 2021).

The probability of using CRP to guide treatment in COVID-19 patients who may require mechanical ventilation in the future due to hyper inflammatory syndrome is increased by the fact that high CRP levels caused by increased IL-6 release strongly predict the need for mechanical ventilation in COVID-19 patients, In COVID-19 patients, CRP must be controlled to prevent negative effects(Wang, 2020). quickly increasing CRP in COVID-19 individuals denotes a severe

immunological response to viral infection, which is linked to degeneration of the heart, kidneys, and lungs. The likelihood of developing ARDS and the extent of lung damage increase with initial CRP levels (acute respiratory distress syndrome). Therefore, in COVID-19 patients, increased CRP indicates lung damage that should be controlled to prevent serious sickness (Tan *et al*., 2020). One possible explanation for the substantial elevation of the inflammatory biomarker CRP in severe COVID-19 patients is the overproduction of inflammatory cytokines (Victor *et al*., 2020).

2.8. D-Dimer and COVID-19:

The D-dimer is a very small protein fragment found in the blood after a blood clot is degraded by fibrinolysis, D-dimer is a fibrin degradation product (FDP). Its name comes from the fact that it is composed of two D fragments of the fibrin protein that are cross-linked together (Adam *et al*., 2009). Increased thrombin generation caused by accelerated fibrinolysis was thought to be a sign of disseminated intravascular coagulation, and the elevation of plasma D-dimers was regarded as a sign of coagulopathy, D-dimer levels are increased along with other acute inflammatory plasma indicators such fibrinogen, C-reactive protein, and serum ferritin (Ruetzler *et al*., 2021). An alternate theory was also put up, which contends that the acute lung injury seen in COVID-19 pneumonia is the primary cause of D-dimers (Hunt & Levi, 2020).

The Inflammatory cytokines may cause an imbalance in coagulation and fibrinolysis in the alveoli, which may stimulate the fibrinolysis process and increase D-dimer levels. This suggests that a rise in D-dimer could be a sign of an inflammatory response (Tang *et al*.,

2020). Raised D-dimer levels and thrombocytopenia (low platelet count) have also been found in severe COVID-19 patients, suggesting that a hypercoagulable condition may contribute to the severity of the illness and mortality(Lippi *et al*., 2020).

2.9. Immune cells and Covid-19:

Three stages of the immune response are normal or hypofunction, hyperactivation, and anergy in patients with fatal severe COVID-19. The patients eventually succumbed to viral infections and perished (Sette & Crotty 2021).

The SARS-CoV-2 must be defended against by the innate immune system, which consists of monocytes, granulocytes, dendritic cells (DCs), and natural killer (NK) cells, as well as the adaptive immune system, which consists of T and B lymphocytes. Patients with severe COVID-19 display lymphopenia with decreased CD4+ and CD8+ T cells, lymphocyte activation and dysfunction, an increase in circulating neutrophils with the appearance of circulating neutrophil precursors, dysfunction of classical monocytes and loss of non-classical monocytes, reduced abundance and dysfunction of DCs and NK cells, and more. Interleukin IL-6 and IL-1cytokine levels throughout the body (Dorward *et al*.,2020). The body's immune response is a significant element impacting the course of the disease and the prognosis because there are no specific antiviral medications available. For the purpose of creating diagnostic indicators and treatment plans for COVID-19, a deeper comprehension of the cellular immune response as the condition progresses from a minor illness to a potentially fatal COVID-19 is essential (Xi & Qing, 2021).

2.10. Transmembrane Protein (*IFITM3*) and SARS-CoV-2 Infected Lung Epithelial Cells:

The interferon-induced transmembrane (*IFITM*) proteins are essential for the immune system's innate and adaptive defense against viruses. On chromosome 11p15.5, the human *IFITM* locus contains five genes, including *IFITM3*.The *IFITM3* protein is mostly expressed on endosomes and lysosomes and is an IFN-stimulated gene (ISG). It stops hemifusion of a wide range of enveloped viruses, such as influenza A, Ebola, Marburg, or SARS-CoV, between the viral membrane and the host cellular membrane (Diamond *et al*., 2013).

The Single nucleotide polymorphisms (SNPs) in the gene *IFITM3* have been linked to increased infection susceptibility and disease severity, according to earlier research (Everitt *et al*., 2012). According to research by Chen et al. (2018), the C-allele of the SNP rs12252 (c.-22T>C) was found to be substantially correlated with the severity of H1N1 and H7N9 influenza A virus infections in Caucasians and Asians (Kim *et al*., 2020). The *IFITM3* protein is projected to be truncated and mislocalized because it loses the first 21 N-terminal amino acids (*21IFITM3*) due to the SNP rs12252, which is predicted to disrupt a splice acceptor site.

The functional effects of the truncation are still hotly debated and need to be proved beyond a reasonable doubt (Makvandi-Nejad *et al*., 2018). In a first preliminary investigation, it was found that patients with severe COVID-19 (N = 24) and patients with mild COVID-19 (N = 56) had considerably greater frequencies of rs12252 C-allele carriers (Zhang *et al*., 2018). A recent study in a Spanish cohort revealed that C-allele bearers of the SNP rs12252 have a 2-fold higher risk of contracting

SARS-CoV-2 infection (N = 311) compared to a control group (N = 440) gathered before to the pandemic (G'omez *et al*., 2020).

The severe influenza A virus infections have also been linked to the A-allele of a second SNP (rs34481144, c.-22-64G>A), which has the highest MAF in Europeans (0.46) and the lowest MAF in East Asians (0.01) (Kim & Jeong 2020; Kim, Jeong, & Jeong 2020). (Allen *et al*., 2017) shown in a study conducted in Europe that the A- allele of this promoter SNP results in lower *IFITM3* mRNA and protein levels, reducing *IFITM3*'s antiviral defensive capabilities. In functional in vitro research, it was possible to show that *IFITM1*, *IFITM2*, and *IFITM3* restrict S protein-mediated entrance for SARS-CoV (Huang *et al*., 2011). Between SARS-CoV-2 and SARS-CoV, there is an 82 percent sequence similarity, It is hypothesized that *IFITM3* may also play a significant role in SARS-CoV-2 infection because SARS-CoV-2 similarly penetrates cells utilizing the S protein, which binds to angiotensin-converting enzyme 2 (ACE2) (Kaur *et al*., 2020)

2.10.1. Localization of *IFITM* gene:

The *IFITM* gene in humans is located in chromosome 11 at position NC_000011.10 (319676-320860), previous literatures referred to the role of *IFITM1*, have been an important restriction factors in viral infections.

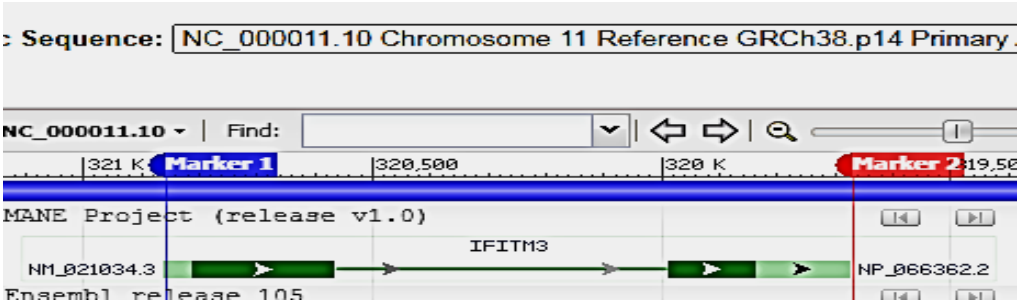


Figure (2-4) Location and boundaries of *IFITM* gene, Marker 1 and 2 delimited this gene on Chr. 11

Chapter Three
Materials and Methods

3. Materials and Methods

3.1. Materials

3.1.1. Instruments and Equipment for Laboratories:

The table below listed all of the instruments utilized in the lab throughout the study (3-1).

Table (3-1): Instruments and Equipment for Laboratories

No	Item	Company	Country
1	Blue tube (EDTA)	Afco	USA
2	Celestial tube(sodium citrate)	Afco	USA
3	Centrifuge	Gemmy	Taiwan
4	Chromate reader	Awarness technology	USA
5	Cool box	Tank	India
6	Cotton	Kardelen	China
7	Cylinder included	DIALAB	Austria
8	Deep freezer	Thermo-Fisher	Germany
9	Distill wator	GFL	Germany
10	Filter paper	ZELPA	Turkey
11	FREND^{TM}	Nano Entek	Korea
12	Gel tube	Afco	USA
13	Gloves	Latex	China
14	Incubator	Memmert	Germany
15	Micropipette from 100-1000 micro liter	Dragon lab	USA
16	Micropipette from 20-200 micro liter	Dragon lab	USA
17	Micropipette from 5-50 micro liter	Dragon lab	USA
18	Microwell plate	DIALAB	Austria

19	Panel tube (test tube)	LAB	China
20	Pipette tips (blue)	Applied Biosysyem	USA
21	Pipette tips (yellow)	Applied Biosysyem	USA
22	Rack	Bioneer	Korea
23	Refrigerator	Vesti	Turkey
24	Sterile material (Ethanol)	Aljoud	Iraq
25	Syringe	SUPER	China
26	Tips (various volumes)	Applied Biosysyem	USA

3.1.2. Biological and Immunological Materials:

The immunological and biological materials were summarized in the following table (3-2)

Table (3-2) List of Kits used in the study

No	Name	Company	Country
1	Blood sugar	BIOLABO	France
2	C-reactive protein	Latex	Italy
3	D-Dimer	Ichromax	Belgium
4	HbA1c	Finecare	Belgium
5	Human Cortisol	Bioassay technology laboratory	China
6	Human Interferon-γ	Bioassay technology laboratory	China
7	Human Interleukin- 6	Bioassay technology laboratory	China
8	SARS-COV-2 IgG	Biomerieux	France
9	SARS-COV-2 IgM	Biomerieux	France

3.2. Methods and Subjects

3.2.1. Participants:

The study's sampling took place on Covid-19, a subject patient in Babylon Governorate, during the period from October 2021 to January 2022. This investigation comprised the collection of blood from 54 Covid-19 patients as well as 41 people who appeared to be healthy. They were for the different ages and genders as the COVID-19 patients.The years of Covid-19 patient and control from (20 to 60) years.

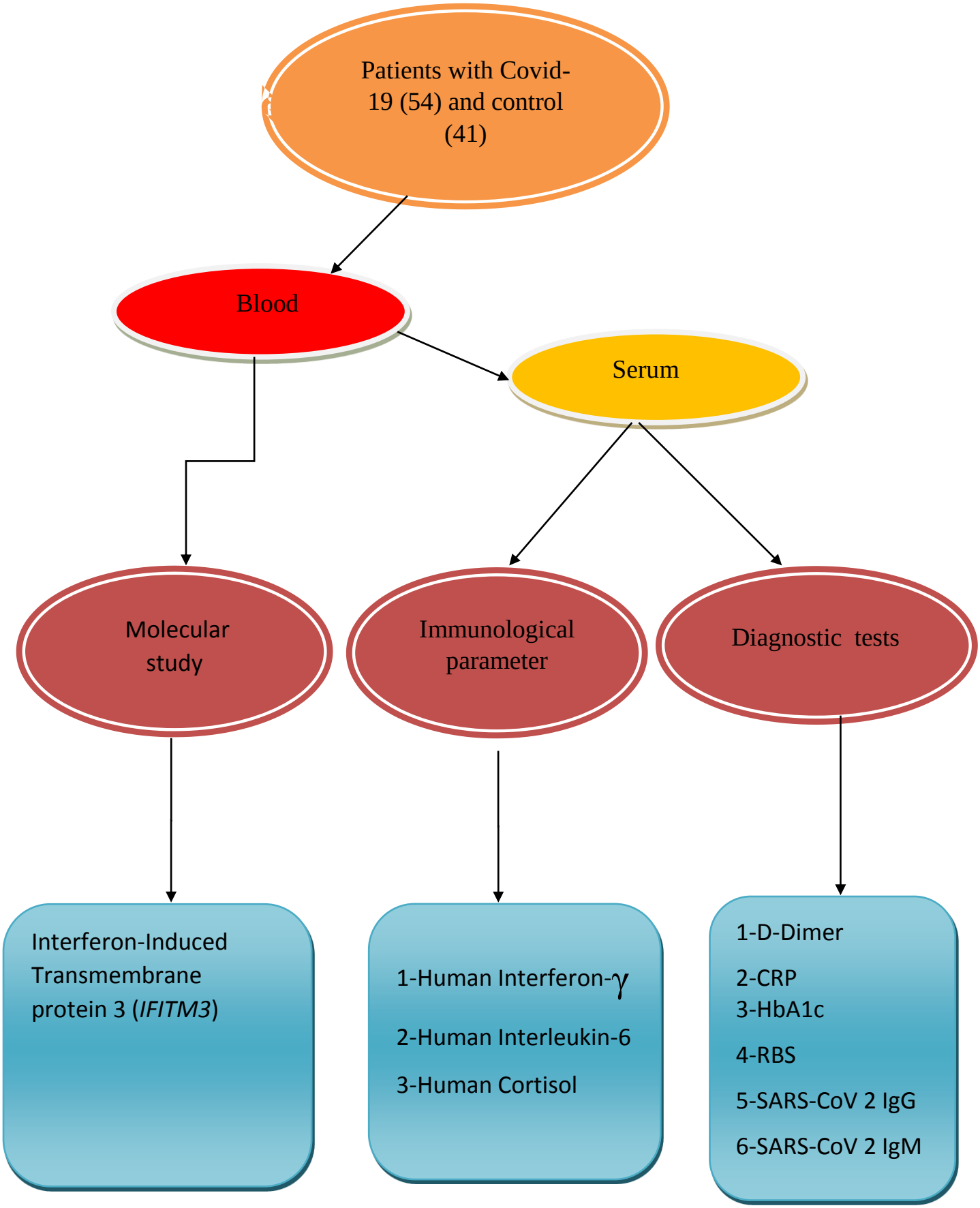
3.2.2. Inclusion criteria: The patients were enrolled in the current study if they had the following criteria**:**

1. Personnel with history of covid-19 infection.

2. The participants must have no history of any chronic diseases**.**

3. Exclusion of models of patients that infection with diabetes.

3.2.3. Study design:



3.2.4. Sample collection:

Atotal of seven milliliters of venous blood was taken from each participant. Three milliliters of venous blood were deposited in a gel tube and allowed to stand for 10 minutes before being centrifuged (3000rpm/15 min) and the serum divided into six eppendorf tubes (200 µl each) and stored in the freezer (-20^{0}C), for using to immunological parameter testing and using to C-reactive protein, SARS-CoV 2 IgG and SARS-CoV 2 IgM testing .Two milliliters of venous blood were put in an EDTA containing tube for molecular testing and stored in the freezer (-20^{0}C). In addition, Two milliliters of venous blood were inserted in a tube containing sodium citrate, which was used to quantify the D-Dimer testing, HbA1c, Random blood sugar testing and Rapid determination of COVID -19 Antibodies (IgM and IgG) testing (Pazzagli *et al*., 2013).

3.2.5. Data collection:

The study's participant data was collected utilizing a data collection sheet created specifically for the purpose of the study (Appendix). For each participant in the research:

1.Personal characteristics: Name, Age, Sex, Chronic Disease History, Habitat and Vaccination.

2.Signs & Symptoms, Date of onset of symptoms, Duration of infection, and treatment method (Home management or hospital), Plasma-based therapy (Yes or No).

3.2.6. Ethical approval:

Before collecting data, the following formal administrative agreements were obtained:

1.Following the presentation of the protocol, the University of Babylon/College of Science/Higher Education committee gave its initial approval.

2.The study protocol was approved by an ethics committee of Babylon University's College of Science's Department of Biology.

3.A formal requisition for the agreement was filed to the Babylon Health Directorate.

4.An official agreement was reached with the Babylon Health Directorate's Department of Development and Training.

3.3. Immunological tests:

By using the manual principle and procedure of Bioassay Technology Laboratory as ELISA kits. IL- 6, Cortisol, and INF-γ were estimation for all studied patients and control, The Specific standard curve was done for each parameter separately (Sheikhzadeh & Elham, 2020).

3.3.1. Human Interleukin 6:

This sandwich kit detects human interleukin 6 (also known as IL-6) in serum and plasma with high accuracy.

Assay principle:

Enzyme-Linked Immunosorbent Assay (ELISA).Human IL-6 antibody was pre-coated on the plate. IL-6 from the sample was introduced to the wells, where it binds to antibodies. The biotinylated Human IL-6 antibody is then added to the sample and binds to the IL-6. Streptavidin-HRP was then added, which binds to the biotinylated IL-6 antibody. During the washing stage after incubation, unbound Streptavidin-HRP is rinsed away. After that, the substrate solution is added, and the color develops in accordance to the amount of Human IL-6 present. The process was stopped with the addition of an acidic stop solution, and the absorbance is read at 450 nm**.**

Assay procedure:

1. Following the instructions for reagents, standard solutions, and samples. Before using, make sure all of the reagents are at room temperature. At room temperature, the assay was carried out.

2.The utilizing strips, place them in the frames. The unused strips should be kept at a temperature of 2 to 8 degrees Celsius.

3.Adding 50 µL standard to standard well. Note: Don't adding biotinylated antibody to standard well because the standard solution contains biotinylated antibody.

4. Exactling 40 µL serum was added to sample wells and then added 10 µL anti-IL-6 antibody to sample wells, as well as 50 µL streptavidin –HRP to sample wells and standard wells (Not blank control well). Mixing well. The plate was covered with a sealer. Incubated for 60 minutes at 37^0C.
5. The sealer was Removed and wash the plate 5 times with wash buffer. Soak wells with 300 µL wash buffer for 30 seconds to 1 minute for each wash.
6. Adding substrate solution A 50 µL to each well and then adding 50 µL substrate solution B to each well. Incubation the plate covered with a new sealer for 10 minutes at 37^0C in the dark.

7. Adding 50 µL stop solution to each well, the blue color will change into yellow immediately.

8.The optical density (OD value) of each well immediately determine using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

9.The calculation of IL-6 concentration was done by using the following standard curve listed in figure (3-1).

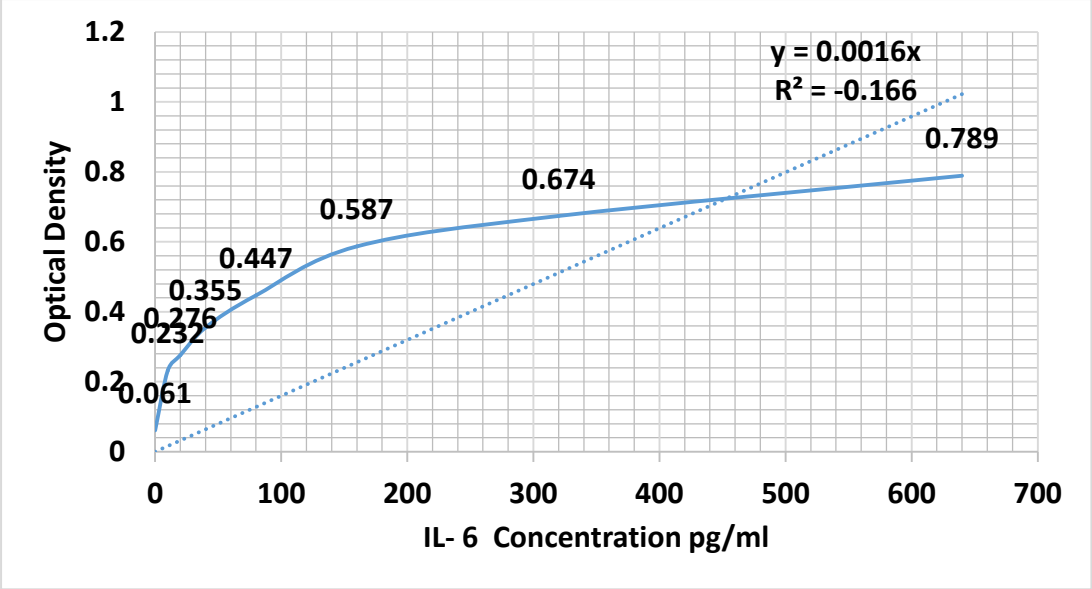


Figure (3 - 1) IL- 6 standard curve

3.3.2. Human Interferon - γ:

As like as IL- 6 , using the same company and manual procedure in assessment of INF-γ. in specific standard curve listed in figure (3 - 2).

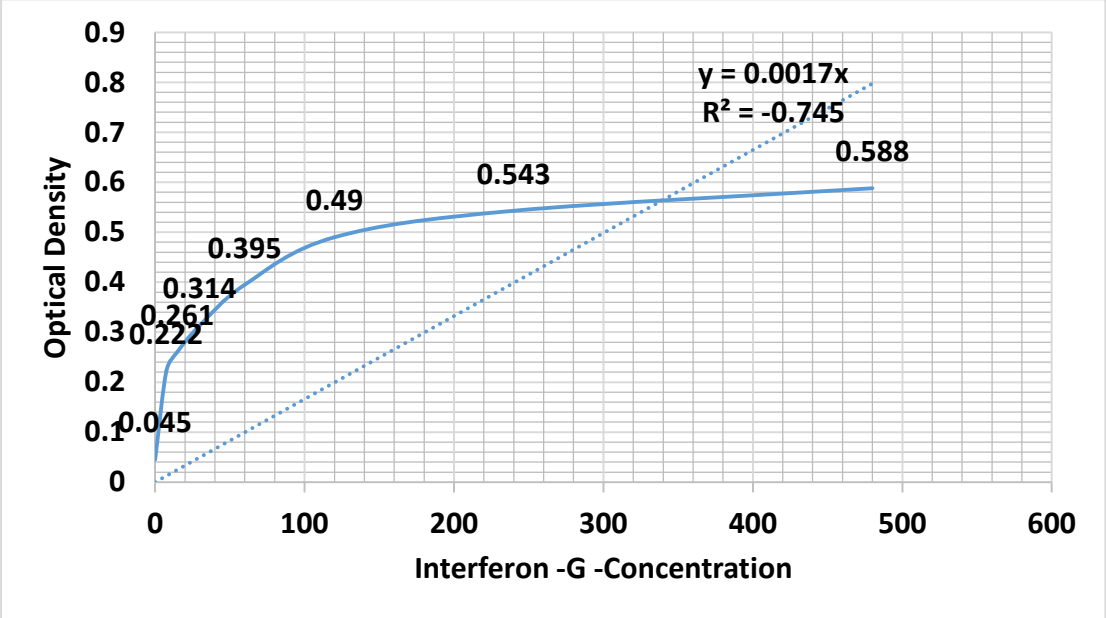


Figure (3 - 2) INF-γ standard curve

3.3.3. Human Cortisol:

As like as IL- 6 and INF-γ , using the same company and manual procedure in assessment of human cortisol hormone , in specific standard curve listed in figure (3 - 3).

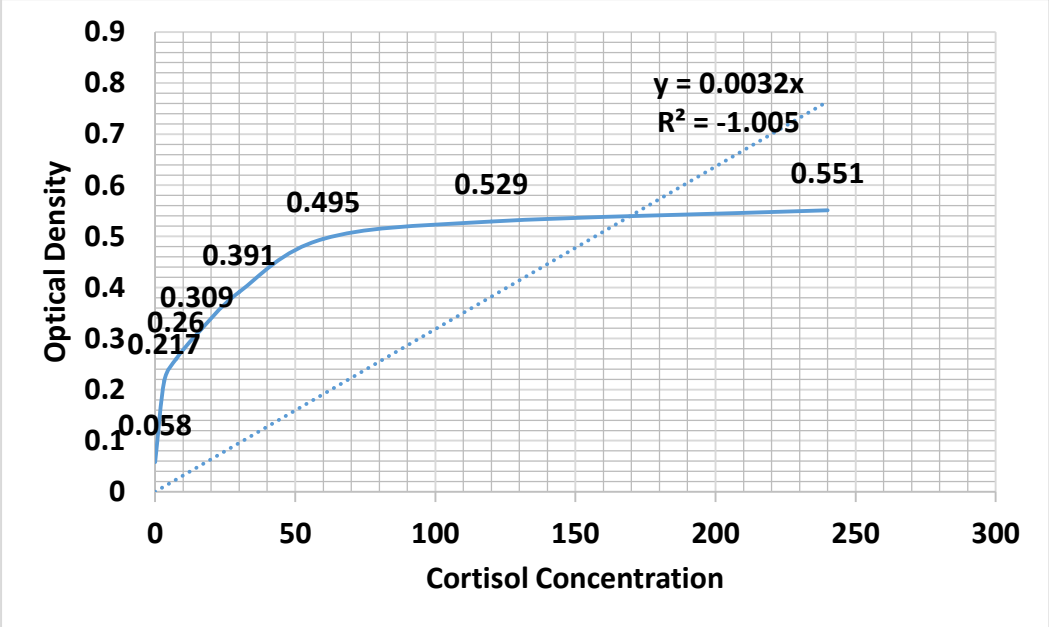


Figure (3 - 3) Human Cortisol standard curve

3.4. Diagnostic tests

3.4.1. HbA1c :

Assay principle:

Fluorescence immunoassay technology is used in the FinecareTM HbA1c Rapid Quantitative Test. The FinecareTM HbA1c Rapid Quantitative Test measures the proportion of HbA1c in human blood using a sandwich immunodetection technique. The sample combination is added to the sample well of the Test Cartridge after mixing with the sample and buffer, and the fluorescence-labeled detector HbA1c antibody binds to HbA1c in the blood specimen. The complexes of detector antibody and

HbA1c are captured to HbA1c antibody that has been immobilized on test strip as the sample mixture migrates on the nitrocellulose matrix of the test strip via capillary action. The fluorescence-labeled detector Hb antibody binds to Hb in the blood samples, and the complexes are collected by the immobilized Hb antibody on the test strip. Fluorescence signal intensity is proportional to HbA1c and Hb concentrations in blood specimens. The ratio of HbA1c and Hb inflorescent signals is the ratio of HbA1c and Hb (Tahara *et al.* , 1995)**.**

Assay procedure:

For detailed instructions on how to use the Test, see the FinecareTM FIA Meter Operation Manual. The test must be performed at room temperature.

Step1: Check/insert the ID Chip into the instrument as part of the preparation process.

Step2: Sampling Using a transfer pipette, draw 10 µL of whole blood and place it in the buffer tube.

Step3: Mixing by tapping or inverting the tube, thoroughly mix the specimen with buffer for 1 minute.

Step4: Loading Take 75 µL of sample mixture and load it onto the sample well of the Test Cartridge.

Step5:Testing

1. Finecare™ FIA meter: Standard test: Insert the Test Cartridge onto the Test Cartridge Holder and click “Test”. 5 minutes later, the result will show in the display and print out when click “Print”. Quick test: Put the Test Cartridge on the operation platform. 5 minutes later, insert the Test Cartridge onto the Test Cartridge Holder and click “Test”. The result will show in the display and print out when click “Print”.

2. Finecare™ multi-channel FIA meter: Insert the Test Cartridge onto the Test Cartridge Holder. 5 minutes later, the result will show in the display and print out when click "Print" (Jeppsson *et al*. , 2002).

3.4.2. Glucose test:

Assay principle:

The glucose oxidase converts glucose to gluconic acid and hydrogen peroxide, which reacts with chloro-4-phenol and PAP to generate a crimson quinoneimine when combined with peroxidase. At 500 nm, the absorbance of the colored complex, which is proportional to the glucose concentration in the material, is measured (Young D, 1995).

Assay procedure:

Allow stand reagent and specimens at room temperature.

Pipette into well identified test tubes:	**Blank**	**Standard**	**Assay**
Reagent	1 ml	1 ml	1 ml
Demineralised water	10 µl		
Standerd		10 µl	
Specimen			10 µl

Mix. Allow stand for 10 minutes at 37°C or 20 minutes at room temperature. Read absorbance at 500 nm (460-560) against reagent blank.using spectrophotometer system for reading the absorbance.

Coloration is stable for 15-20 minutes at 37°C, and then slowly decreases.

Calculation:

$$\text{Result} = \frac{\text{Abs(Assay)}}{\text{Abs(Standard)}} \times \text{Standard concentration}$$

3.4.3. D-Dimer:

Assay principle:

The test employs a sandwich immunodetection approach, in which detector antibodies in the buffer bind to antigens in the sample, generating antigen-antibody complexes, which then move onto the nitrocellulose matrix, where they are caught by additional immobilized antibodies on the test strip. With more antigens in the sample, more antigen-antibody complexes form, resulting in a greater fluorescence signal from detector antibodies, which is processed by the equipment for ichromaTM tests to determine D-Dimer concentration in the sample (Kyrle *et al*. , 2005).

Assay procedure:

1.Using a pipette,transfer 10 µL of the sample (Human whole blood/plasma/control) to a tube containing the detection buffer.

2.Lock the detection buffer tube's lid and thoroughly mix the sample by shaking it 10~15 times.

3.Fill the sample well on the cartridge with 75 µL of a sample mixture pipette out.

4.Allow 12 minutes for the sample-loaded cartridge to cool to room temperature.(As soon as the incubation time is up, scan the sample-loaded cartridge Otherwise, the test result will be erroneous).
5.Put in the sample-loaded cartridge into the ichromaTM test instrument's cartridge holder to scan it. Before inserting the cartridge all the way into the cartridge holder, double-check its orientation. An arrow is specially marked on the cartridge for this reason.

6. To begin the scanning procedure, press the 'Select' or 'START' button on the Instrument for ichromaTM testing.

7.The Instrument for ichromaTM testing will immediately begin scanning the sample-loaded cartridge.

8.Read the test result on the display screen of the Instrument for ichroma™ tests.

3.4.4. Rapid determination of COVID-19 antibodies (IgM and IgG):

Assay principle:

The COVID-19 (SARS-CoV-2) The nitrocellulose membrane is coated with mouse anti-human IgM, mouse anti-human IgG, and sheep anti-mouse polyclonal antibodies, and the IgM-IgG test is used to detect the recombinant new coronavirus (COVID-19) antigen. The compounds in mouse anti-human IgM antibody were collected when specimens with IgM antibody marked a novel coronavirus antigen from compounds with colloidal gold. demonstrates the colorful lines If the sample has an IgG antibody, a new coronavirus antigen was identified by colloidal gold from chemicals, The substances were able to capture the mouse anti-human IgG antibody. demonstrates the colored line that forms as a quality control line when specimens containing both IgG and IgM at the same time create two lines at T1 and T2, along with additional colloidal gold complexes. Only the quality control line displays the color, which is unfavorable when the sample lacks both IgG and IgM antibodies. (Tian *et al*. , 2020).

Assay procedure:

1. Adding 10 µl of serum to the specimen hole (S).

2. One drop of sample diluents was added to the specimen hole on the test card(S).

3.Waiting for the colored line (s) to appear. At 10~15 minutes, the

outcome should be read. After 30 minutes, the outcome should not be interpreted.

4. Positive results:

1.Two or three separate lines that appear. The control line zone (C) should always include one line, and the test line section (S) should always contain one or more obvious colored lines (T1 and T2).

2. The presence of an IgG antibody in the specimen and the absence of an IgM antibody are both indicated by lines on the quality control line, test line T1, and test line T2, respectively.

3. The presence of an IgM antibody in the specimen and the absence of an IgG antibody are both indicated by lines on the quality control line, test line T2, and test line T1, respectively.

5. Negative results:

The control displays one colored line (C). In the test line regions, there are no visible colored lines (T1 and T2).

3.4.5. COVID-19 AB Automated Fluorescent Immunoassay System (AFIAS):

Assay principle:

A sandwich immunodetection method is used in the test, in which fluorescence-labeled conjugates in dried detection buffer (DB) bind to antibodies in the sample to form antibody-antigen complexes, which then migrate onto the nitrocellulose matrix and are captured by immobilized anti-human IgG and anti-human IgM on the test strip. Antigen-antibody complexes multiply as the number of antibodies in a sample increases, producing a stronger fluorescence signal on the detector antigen, which

was then processed to determine the amounts of anti-COVID IgG and IgM in the sample (Trivedi *et al*.; 2019).

Contents:

The cartridge box includes a cartridge, a pipette tip, a C-tip, a spare cartridge, a zipper bag, an ID chip, and usage instructions.

Assay procedure:

1. The instrument's general mode was chosen for AFIAS tests.

2. Using a pipette, 100µ l of the sample (serum) was collected and poured into the cartridge's sample well.

3. The cartridge was inserted into the cartridge holder.

4. A tip was put into the cartridge's tip hole.

5. The screen's "START" button was taped.

6.After 10 minutes, the test results were shown on the screen.

testing result interpretation:

The test outcome is measured instantly by the AFIAS measurement apparatus, which displayed the results as Positive, Negative, or Indeterminate.

A cut-off index (COI), as shown in the tables, provided ancillary tables (3-3).

Table (3-3): Cut-off index (COI) for IgG and IgM

Note	Cut-off index (COI)	Result
No need to retest	< 0.9	Negative for IgG and IgM
Negative for IgG and IgM	0.9 ≤ Titer < 1.1	In determinate
In determinate	≥ 1.1	Positive for IgG and IgM
Positive for IgG and IgM		

3.4.6. C-Reactive protein Automated Fluorescent Immunoassay System (AFIAS):

Assay principle:

A sandwich immunodetection methodology is used in the test, in which the indicator antibody in the buffer binds to the antigen in the sample to produce antigen-antibody complexes that migrate to the nitrocellulose matrix and are captured by a different immobilized antibody on the test strip. An more antigen in a sample causes the formation of more antigen-antibody complexes, which strengthens the fluorescence signal on the sensor antibody. This allows the equipment to do an AFIAS analysis to determine the concentration of CRP in the sample (Torti *et al.*, 2002; Pepys and Hirschfield, 2003).

Contents:

The AFIAS CRP includes (Cartridge, Pipette tip, ID chip, Instruction for use). A detector and a cartridge part are the two parts of each cartridge that is wrapped in an aluminum pouch. A test strip, a membrane with anti-human CRP on the test line and rabbit IgG on the control line, are both included in the cartridge component. Anti-human CRP-fluorescence conjugate, anti-rabbit IgG fluorescence conjugate, bovine serum albumin (BSA) as a stabilizing agent, and sodium aside in phosphate buffer saline (PBS) as a preservative are all components of the detector section.

Assay procedure:

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

Testing Results Interpretation:

1.The CRP concentration of the test sample is shown in terms of mg/L using a tool for AFIAS assessment that automatically calculates test results.

2.Ten mg/L was the cutoff.

3. The AFIAS CRP's operational range was 0.5-200 mg/L.

3.5. Molecular study:

3.5.1. The DNA extraction kit:

Table (3-4):The Contents of the DNA Extraction Kit (FAVORGEN)

Seq.	Material	Volume
1.	FA Buffer	120 ml
2.	FB Buffer	65 ml
3.	TG1 Buffer	45 ml
4.	TG2 Buffer	30 ml
5.	W1 Buffer	44 ml
6.	Wash Buffer	20 ml
7.	Elution Buffer	15 ml
8.	Proteinase K	11 mg

3.5.2. Primers used in DNA amplification:

Table (3-5): Primers Used in this Study

Primers	Primer sequence (5' → 3')	Product size
IFITM3 rs6598045 *	F: TTCATGGTGTCCAGCGAAGA R: TGTGGAGACCCCCAACACAG	305 bp

*** Designed in this study for IFITM3 rs6598045(The primer designed by Prof. Dr. Zaidan Khliaf Imran Al-maamouri at University of Babylon College of Science for women Biology department).**

3.5.3. Materials and Procedures for DNA Extraction:

Using the FAVORGEN kit, which includes the solutions in the table(3-7), DNA was extracted and purified. Several extraction and purification solvents were kept at 28 °C until needed. Other substances were utilized in the extraction and purification of DNA in addition to the solution already present in a number of extraction solvents.

3.5.4. DNA extraction from blood:

Step 1: RBC Lyses

1. Frozen human blood was collected in an anticoagulant-treat collection tube and waited at room temperature until it thawed.

2.Transferring to a microcentrifuge tube, up to 300µl of blood .

3. Add RBC Lyses Buffer volume 600 and combined by inversion .

4. Incubate for 10 minutes, at room temperature .

5.Centrifuge for 5 minutes at 3000 x g, and extract the supernatant completely .

6.Resuspend the pellet with 100 µl RBC lyses buffer and added 20 µl protenase k Mix thoroughly by Incubate water bath at 56ºC for 15 minutes.

Step 2: Cell Lyses

7. Adding 200µl FABG Buffer and vortex combined .

8. Incubated at 70oC to lyses the sample for 15 minutes. Invert the sample every 3 ~ 5 minutes, during incubation .

9.Turn the tube quickly to extract droplets from within the cap.

10. Preheat the buffer with the elution in a 70oC water bath (DNA Elution for step 5 .

11.Five µl of RNase A was applied to the sample and vortex, then incubated at room temperature for 5 minutes.

Step 3: Binding

12. The sample was supplemented with 200µl ethanol (96 ~ 100 per cent). Mix vigorously for 10 seconds using pulse vortexing.

13.Turn the tube quickly to extract droplets from within the cap.

14. The sample collection tube mixture (including any precipitate) was carefully transferred to FABG Column. 5 Minute centrifuge.

Step 4: Washing

15.Wash FABG Column with 400µl W1 Buffer (ethanol added) by centrifuge for 30 seconds.

16.Wash FABG Column with 600µl Wash Buffer (ethanol added) by centrifuge for 30 seconds.

17.Centrifug for an additional 3 min to dry the column.

Step 5: Elution

18. Putted FABG Column to a new 1.5ml microcentrifuge tube.

19. Added 100µl of Preheated Elution Buffer or TE to the membrane center of FABG Column. Stand FAGB Column for 3~5 min or until the buffer is absorbed by the membrane.

20. Centrifuged for 30 seconds to elude the pure DNA .

21. Stored the DNA fragment at 4ºC or -20ºC.

3.5.5. Material and solution used in Electrophoresis:

1. Six X DNA loading dye supplied by promega Corporation.

2. Solution of Ethidium Bromide stain supplied by BDH- Chem –Ltd .boo (0.5) µl.

3. Agarose used from Norgen Biotic Comp.

4. DNA ladder 100 bp supplied by Intron Comp.

5. T.B.E buffer solution supplied by Promega Comp.

3.5.6. DNA electrophoresis:

1. In the beginning, 100 ml of the T.B.E buffer was placed in a beaker.
2. Then 1 g weight of agarose was added to the buffer.
3. the buffer with the agarose was heated on a hot plate to boiling point so that all of its components were solvent.
4. The agarose mixture was cooled by leaving it between 50-60˚C.
5. Ethidium Bromide dye was added at (0.5) µl to the agarose before solidification of the liquid and mixed it well.
6. The comb was put into one of the ends of the agarose gel template.
7. Agarose was poured into the template to prevent the formation bubbles and left to cool at room temperature for 30 minutes.
8. The migration electric basin was filled with the T.B.E buffer solution so that it rose from the gel surface.
9. The samples were placed in the pits with the addition of the agarose gel loading dye so that the dye was linked to the DNA .
10. The electrophoresis was performed in 70 V to 45 min.
11. The agarose gel was exposed to UV trans illuminator for DNA bands visualized and documented.

3.6. PCR assay:

3.6.1. Preparation of Primers for the Polymerase Chain Reaction Technique (PCR):

The primers were prepared by adding distilled water free of nuclease in the a different volume according to the manufacturing company instructions to obtain a solution of base stock with a concentration of 100 Pico mole / µl, mixed by vortex, then centrifuged for 10sec at 4000 rpm. Then 10 µl of each primer was taken and putt in the micro centrifuge tube with 90 µl of nuclease free distilled water to prepare the working solution.

3.6.2. PCR mixture:

PCR mixture for all primers used in this study was prepared according to the table (3-6).

Table (3-6): Volumes of chemical materials uses in PCR assay

Chemical materials	Volumes
Master Mix	5 µl
DNA	1-2 µl
Forward Primer	1 µl
Reveres Primer	1 µl
Deionizer D. W	16 µl
Total	25 µl

3.6.3. PCR conditions:

The conditions of PCR reaction for three primers used for polymorphism of human genes as shown in table (3-7).

Table (3-7): The conditions of PCR for two primers

No.	**IFITM3 rs6598045**			
	Stages	**Temperature**	**Time**	**Cycles**
1.	Pre denaturation	95°C	5 min	1
2.	Denaturation	95°C	30 sec	30
	Annealing	55°C	30 sec	
	Extension	72°C	40 sec	
3.	Final extension	72°C	5 min	1
4.	Cooling	4°C	∞	

3.6.4. PCR gel Electrophoresis:

The amplified PCR products were detected by agarose gel electrophoresis which was visualized by staining the Ethidium bromide. The electrophoresis result was detected by using gel documentation system. The positive result was distinguished when the DNA band base pairs of sample equal to the target product size, or the size of amplified DNA fragments which were identified by a comparison with molecular size marker DNA (100 - bp DNA ladder).

3.7. Detection of genes polymorphisms of PCR products

3.7.1. Detection of IFITM3 rs6598045 by sequencing analysis:

The 60 PCR products of IFITM3 rs6598045 gene (305 bp) were directly sequenced by Macrogen Company (Korea). After received the sequencing data for PCR products which are compared with gene bank by using NCBI Blast nucleotide database. Only clear chromatographs obtained from ABI sequence files were further analyzed to ensure that them annotation and variations are not due to PCR or sequencing artifacts. The sequencing results of the PCR products of different samples were edited, aligned, and analyzed as long as with the respective sequences in the reference database using BioEdit for multiple alignment sequence based on editor Software version 7.1 (DNASTAR, Madison, WI, USA). The observed variations in each sequenced sample were numbered in PCR amplicons as well as in its corresponding position within the referring genome.

3.8. Statistical analysis:

By using statistical program (SPSS) version, the ANOVA analysis were done as well as , Chi- Square , T- Test , Regression and Correlation for each studied parameters in type of patients and control . The significant value as P. Value < 0.05 . Scattered correlation between different studied parameters (Alan C. *et al*.; 2007 , Seetharaman & Selvaraj 2020).

Chapter Four

Result and Discussion

4.1. Immunological parameters and age groups:

The results of the study demonstrate a substantial rise in the levels of (CRP, D. Dimer, R.B.S., Interferon -ɤ, HBA1C, AntiSars-Cov2 -IgG and IgM antibodies), but Interleukin -6 levels were not significantly different from controls as shown in table (4-1).

Table (4-1) Immunological parameters and age groups

Studied groups	Age Groups	No	Mean ± SD				
			CRP	D.Dimer	IL-6	INF-γ	Cortisol
Patients	20 – 29	9	13.35 ±2.90	1812.3 ± 225.6	148.8± 36.3	129.1± 16.2	79.4 ±16.5
	30 – 39	10	12.48± 2.10	1686.7± 207.6	159.9± 58.8	137.8± 39.4	79.5± 24.8
	40 - 49	13	12.76± 4.81	1759.2 ±267.8	179.5± 87.8	125.7± 18.2	81.2± 22.1
	50 - 59	10	14.18±6.00	1925.6± 331.4	167.9± 53.8	152.5 ±58.9	78.7 ±20.3
	> 60	12	12.09 ±2.11	1268.6 ±213.3	161.4± 43.9	143.0 ±20.9	75.2 ±11.7
Control	20 - 29	18	1.38± 0.24	224.2± 32.8	121.3 ±78.9	168.3± 45.3	85.4 ±19.4
	30 - 39	6	1.35± 0.42	251.4 ±19.6	166.3± 40.2	139.2±25.2	79.1±16.4
	30 - 39	14	1.31± 0.18	235.0± 6.81	149.6± 12.1	137.1± 13.2	74.6± 10.2
	40 - 49	3	1.35 ±0.02	228.3± 11.02	177.5± 31.8	136.1 ±17.1	77.9 ±20.1
LSD Value			1.26*	53.08*	11.04*	8.04*	1.86*

(*) LSD (Less significant differences in Mean results),The difference value < LSD refer to No significant,While in > LSD it was significant.

The result of table (4 -1) shows that the adult age group (50 - 59 years) have higher level (Mean: 14.18) of CRP in comparison with other age groups as well as control groups , with noted that all age groups have higher level of CRP in comparison with control. The CRP level were increased in Sars-Cov2 infected patients and used as diagnostic criteria of infection. A number of recent series have reported an association between higher CRP concentrations and greater disease severity in COVID-19 (Petrilli *et al*., 2020 ; Luo *et al*., 2020 ; Wu *et al*., 2020). CRP is associated

with extra-pulmonary disease in COVID-19, and correlations between CRP concentrations and myocardial injury have been reported in multiple series (Guo *et al*., 2020 ; Basso *et al*., 2020).

The adult age group (50 -59 years) has higher level(Mean:1925.60) of D-Dimer in comparison with other age groups as well as control groups , with noted that all age groups have higher level of D-Dimer in comparison with control as shown in table (4 - 1). The D-Dimer level were increased in Sars-Cov2 infected patients and refers to thrombotic complication in infected patients. D-dimer is a byproduct of fibrin degradation. It is widely recognized as a biomarker for thromboembolism and as a prognostic marker for critical patients. COVID-19 being a procoagulant state thus D-dimer has been studied as a biomarker for predicting disease severity. Studies in COVID-19 patients have demonstrated coagulation abnormalities with marked elevation in D-dimer levels in patients with severe disease. Recent studies have correlated D-dimer more than 2000 ng/mL was associated with increase fatality in COVID-19 (Zhang *et al*., 2020).

The result of table (4-1) show that the young age group (30 -39 years, followed by 40-49 years) have higher level (Mean:83.50) of Cortisol hormone in comparison with other age groups as well as control groups , with noted that all age groups have cortisol level more than control groups. The cortisol hormone level was increased in Sars-Cov2 infected patients due to the stress conditions of infected patients , there is some patients might show lower level than normal range of Cortisol hormone. The effects of COVID-19 on cortisol are still uncertain. It has been recommended that SARS-CoV, the precursor of SARS-CoV-2, could activate an immunogenic reaction to adrenocorticotropic hormones(Tan *et al*.,2020) .SARS-CoV-2 could employ similar pathways to increase morbidity and mortality by causing a cortisol synthesis

insufficiency linked to a serious disease(Tan *et al*., 2020 ; Khodeir *et al*., 2021) . It can be said that stress during the disease leads to the HPA axis activation and as a result elevates the level of serum cortisol in patients (Ramezani *et al*., 2020). It was concluded that the increased evening and nocturnal but not morning cortisol secretion may occur in even clinically mild COVID-19. In the context of acute viral infection (COVID-19), IL-6 may partially replace ACTH as a stimulus of the glucocorticoid-secreting adrenal zona-fasciculata without influencing the secretion of DHEA and aldosterone (Maria *et al*., 2022).

The results show that there are no differences were mentioned in the Interferon -γ concentration among all age groups in comparison with control groups as in table (4- 1). The level of Interferon - γ was changing in viral response rather than other infection especially at active state of infection. IFNs were initially described as molecules that interfere with viral replication (Seo & Hahm 2010).The excessive level of released cytokines (cytokine storm) in response to SARSCoV2 infection is related to COVID-19 severity complications, hospitalization, and even mortality (Skinner *et al*., 2019 ; Sanli *et al*., 2021). Patients with the severe progression of COVID-19 showed signs of hyperinflammatory secondary hemophagocytic lymphohistiocytosis (HLH) syndrome. This syndrome is accompanied by a fatal cytokine storm and multiorgan failure (Skinner *et al*., 2019 ; Ruan *et al*., 2020).

There are no differences were mentioned in the interleukin-6 concentration among all age groups in comparison with control groups as in table (4 - 1). The level of IL-6 were increased in response to viral response in association with cytokine stroma patients , certain cases have no IL-6 differences depending on the immunity status as well as prognostic condition of infected patients. Previous studies have found a negative correlation between IL-6 and respiratory function in patients

with coronavirus disease 2019 (COVID-19) (Jørgensen *et al*., 2020) , and IL-6 antagonist tocilizumab is shown to increase survival in COVID-19 patients with severe or critical disease (Gordon *et al*., 2021). These findings suggest that IL-6 is not only a marker of respiratory failure but also contributes to pulmonary inflammation and injury in this disease.

Table (4 - 2) Immunological parameters and age groups

Studied groups	Age Groups	No	Mean ± SD			
			RBS	HbA1c	IgG	IgM
Patients	20 -29	9	134.7± 10.7	10.4± 3.3	10.9± 2.20	4.77± 2.6
	30 -39	10	134.7± 17.8	8.1± 2.4	7.2±14.96	3.11± 1.8
	40 - 49	13	163.3± 29.7	8.8 ±2.2	5.4 ±8.40	5.47± 2.4
	50 - 59	10	163.7±19.3	9.6 ±3.1	12.3± 12.02	3.97±1.2
	> 60	12	177.5± 16.6	8.8 ±1.8	11.2± 10.38	1.53± 0.6
Control	20 – 29	18	106.9± 11.0	4.9± 0.2	0.06 ±0.11	0.05± 0.06
	30 -39	6	103.1± 16.4	4.8± 0.2	0.04± 0.08	0.10± 0.10
	30 -39	14	106.5 ±13.6	5.0± 0.2	0.17±0.12	0.01± 0.00
	40 -49	3	109.0± 12.6	5.2 ±0.5	0.22± 0.29	0.12 ±0.05
LSD Value			11.6*	1.62*	5.41*	1.66*

(*) LSD (Less significant differences in Mean results),The difference value < LSD refer to No significant,While in > LSD it was significant.

The results of table (4-2) indicate that all age groups had greater levels of RBS in comparison with controls, with the exception of the adult age group (> 60 years), which has the highest level (Mean:177.50) of RBS. Due to stress, variations in metabolism, and some hormones, the blood sugar level was elevated in connection to Sars-Cov2 infection. Diabetes mellitus has been one of the most consistent risk factors for severe disease in patients with COVID-19 and uncontrolled hyperglycemia has been associated with poor outcomes and mortality. This could be due to diabetes being associated with other risk factors like

age, hypertension and obesity (Apicella *et al*., 2020).

The young age group(20 - 29 years) has higher level (Mean: 10.43) of HBA1C in comparison with other age groups as well as control groups , with noted that all age groups have HBA1C level more than control groups table (4- 2). The percentage of HBA1C was increased in some patients infected with Sars-Cov2 infection due to the stress conditions of infected patients.The relationship between HbA1c and severe COVID-19 in diabetic and non-diabetic individuals is still unclear one showed that COVID-19-related mortality was higher in people with HbA1c>7.5% (Williamson *et al*., 2020). and another identified that mortality in people with type 1 and type 2 diabetes was significantly and independently related to the HbA1c (Holman *et al*., 2020). In addition, a study of 80 patients with type 2 diabetes from India even found that COVID-19 patients with HbA1c <8 % exhibited a excessive uncontrolled inflammatory responses, hypercoagulable state, and severe symptomatic presentation (Bhandari *et al*., 2020) Besides, A similar outcome was found in 44 COVID-19 patients admitted to an intensive care unit in Austria (Klein *et al*., 2020). From another point of view, based on the results of a study of 806 diabetes patients in Saudi Arabia, it was found that high HbA1c level were independently associated with hospitalization, compared with low HbA1c level (Al Hayek *et al*., 2020).

The result of table (4-2) shows that the young age group (50 -59 years followed by >60 years) has higher level of Anti Sars-Cov 2-IgG Antibody in comparison with other age groups as well as control groups, with noted that all age groups have Anti Sars-Cov 2 - IgG Antibody level more than control groups.

The result of table (4 - 2) shows that the young age group(40 - 49 years) has higher level of Anti Sars-Cov2–IgM Antibody in comparison with other age groups as well as control groups , with noted

that all age groups have Anti Sars-Cov 2– IgM Antibody level more than control groups.

The IgM antibodies form during the acute stage of the illness, reach their peak at 14 to 35 days, and then start to drop during the following 21 to 35 days. IgG antibodies reach their peak at about 21 to 49 days after infection, and they can last for up to four months when combined with neutralizing antibodies (Dan *et al*., 2021).The majority of immunoassays that are used to check people for COVID-19 exposure identify particular antibodies (IgG and IgM) in the serum that react with SARS-CoV-2 proteins. IgG is a late antibody that is only detectable after around 8 days, while IgM is released sooner during an infection (between 3 to 6 days). IgG has a higher affinity for a protein antigen than IgM, and its specificity for the antigen (viral) is typically higher (Janeway *et al*., 2001).

4.2. Immunological parameters and sex groups:

Table (4-3) Immunological parameters and sex groups

Studied groups	Six Groups	No	Mean ± SD				
			CRP	D.Dimer	IL-6	INF-γ	Cortisol
Patients	Male	20	13.4 ±3.8	2217.8 ±305 .2	150.7 ±29.3	38.5± 8.6	76.46± 19.8
	Female	34	12.9± 3.4	1402.5± 203 .4	173.6± 25.5	31.8 ±5.4	80.56± 18.9
Control	Male	26	1.4±0.26	230.6 ±53.5	115.0± 31.4	42.2 ±8.2	78.10± 16.3
	Female	15	1.2 ±0.20	235.8 ±4.4	116.6 ±21.4	18.2± 4.7	83.50 ±16.2
LSD Value			0.85*	21.2*	9.88*	11.43*	4.62*

(*) LSD (Less significant differences in Mean results),The difference value < LSD refer to No significant,While in > LSD it was significant.

Table (4-4) Immunological parameters and sex groups

Studied groups	Six Groups	No	Mean ± SD			
			RBS	HbA1c	IgG	IgM
Patients	Male	20	163.7± 19.2	9.8 ±3.2	7.23 ±8.7	4.01±4.70
	Female	34	142.5± 14.3	8.7±2.3	10.62 ± 12.1	3.78± 4.56
Control	Male	26	105.7± 13.5	4.9± 0.30	0.08 ±0.12	0.04 ±0.07
	Female	15	107.7 ±11.5	5.2 ±0.26	0.08± 0.13	0.04± 0.06
LSD Value			21.3*	3.71*	3.39*	2.73*

(*) LSD (Less significant differences in Mean results),The difference value < LSD refer to No significant,While in > LSD it was significant.

The table (4-3) show that significant differences in CRP level between male and female , although it increased in CRP level after comparison with control at LSD value (0.85).

The result of table (4-4) show that significant increased in blood sugar level in male more than female of Sars-Cov2 infected patients ,as well as after comparison with control at LSD value (21.3).

There was a significant increase in D. Dimer level among male patients rather than female , and shows that there is a significant increased in patients after comparison with control , the LSD value (21.2) as shown in table (4 - 3).

When studying the relationship between cortisol hormone and sex distribution, has been shown significant decreased in male rather than female , at LSD value (4.62) as mentioned in table (4 - 3).

There is no significant difference in interferon– γ level in association with sex distribution , although it increased after comparison with control , LSD value (11.43) as shown in table (4 - 3).

The result of table (4- 3) show that decreased level of IL- 6 in male patients after comparison with female as well as the control groups.

The HBA1C percentage revealed that elevation in male patients than female,the overall result of patients groups were increased in comparison with control,This result might be due to that the female have low hemoglobin level than male , so the glycosylated hemoglobin (HBA1C) well be reduced accordingly. This result mentioned in table (4-4).

The level of Anti Sars –Cov2 –IgG antibody was elevated in female rather than male as well as higher level in comparison with control groups , LSD value (3. 39) as mentioned in table (4 -4) , this result might refer to the increased level of IgG against virus infection used as a monitor to evaluation of diseases activity and immunological status of patients with noted that , not all patients revealed antibody elevation.

The level of Anti Sars –Cov2 –IgM antibody was increased in male rather than female as well as higher level in comparison with control groups , LSD value (2.73) as mentioned in table (4 - 4), this result might refer to the increased level of IgM against virus infection used as a monitor to evaluation of diseases activity and immunological status of patients at the first or second week of infection.

Females and males differ in the energy consumption and nutritional requirements which are based on the interactions between environmental factors and sex hormones (Wu *et al*., 2011).

In general, females have stronger innate and adaptive (humoral and cellular) immune responses in comparison to males. The factors responsible for the stronger immune response in females than males may be due to biologic factors (i.e., sex differences, such as genetic and epigenetic factors, sex hormones) and to psychosocial factors (i.e., gender differences) (Elena *et al*., 2019). Males and females have similar proportions of COVID-19 cases, suggesting similar infection rates (Peckham *et al*., 2020). However, males exhibit higher disease severity and are at a higher risk of succumbing to the disease (Lipsky & Hung

2020 ; Gomez *et al*., 2021). Mortality rates were higher amongst males across all age groups above 20 years in multiple European countries (Scully *et al*., 2020).

There are many possible explanations for the difference in COVID-19 outcomes between men and women, including certain behavioral and social factors. However, biological factors such as sex-related genes and sex hormones that influence immune system regulation may also play an important role(Sharma, Volgman & Michos 2020).

The weaker male immune system leaves men more susceptible to a range of infectious diseases. One potential explanation is that sex hormones are involved in the way the immune system triggers an inflammatory response to pathogens. Some researchers think these differences may also explain why men have been hit harder by COVID-19 (Zhang *et al*., 2020).

4.3. Qualitative measurement of serological Antibodies IgM and IgG:

The qualitative serological measurement of anti Sars-Cov2 antibodies (IgM and IgG), shows that the Anti-Sars-Cov 2 –IgM antibody and the Anti-Sars-Cov 2–IgG antibody was associated with differences of many studied parameters , Increased level of CRP , Cortisol level , Interferon – γ , IL-6, Quantities of specific IgM and IgG against Sars-Cov2 virus. The D. Dimer level show that decreased in qualitative IgM positive patients in comparison with IgM negative,while no significant changes in HBA1C at both positive and negative IgM antibody. The level of Cortisol and IL-6, show that decreased in qualitative IgM positive patients in comparison with IgM negative The Table (4 - 5) was monitoring these results:

Table (4-5) Qualitative serological detection of anti- SARS-CoV 2 IgM and IgG

Parameter	Qualitative detection of anti- SARS –CoV 2 IgM and anti-SARS- C0V 2 - IgG (Mean ±SD)				
	IgM +ve (41)	IgM –ve (13)	IgG +ve (30)	IgG –ve (24)	Control (41)
CRP	13.21 ±3.87	12.89 ±2.47	13.47 ±4.09	12.70 ±2.79	1.35 ± 0.24
P.Value	**0.00**		**0.00**		
RBS	152.09 ±63.30	145.00 ±52.56	151.76 ±56.84	148.66 ±65.98	106.48 ±12.74
P.Value	**0.00**		**0.00**		
D. Dimer	1668.82 ±259.68	1817.00 ±209.28	1837.03 ±261.48	1538.83 ±231.31	232.58 ±42.50
P.Value	**0.00**		**0.00**		
Cortisol	80.85 ±24.33	76.44 ±12.44	76.43 ±15.22	83.98 ±28.17	80.56 ±18.12
P.Value	**0.00**		**0.00**		
Interferon –γ	139.51 ± 37.95	129.27 ±15.97	137.64 ±37.33	136.29 ±30.42	109.85 ±39.04
P.Value	**0.00**		**0.04**		
IL- 6	175.44 ±65.59	132.88 ±19.96	153.62 ±47.16	179.66 ±72.60	176.11 ±59.07
P.Value	**0.78**		**0.09**		
HBA1C	9.08 ±2.45	9.48 ±3.39	9.61 ±3.03	8.64 ±2.09	5.00 ± 0.28
P.Value	**0.13**		**0.13**		
IgG	6.34 ±10.13	18.90 ±7.98	15.46 ±9.27	1.74 ±7.88	0.08 ±.01
P.Value	**0.05**		**0.19**		
IgM	5.04 ±4.67	0.16 ± 0.13	2.94 ±4.16	5.02 ±4.87	0.04 ± 0.02
P.Value	**0.00**		**0.00**		

4.4. Sex distribution of Sars-Cov2 infected patients:

In the present study the figure (4 -1) show that the female infected patients were have higher level than male at (63% and 37 %) respectively.

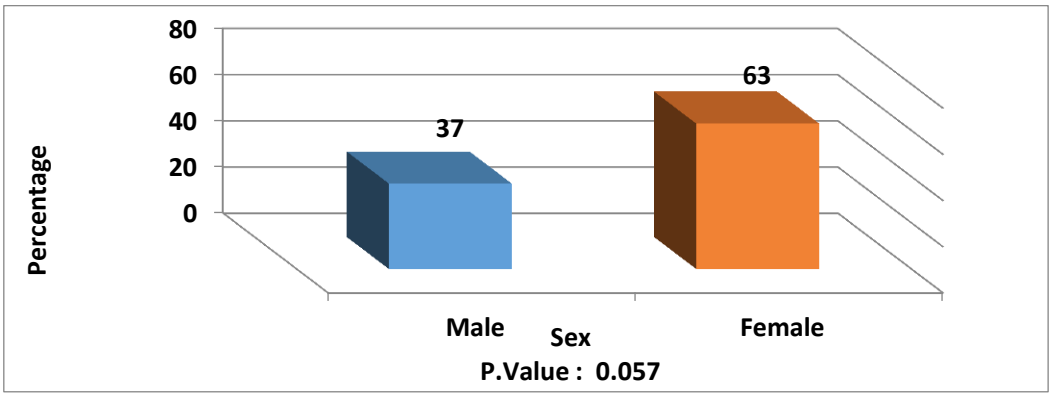


Figure (4 - 1) Sex Distribution of SARS –COV-2 infected patients

4.5. Qualitative measurement of anti - Sars-Cov2 IgM infected patients:

The serological detection of anti Sars-Cov2 –IgM antibody shows that 75.9 % of patients have positive reaction in comparison with 24.1 % as negative result, the negative result might be due to very early infection or late infection more than 2 week of infection. These results were show in the figure (4 - 2).

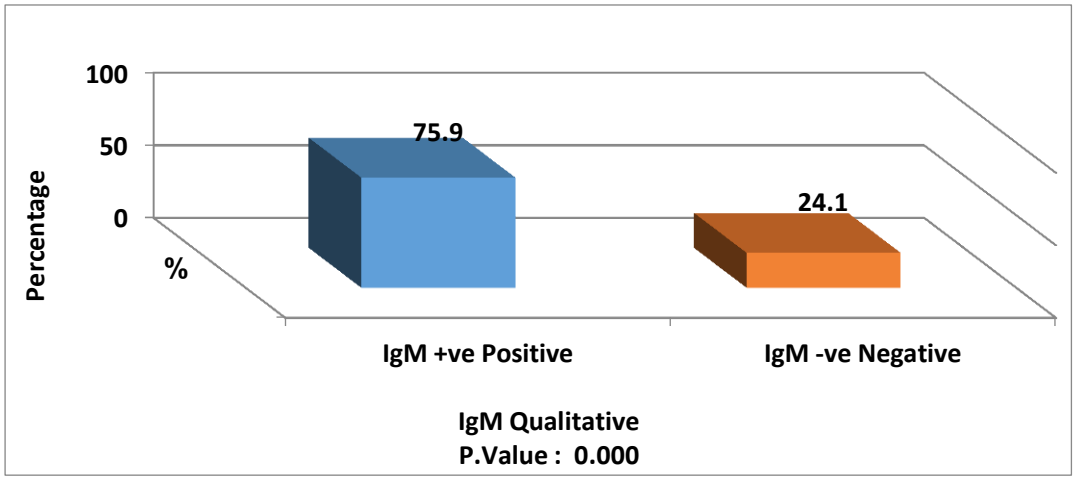


Figure (4 -2) Qualitative measurement of anti - Sars-Cov2 IgM infected patients

4.6. Qualitative measurement of anti - Sars-Cov2 IgG infected patients:

The serological detection of anti Sars-Cov2 –IgG antibody show that 55.6 % of patients have positive reaction in comparison with 44.4 % as negative result; the negative result might be due to very early infection or late infection more than 2 week of infection. These results were show in the figure (4 -3) .

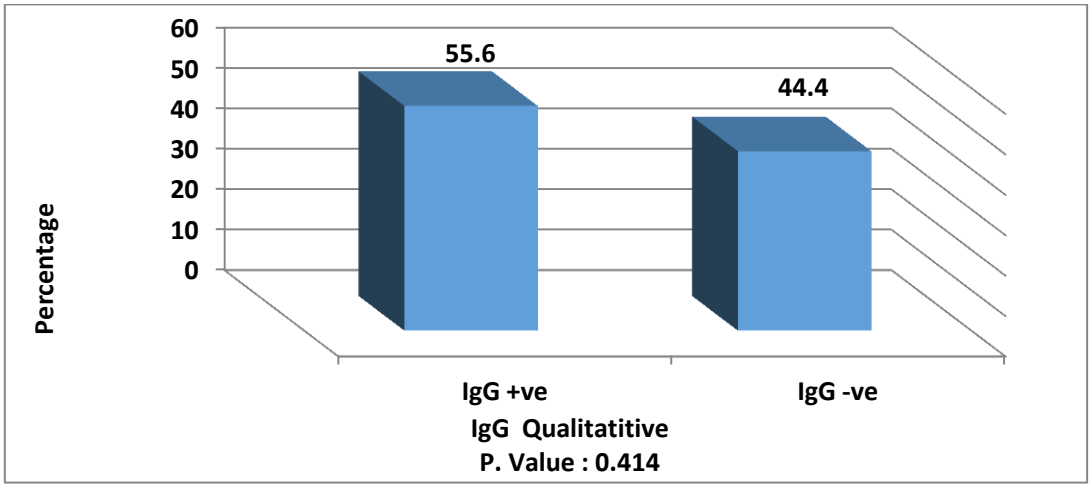


Figure (4 -3) Qualitative measurement of anti - Sars-Cov2 IgG infected patients

4.7. Correlation of HBA1C level and cortisol hormone:

The result of figure (4-4) shows that negative correlation between HBA1C percentage with cortisol level , this result might show that increased blood sugar and HBA1C lead to increased consumption of cortisol hormone and lead to decreased it level . The figure (4 - 4) shows this result.

Hemoglobin A1c (HbA1c) Interpretation: A 100% increase in cortisol is associated with an average beta-coefficient unit change in HbA1c (%). In the continuous unadjusted model, a 100% increase in serum cortisol is associated with a 0.26 increase in HbA1c (Ortiz *et al*., 2019). Cortisol has been associated with components of the molecular pathogenesis of type 2 diabetes (T2D) including insulin resistance and

impaired β-cell function. Insulin resistance and T2D are comorbities commonly associated with Cushing's disease (Anagnostis *et al*., 2009).

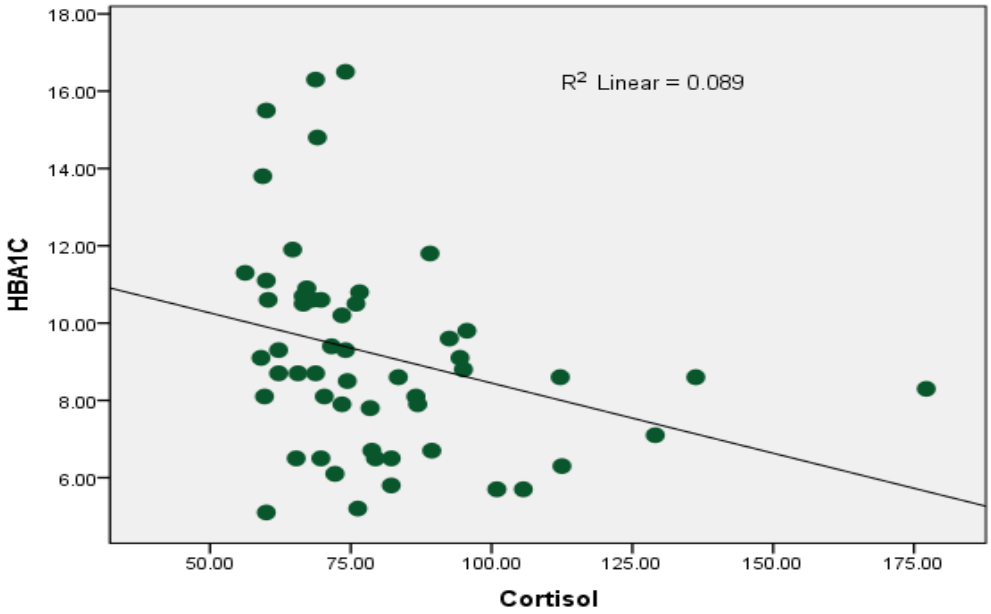


Figure (4-4) Correlation between HBA1C and Cortisol hormone

4.8. Correlation of HBA1C level and IL- 6 concentration:

There is no significant correlation was shown between HBA1C and IL-6 in Sas-Cov2 infected patients, they increased in parallel manner according to infectivity rate or viral activity at covid -19 patients with high blood sugar level either before or during infection,as in figure (4- 5).

Interleukin-6 (IL-6) is a pleiotropic proinflammatory cytokine that regulates the acute-phase inflammatory response and plays a central role in immune-mediated diseases, being a therapeutic target in various inflammatory conditions (Jones, Scheller & Rose-John 2011 ; Garbers *et al*., 2018). Several lines of evidence supported a potential prognostic role for IL-6 for severe outcomes in hospitalized COVID-19 patients, showing that high IL-6 serum levels at admission predicted the development of hypoxemia requiring hospitalization (Al Barzin *et al*., 2020 ; Giannakodimos *et al*., 2021). IL-6 may also affect the function of

endocrine tissues including stimulation of the hypothalamic-pituitaryadrenal (HPA) axis in healthy volunteers (Tsigos *et al*., 1997) and patients with sepsis (Torpy, Bornstein & Chrousos 1998) and cancer (Spath-Schwalbe *et al*., 1994).

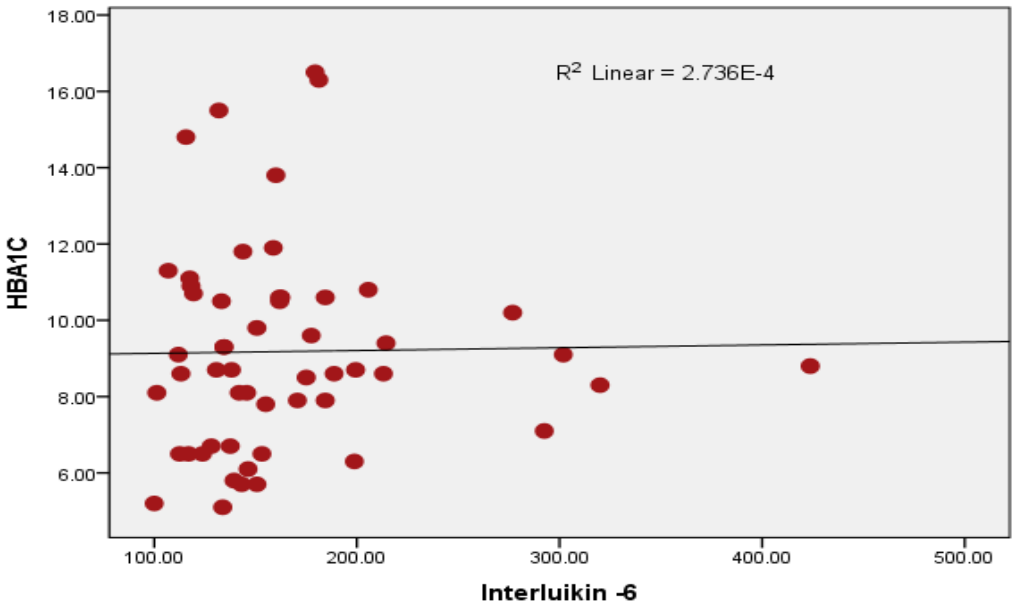


Figure (4 -5) Correlation of HBA1C with IL -6 concentration

4.9. Correlation of HBA1C level and anti Sars-Cov2 - IgG titer:

There is a direct correlation between HBA1C and anti Sars-Cov2-IgG level among infected patients, increasing the HBA1C percentage has no effect on antibody production against Sars-Cov2 virus as well as there is an increase in IgG level during infection. Figure (4 - 6) shows these results.

It is more likely that the link between hyperglycemia and diabetes is multifaceted, not only that HbA1c is a marker of overall health, but that acute and chronic hyperglycemia also actively effects the immunologic response to viral infection. It is likely that patients with elevated HbA1c would have changes associated with both acute and chronically elevated blood glucose. Acute blood glucose changes could be induced by cytokines and endogenous steroid release seen in many stress states.

The Beta-cells in the pancreas have been shown to have ACE-2 receptors integral to viral entry, and these may become up regulated with COVID-19 infection (Fignani *et al*., 2020).

Acute hyperglycemia can affect the innate immune system through neutrophil dysfunction, inhibition of circulating complement and immunoglobulin function, and stimulation of cytokine release(Jafar, Edriss & Nugent. 2016)In COVID-19, neutrophils are thought to react infection in a multitude of ways, with the production of neutrophil extracellular traps as an important mechanism for viral clearance (Borges *et al*., 2020).

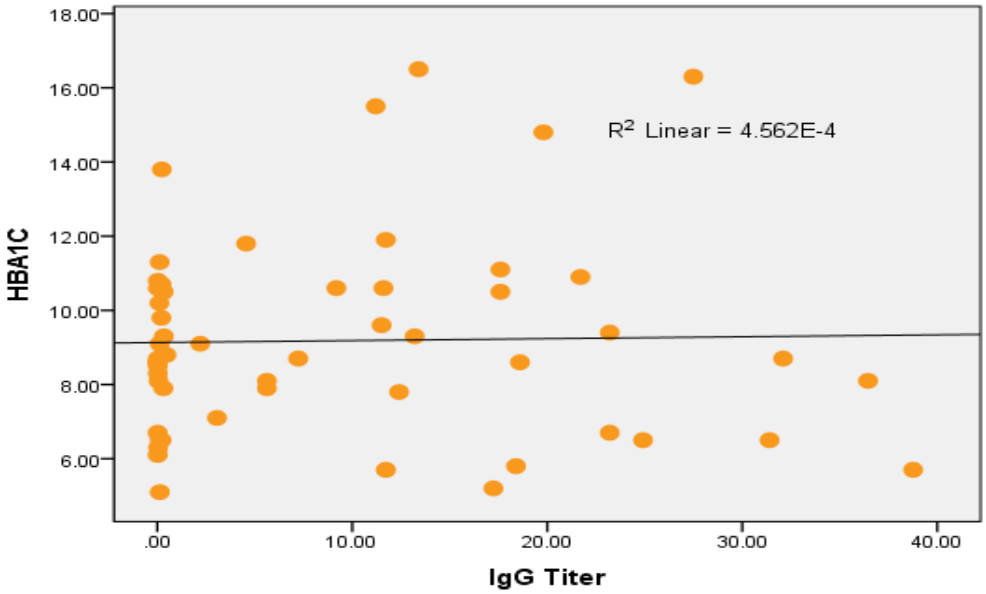


Figure (4 -6) Correlation of HBA1C and Anti –Sars–Cov 2 –IgG antibody level

4.10. Correlation of HBA1C level and anti Sars-Cov2 - IgM titer:

There is a direct correlation between HBA1C and anti Sars-Cov2-IgM level among infected patients, increasing the HBA1C percentage has no effect on antibody production against Sars-Cov2 virus as well as there is an increase in IgM level during infection. Figure (4 - 7) shows this result.

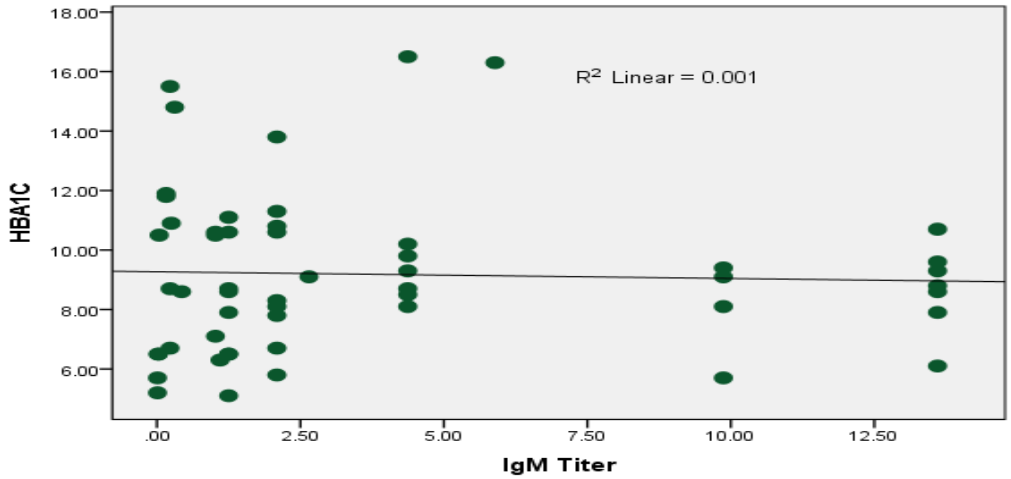


Figure (4 - 7) Correlation of HBA1C and Anti –Sars –Cov 2 –IgM antibody level

4.11. Correlation of Cortisol level and anti Sars-Cov2 - IgG antibody:

The result of figure (4- 8) shows that there is no significant effect of cortisol level on antibody production in high blood glucose level of Sars-Cov2 infected patients , The level of Anti Sars-Cov 2 -IgG antibody was slightly reduced in association with cortisol level .

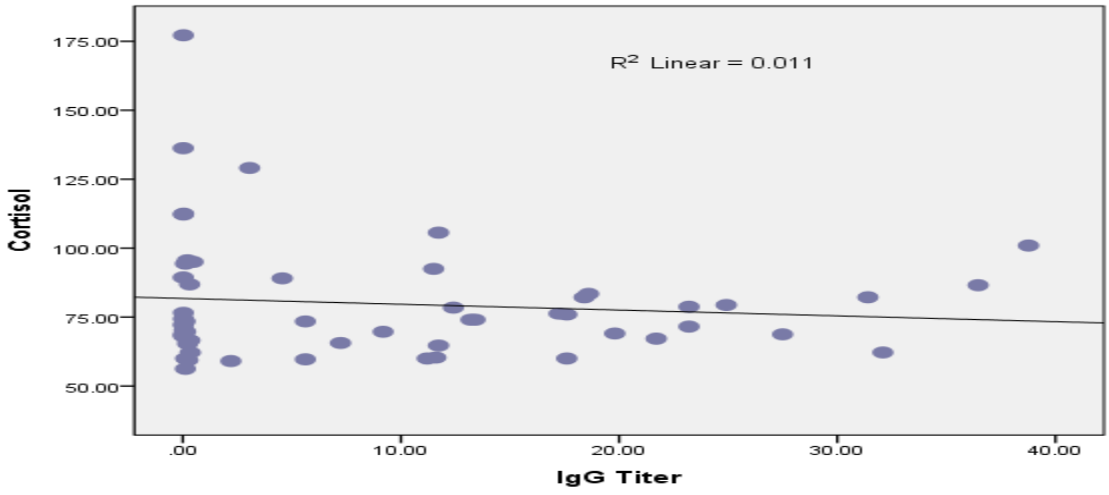


Figure (4 – 8) Correlation of Cortisol and Anti –Sars –Cov 2 –IgG antibody level

4.12. Correlation of Cortisol level and anti Sars-Cov2 - IgM antibody:

The result of figure (4 - 9) shows that there is no significant effect of cortisol level on antibody production in high blood glucose level of

Sars-Cov2 infected patients , The level of Anti Sars-Cov 2 -IgM antibody was slightly elevated in association with cortisol level .

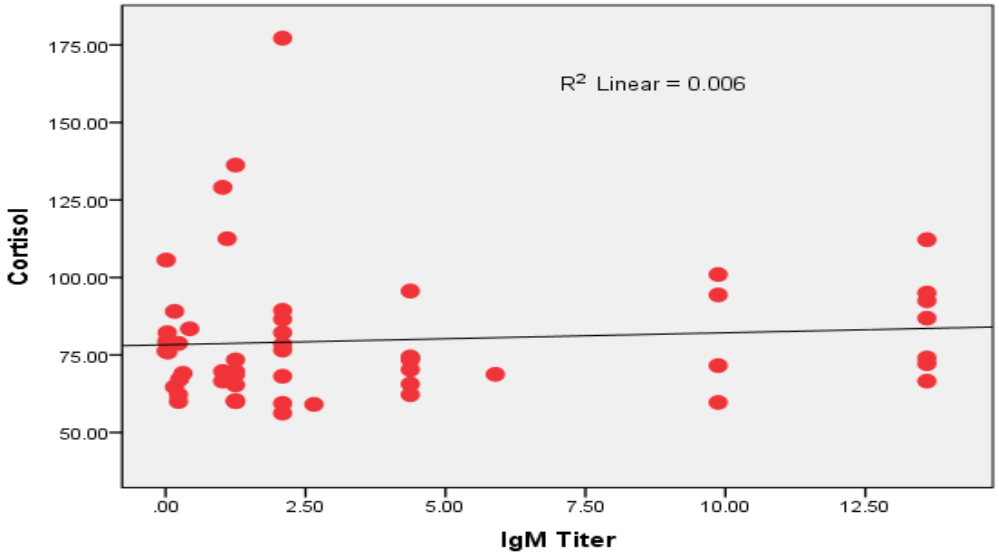


Figure (4 – 9) Correlation of Cortisol and Anti –Sars –Cov 2 –IgM antibody level

4.13. Correlation of Interferon-γ and anti Sars-Cov2 - IgG titer:

The result of figure (4 - 10) shows that there is no significant effect of interferon - γ level on antibody production in high blood glucose level of Sars-Cov2 infected patients , The level of Anti Sars-Cov 2 -IgG antibody was slightly reduced in association with interferon - γ level .

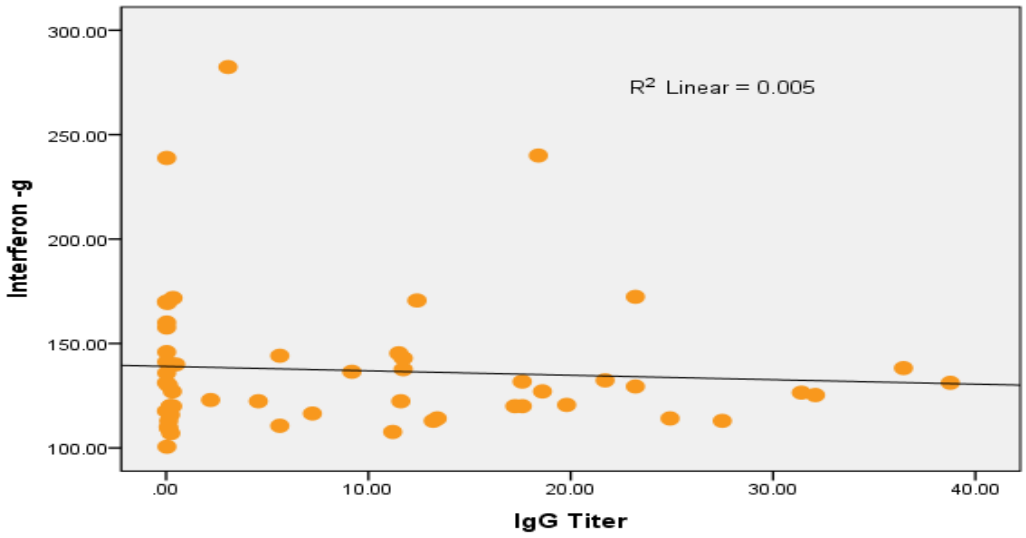


Figure (4 - 10) Correlation of Interferon - γ and Anti –Sars –Cov 2 –IgG antibody level

4.14. Correlation of Interferon - γ and anti Sars-Cov2 -IgM titer:

The result of figure (4- 11) shows that there is no significant effect of interferon - γ level on antibody production in high blood glucose level of Sars-Cov2 infected patients , The level of Anti Sars-Cov 2 -IgM antibody was slightly reduced in association with interferon - γ level.

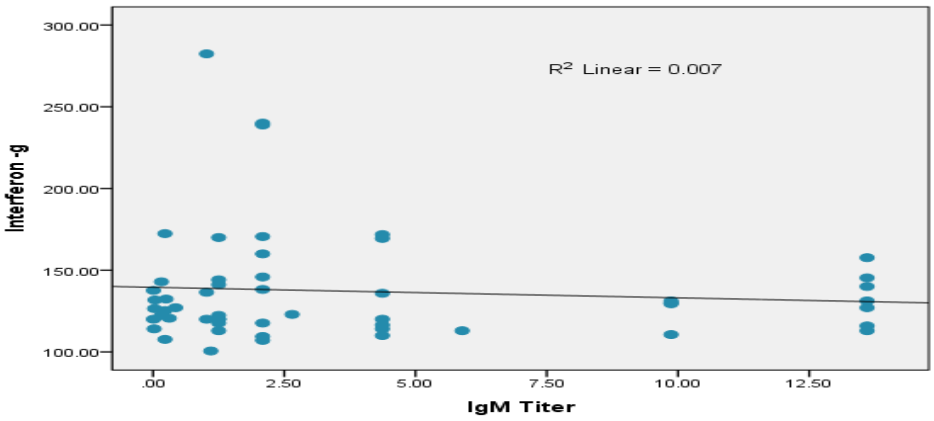


Figure (4 - 11) Correlation of Interferon - γ and Anti –Sars –Cov 2 –IgM antibody level

4.15. Correlation of Interleukin-6 and anti SARS-COV-2 IgG antibody:

The result of figure (4 -12) shows low IgG level in comparison with IL-6 , this result might show that early production of IL-6 in counter to viral infection not necessary accompanied with antibody production because the antibody need more time to production may belonging to 10 days or more.

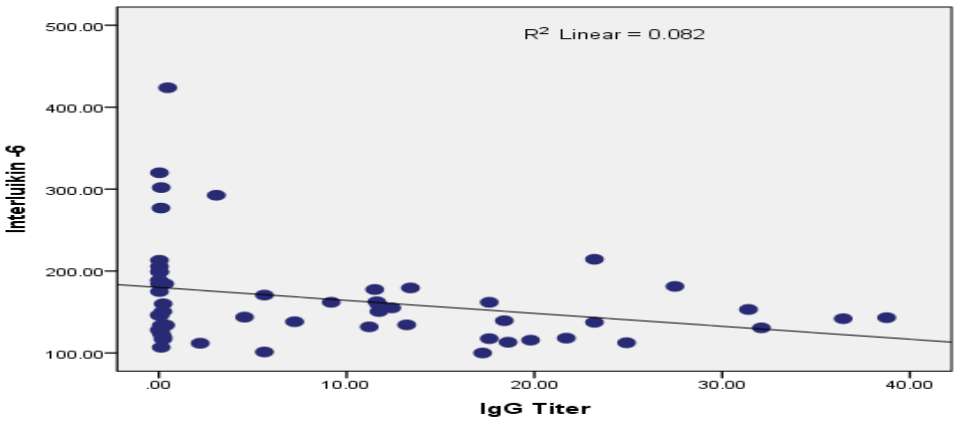


Figure (4 -12) Correlation of Interleukin - 6 and Anti –Sars –Cov 2 –IgG antibody level

4.16. Correlation of anti Sars -Cov2-IgG antibody and anti Sars -Cov2 -IgM antibody:

There is asignificant correlation between Anti-Sars-Cov-2 antibody (IgM and IgG) among diabetic population, in which that the IgG level revealed more than IgM level as shown in figure (4 -13) . This result might be show that the anti-Sars-Cov2 IgG more prolonged that IgM as well as higher concentration than.

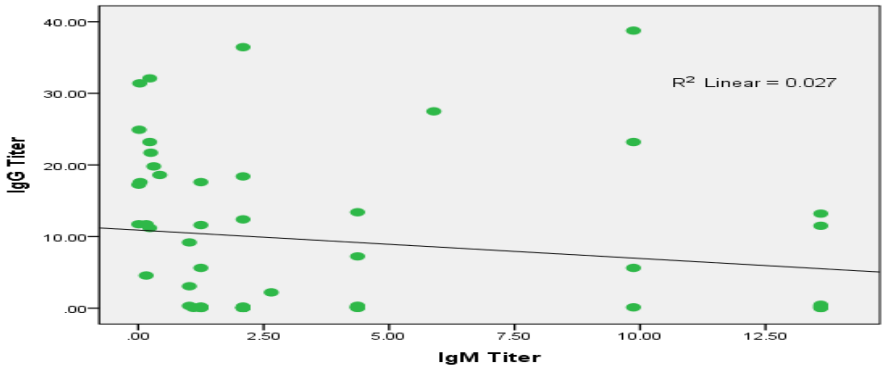


Figure (4 -13) Correlation of Interleukin - 6 and Anti –Sars –Cov 2 –IgM antibody level

4.17. Correlation of CRP level in relation to anti -Sars-Cov2-IgM antibody:

The result of figure (4-14) shows that a direct relationship or correlation between CRP level and anti Sars-Cov2 –IgM antibody. IgM antibody might be elevated during the onset of infection by Sars-Cov2 virus The CRP and IgM antibody were elevated at the same time of Infection although the CRP increased earlier that IgM.

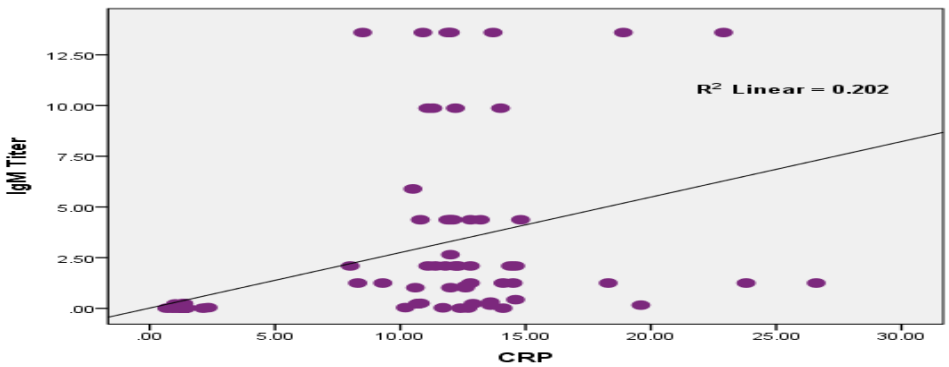
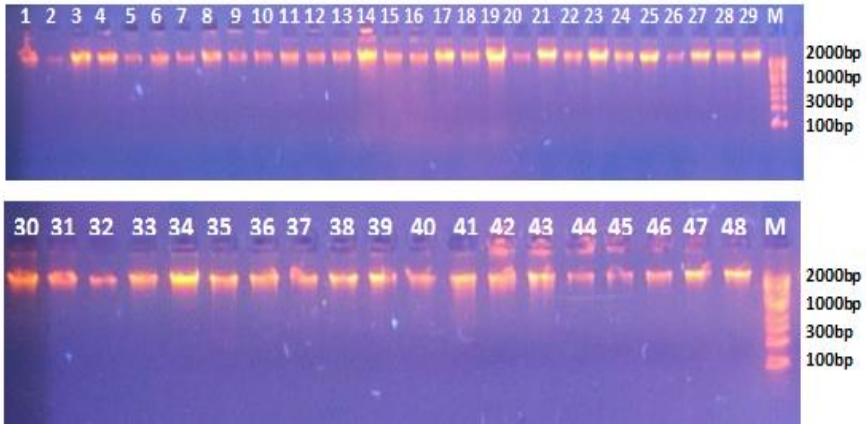


Figure (4 -14) Correlation of CRP concentration with Anti –Sars-Cov2 – IgM antibody level

4.18. Evaluation the polymorphism of IFITM3 gene and it role in Covid 19 severity infection

4.18.1. Quality DNA extracted from blood of Covid 19 patients:

The profile gel-electrophoresis 48-blood samples of Covid- 19 patients Figure (4-15) was shown that all samples gave huge bright bands of DNA (Note: Were lost 6 blood samples of Covid-19 patients during DNA extraction).



Figure(4-15) :Gel-electrophoresis illustration quality and quantity of DNA extracted from Covid -19 patients group, 1-48 patient samples, M=molecular marker 100bp for each step.

4.18.2. The profile gel-electrophoresis 10 blood samples of control group:

The figure (4-16) shows that all samples gave huge bright bands of DNA except sample 3 shown faint band.

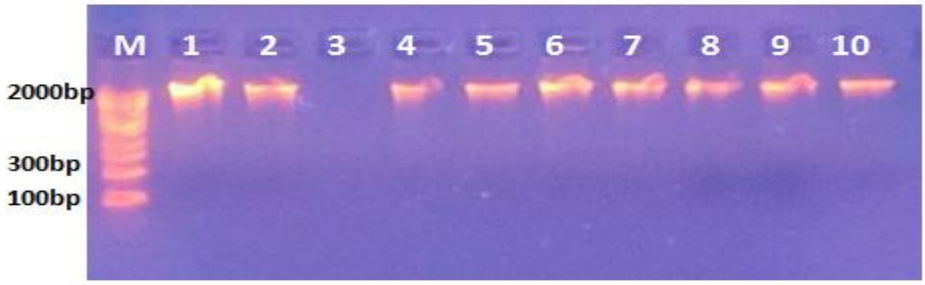


Fig (4-16):Gel-electrophoresis illustration quality and quantity of DNA extracted from healthy group, 1-10 control samples, M=molecular marker 100bp for each step

4.18.3. Targeted region in *IFITM3* gene and SNPs validity:

The figure (4-17) represents the boundaries primer pair F3 sites (320809 &321113) on the region of *IFITM3* gene at Chr:11 on reference strain NO. NC_000011.10. The length of the gene segment was 310bp marked by the Forward and revers primers at sites on 320809 and 321113 respectively.

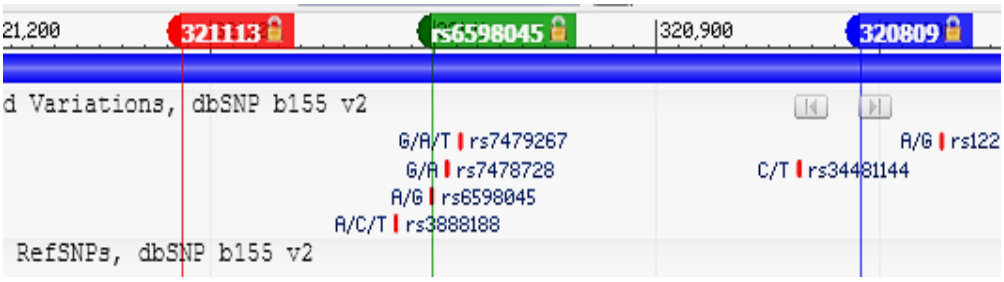


Figure (4-17) Targeted region of partial sequence of *IFITM3* gene amplified by primer pair F1&F2 covering the many SNPs

4.18.4. Amplification of targeted of partial sequence of *IFITM3* gene

4.18.4.1. Amplification of targeted of for Covid-19 cases:

The results show success the primer pair efficiency to amplification region 320809-321113 as target DNA region of *IFITM3* gene included many SNPs, the amplification region with flanking primers 305bp for patient group Figure **(4-18)** and for healthy group figure (4-19).

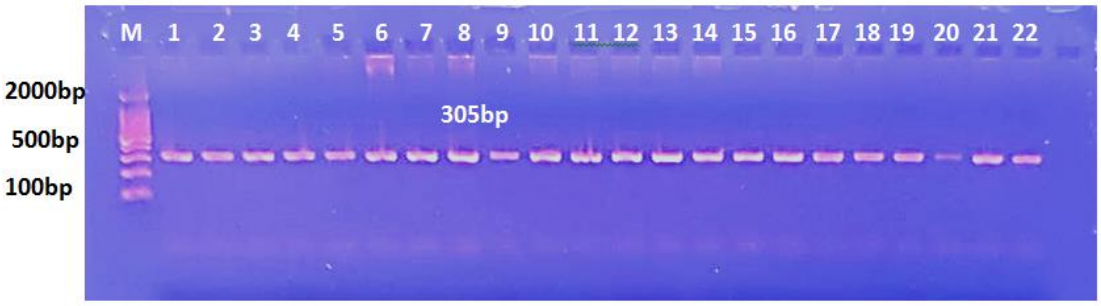


Figure (4-18) Gel electrophoresis of amplicon span from 320809-321113 as target DNA region of *IFITM3* gene amplification region with primer flanking regions, 1-22 patient Covid patients PCR products 305bp, M1= molecular marker 100bp for each step.

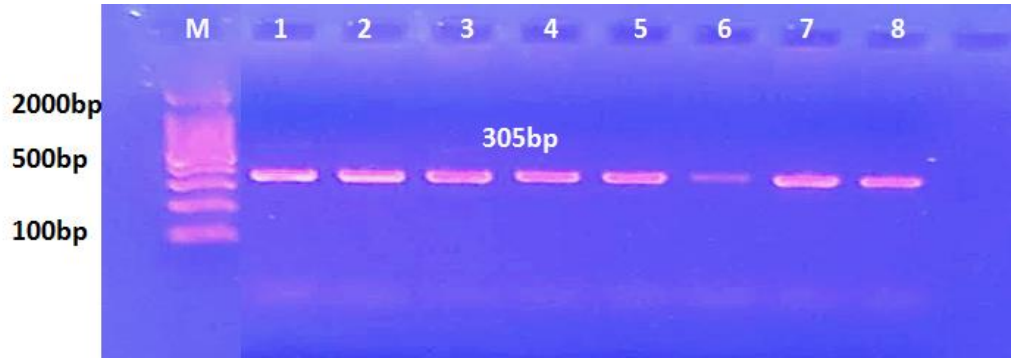


Figure (4-19) Gel electrophoresis of target DNA region for *IFITM3* gene amplification region with flanking region, 1-8 control group, PCR products 305bp, M=molecular marker 100bp for each step.

4.18.5. Genotyping and hereditary investigation:

Four single-nucleotide polymorphisms (SNPs) were genotyped in the 305 base-pair PCR results of the *IFITM3* center advertiser locale around record start site (rs6598045), (rs7478728), (rs71452596), (rs7479267). Every one of these SNPs was in Hardy-Weinberg harmony (P.0.05) both in the Covid -19 and control gatherings. Was found among three SNPs, rs7478728, rs71452596 and rs7479267.

4.18.5.1. Multiple Sequence alignment of *IFITM3* gene for patients based on BioEdit software:

The results of multiple alignment of partial sequence of gene *IFITM3* in figure (4-20) showing 4 mutants located on sites 155 G>A, 158 G>T, 161 G>A and 168 A>G. these mutants show high frequent ranged this indicated to be SNPs. After detected validity of these mutants, the results show that mutant on site 155 was rs7479267 G>A; on site 158 was rs71452590 G>T, on site 161 was rs7478728 G>A and on 168 was rs6598045 A>G.

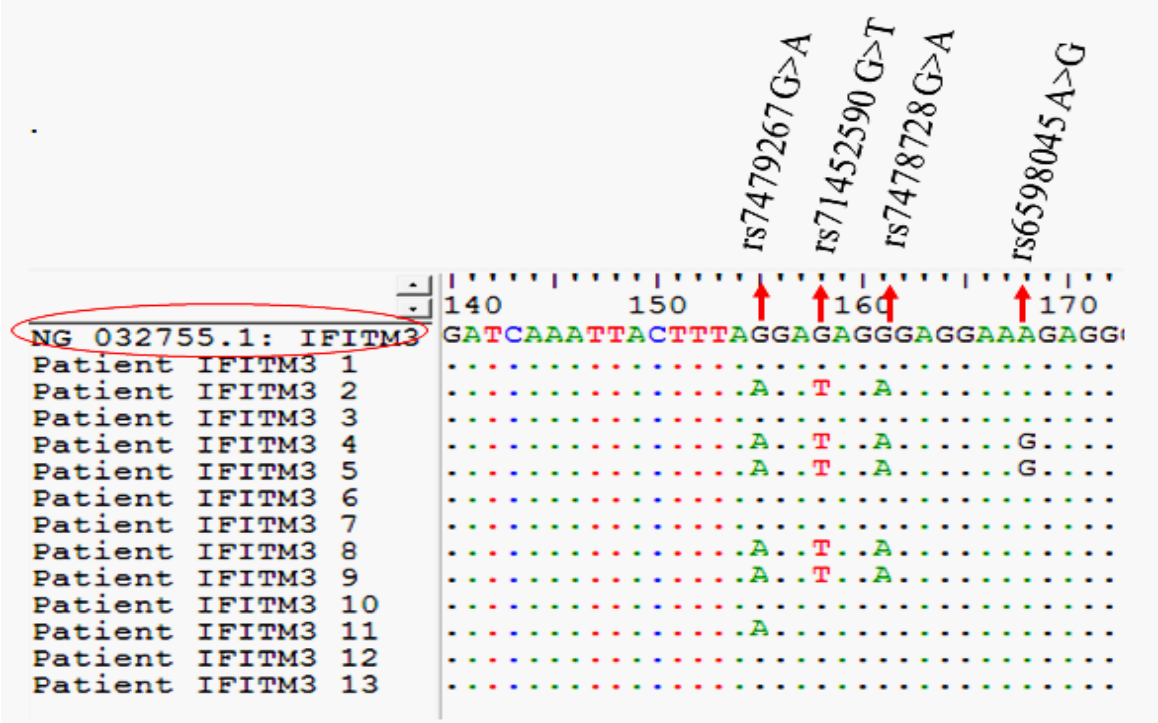


Figure (4-20) Multiple alignment of partial sequence of Targeted region of Chromosome11- *IFITM3* gene, shown SNPs on sites: on 155 was rs7479267 G>A; on site 158 was rs71452590 G>T, on site 161 was rs7478728 G>A and on 168 was rs6598045 A>G based on BioEdit software

The properties of the BioEdit software was illustration only homozygous SNPs but neglected the heterozygous SNPs.

4.18.5.2. The multiple alignments of chromatograms of Covid-19 cases and control based on genius software:

The properties of genius software more advance software was illustration all types of SNPs: homozygous wild type allele, homozygous mutant allele and heterozygous allele. The results of targeted region 320809 &321113 show four SNPs : rs4479267, rs71452596, rs7478728 and rs6598045.Figure (4-21,22,23) shwing this results.

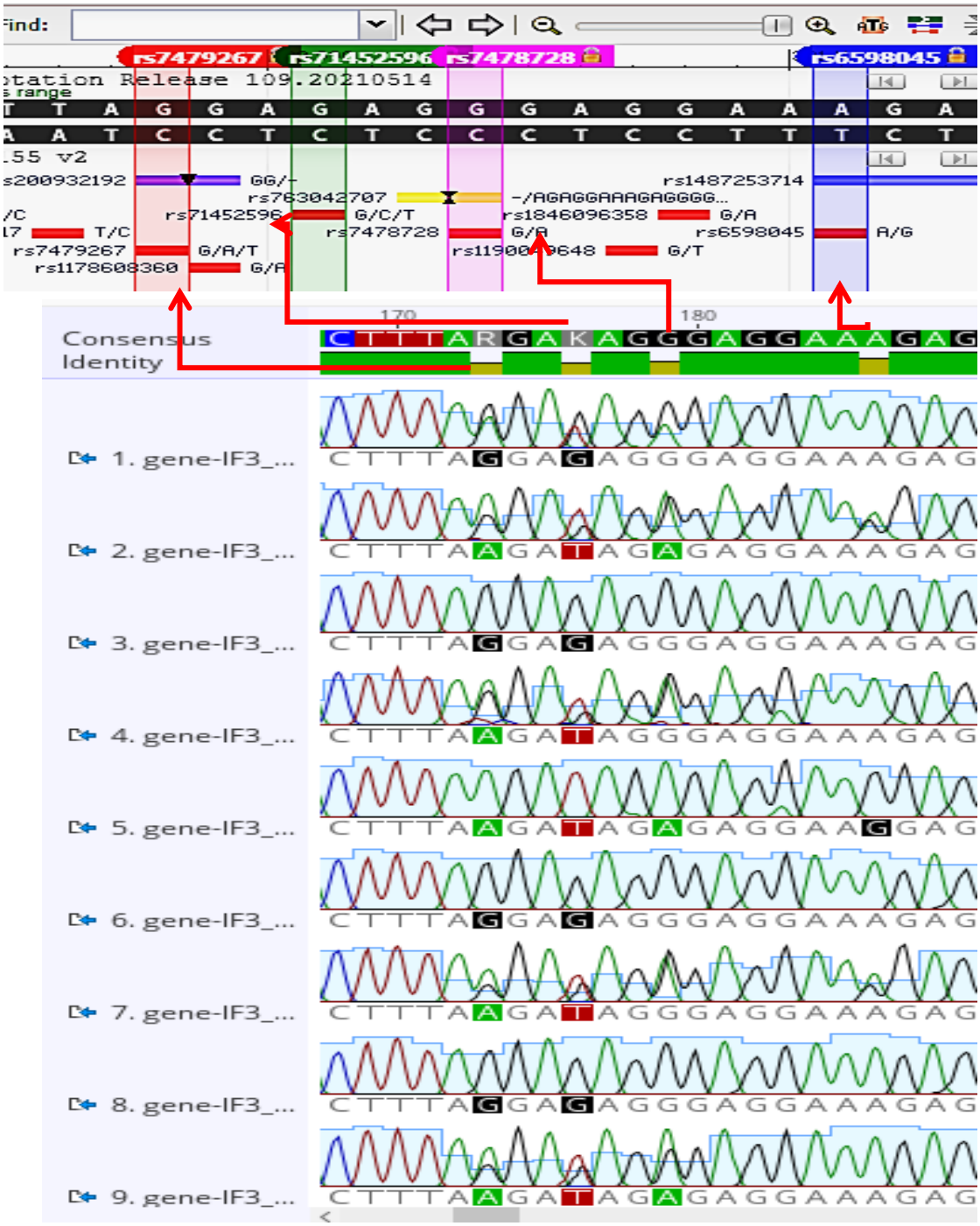


Figure (4-21) The multiple alignment of chromatograms of targeted region 52185415-52186396 show four SNPs: rs7479267, rs71452596, rs7478728 and rs6598045. 1-16 Covid-19 patient disease cases.

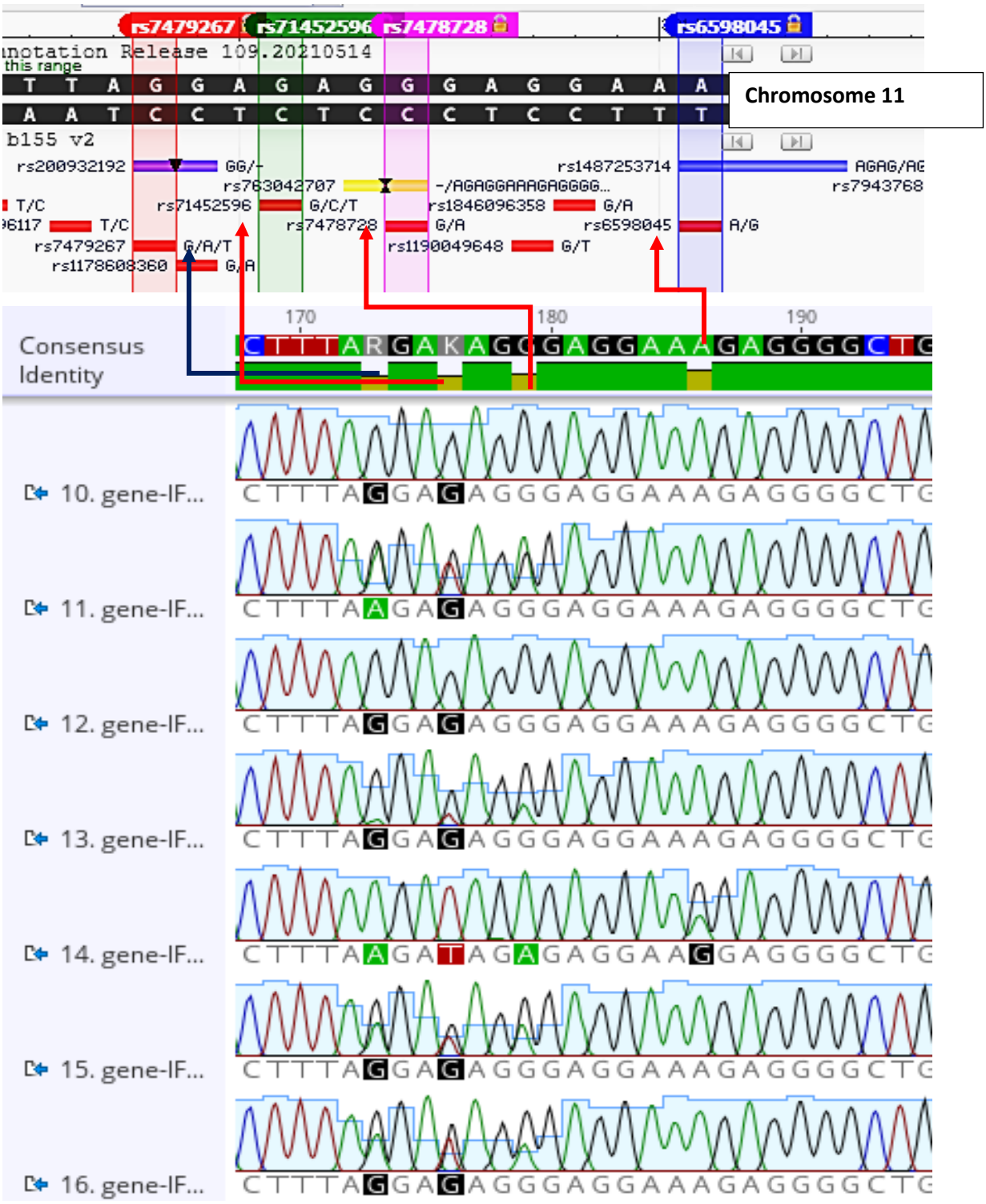


Figure (4-22) (continue):The multiple alignment of chromatograms of targeted region 52185415-52186396 shown four SNPs : rs7479267, rs71452596, rs7478728 and rs6598045. 1-16 Covid-19 patient disease. Alignment of control cases performed by Geneious prime software.

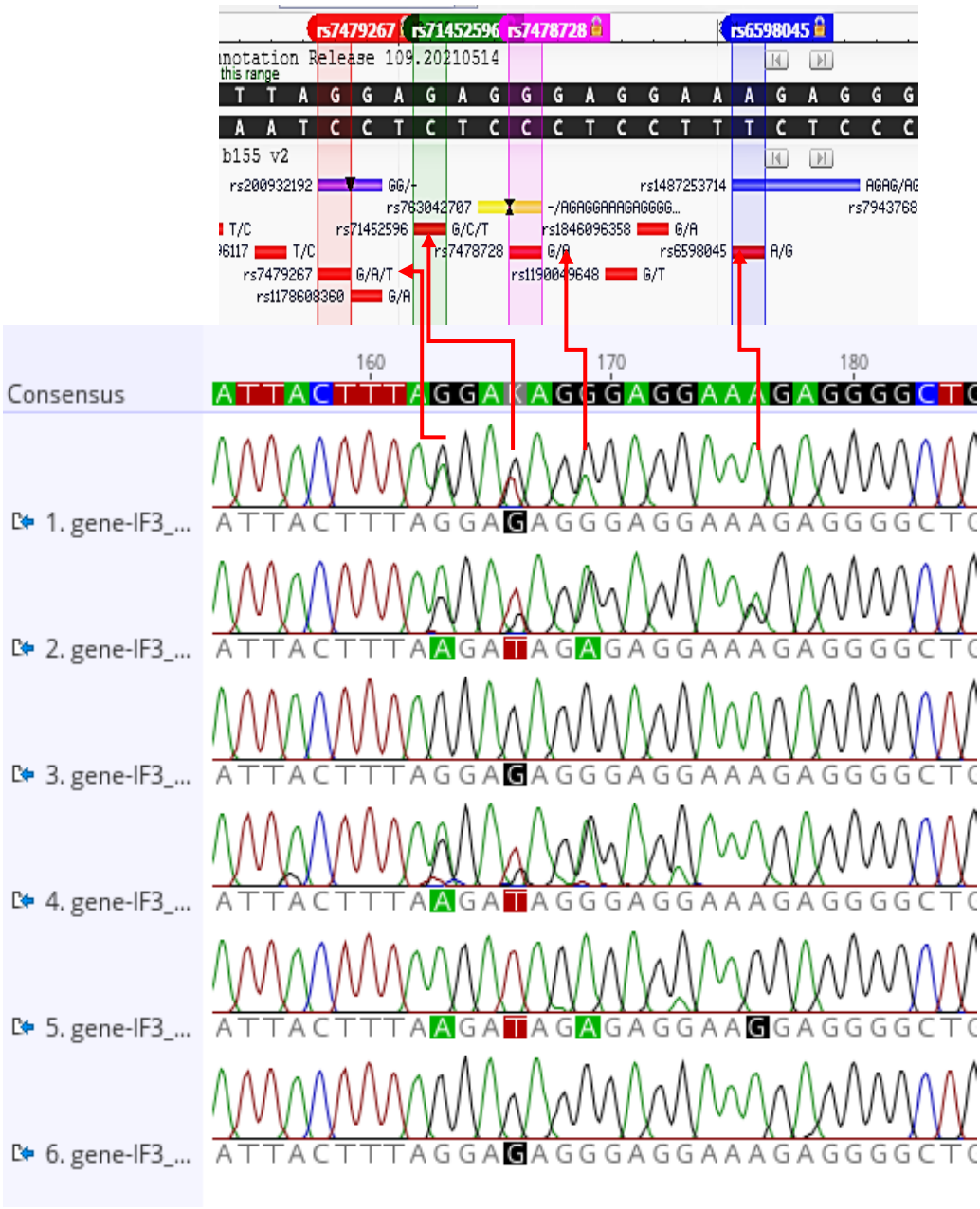


Figure (4-23) The multiple alignment of chromatograms of targeted region 52185415-52186396 shown four SNPs: rs7479267, rs71452596, rs7478728 and rs6598045. 1-6 healthy group

The results of multiple alignment based on genius software.The rs4479267 shown high frequent compared with two SNPs: rs71452596, rs7478728, this result paved away to considers these SNPs to be correlated risk with Covid -19 disease.

4.18.6. Determination the allele risk among SNPs valid in this study:

Due to the results of statistical analysis based on Chi-Square test and genotype distribution, percentage and allele frequency, and evaluated the Odd ratio values (OR) for each genotype of SNPs under interest, the results ware clearest in tables (4-6,7, 8,9).

Table(4-6) Genotypes of *IFITM3* gene SNP (rs7479267G>A and polymorphisms

IFITM3 gene SNP (rs7479267 G>A>T; polymorphisms					
SNPs	Genotype	Patients N=50	Control N=50	OR(95%CI)	P-value
rs7479267 G>A>T	GG	16(32%)	25(50%)	0.45(0.199-1.02)	0.5
	GA	12(24%)	16(32%)	0.4(0.19-1.17)	0.1
	AA	22(44%)	8(16%)	*4.02(1.5-10. 3)	0.03
Allele Frequency	G	44(44%)	66(67.3%)	0.35(0.2-0.63)	0.0004
	A	60(56%)	32(32.4%)	2.81(1.58-4.9)*	

***High value of OR explained that Allele A was risk allele.**

Table(4-7) Genotypes of *IFITM3* gene SNP (rs71452596G>Aand polymorphisms

IFITM3 gene SNP (rs71452596 G>C>T; polymorphisms					
SNPs	Genotype	Patients N=50	Control N=50	OR(95%CI)	P-value
rs71452596 G>C>T	GG	15(30%)	17(34%)	0.83(0.36-1.9)	0.6
	GT	16(32%)	8(16%)	2.5(0.9-6.5)	0.06
	TT	19(38%)	25(50%)	0.61(0.27-1.36)*	0.22
Allele Frequency	G	46(46%)	42(42%)	1.38(0.7-2.4)	0.22
	T	53(54%)	67(58%)	0.7(0.4-1.2)	

*** T allele not considered risk allele based on the low value of OR.**

Table(4-8) Genotypes of *IFITM3* gene SNP (rs7478728G>A and polymorphisms

IFITM3 gene SNP (rs7478728G>A; polymorphisms					
SNPs	Genotype	Patients N=50	Control N=50	OR(95%CI)	P-value
rs7478728G>A	GG	15(30%)	17(34%)	0.83(0.35-1.9)	0.6
	GA	22(44 %)	17(34%)	1.43(0.63-3.2)	0.3
	AA	13(26%)	16(32%)	0.7(0.3-1.8)*	0.5
Allele Frequency	G	52(52%)	51(51%)	1.04(0.59-1.8)	0.8
	A	48(48%)	49(49%)	0.75(0.3-1.8)	

*** A allele not considered allele risk based on low value of OR.**

Table (4-9) Genotypes of *IFITM3* gene SNP (rs65598045 A > G and polymorphisms

IFITM3 gene SNP (rs65598045 A>G; polymorphisms					
SNPs	Genotype	Patients N=60	Control N=60	OR(95%CI)	P-value
rs65598045 A>G	AA	37(74%)	34(68%)	1.34(0.56-3.2)	0.5
	AG	9(18.4%)	8(16%)	1.15(0.41-3.3)	0.7
	GG	4(8%)	8(16%)	0.45(0.12-1.63)*	0.22
Allele Frequency	A	74(83%)	76(76%)	0.95(0.5-1.58)	0.8
	G	51(17%)	50(24%)	1.05(0.63-1.7)	

***G allele not considered as risk allele based on low value of OR.**

The genotyping results of Allele AA, AG and GG of SNP rs65598045 A>G, show that A allele shows high percentage in patient and healthy groups, with high Odd Ratio (OR=1.34(0.56-3.2) more than mutant allele G with low Odd Ratio (OR=0.45(0.5-1.63). these results indicated that wild allele A defiance against disease.

The Allele Frequency Calculator shows high percentage of A wild allele in both patients and control groups 83% and 76% receptacle, while the mutant allele G shown low percentage 17% and 24% receptively

The A allele frequency percentage was high that used to reflect the genetic diversity of a population species. It is also referred to as allele frequency. It is a measure of relative frequency of allele on a genetic locus in a population. The frequency is expressed in terms of percentage. Hardy-Weinberg Equation can be used to find the frequency of allele.

The results of detection the main genotypes in Covid 19 patients presented with higher frequencies of AA genotype in rs65598045 and TT genotype in rs65598045 of *IFITM3* gene than healthy controls (both $P<0.001$).

The *IFITM3* gene SNPs polymorphism the presence of TT genotype of rs7478728G>A and AA genotype in rs7478728G>A of *IFITM3* was fundamentally higher in control bunch than Covid 19 patients . Moreover, the strategic relapse model showed a diminished danger for Covid 19 improvement among people with AA genotype (OR 1.43(0.63-3.2), and transporters of An allele (AG+GG) (changed OR = 1.05).

4.18.7. Genotypes illustration based on phylogeny tree:

The phylogeny tree constructed based on sequence data of IFITM3 gene was shown five main genotypes: genotype 1 include cases 3 and 6; genotype 2 include 15, 20, 25; genotype 3:31, 32; genotype 4:10, 14; genotype 5:11, 19, 21, 24. Figure (4 -24).

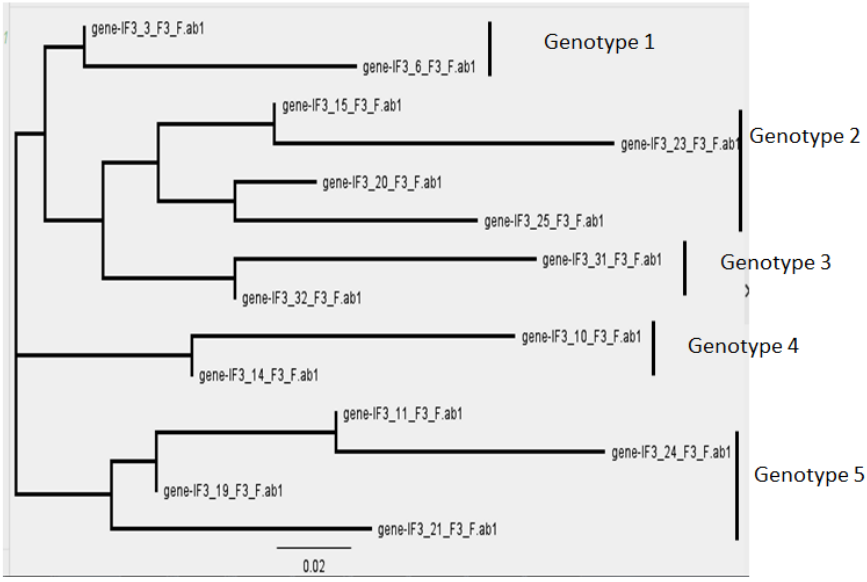


Figure (4 -24) Genotyping closeness among patients constructed based on sequences of *IFITM 3* gene performed by phylogeny tree

In few years ago , interferon- induced transmembrane (*IFITM*) proteins have been distinguished as significant antiviral variables. Newly, *IFITM3* is found in the early and late endosomes, individually, and is intense inhibitors of infections that rely upon endosomal pathways for disease.

The examples of limitation of *IFITM3* is comparative; albeit most examinations report a more strong restraint of disease by *IFITM3* (53, 54, 70).Concentrates in vitro, show that exhaustion of *IFITM 3*, while overexpression of *IFITM 3* proteins hinders viral replication (Brass et al.,2009). Sanger approval of a subset of variations for the following stage of the undertaking, I chose to approve four SNPs inside the locale: Chr11:320,988-321,138 by Sanger sequencing. This locale is situated at the 5' finish of the *IFITM3* quality and it is for the most part inadequately addressed by sequencing information.

The genotypes for three SNPs rs7479267, rs71452596, rs7478728 were mistakenly called as heterozygous in the 1000 Genomes Project and

the Illumina designated sequencing dataset. Sanger it are truth be told homozygous to succession affirmed that these SNPs at those positions.

The results consonant with results of Shen et al.,2013, the conducted that rs65-shown low OR =0.92 while the rs7479267, rs71452596, rs7478728 with OR=1.34, wich conflict with Shen et al.,2013, some of these SNP show low OR, these conflicts may rise small sample size.

Final finding: It has been obtained the local hospitals in Babylon province case statistical indicators based on Odd Ratio of COVID-19 and genetic information on the *IFITM3* gene. It has been performed genotypes and allele frequency analysis between the case of COVID-19 and allele frequencies of the polymorphisms of the *IFITM3* genes in patients group and compared with healthy one.It has been identified a weak to strong effects between the case group of COVID-19 and the allele frequency of the rs6598045 SNP *IFITM3* gene. To the best of our knowledge, this report is the first to describe relationship between COVID-19 and many SNPs: rs6598045 SNP of the *IFITM3* gene at the population-level.Because *IFITM3* plays a pivotal role in IFN-c signaling and successful immunity against M. tuberculosis requires strong IFN-γ (Shen *et al*., 2013).

Interferon-instigated layer protein that hinders the section of infections into the host cell cytoplasm by forestalling viral combination with cholesterol exhausted endosomes is encoded by the *IFITM3* quality (Zhao *et al*., 2019). It has an ability to inactivate new encompassed infections, which bud out of the tainted cell. It has been demonstrated to be dynamic against different infections, including flu An infection, SARS Covid (SARS-CoV), Ebola infection (EBOV), Dengue infection (DNV), human immunodeficiency infection type 1 (HIV-1), and so on. (Lu *et al*.,

2011). Pathways through which *IFITM3* capacities are: Innate Immune System what's more, Interferon gamma flagging. Studies have shown that the initial 21 amino acids of the N-end of *IFITM3* quality are expected for constriction of vesicular stomatitis infection replication, also, that shortened *IFITM3* protein neglects to confine the replication of different kinds of flu infection, as well as HIV-1 (Jia *et al*., 2012; Bailey *et al*., 2014). (Williams *et al*., 2014; Kim *et al*., 2019) showed that even full-length *IFITM3* confines passage and replication of H1N1.

Polymorphisms of this quality have been concentrated in a few diseases. The polymorphism rs6598045 c.−188T > C (4.85% in our partner) prompts a contrast in the limiting limit of the record factor causing a distinction in the record proficiency of the *IFITM3* quality, which was accounted for to show a solid relationship with flu H1N1 2009 pandemic infection contamination (Shen *et al*., 2013). Another practical polymorphism rs3888188, showed that fringe blood mononuclear cells conveying GG genotype had decreased *IFITM3* mRNA level contrasted with those with TT or GT genotype, which inclines toward aspiratory tuberculosis in Iranian and Han Chinese populaces (Shen *et al*., 2013).

Past examinations anticipated that rs12252 C allele could create another joined record that encodes a deviant shortened protein 121 of *IFITM3*, which lessens the cell obstruction to flu infections by obstructing beginning phase of viral replication (Everitt *et al*., 2012; Compton *et al*., 2016). Relationship of this polymorphism has been seen in Chinese populace with pandemic flu (H1N1 09pdm), occasional flu (H3N2 what's more, flu B), and avian flu (H7N9) (Carter *et al*., 2018). In our partner, 11.65% of the people conveyed this allele. Occasional flu medical clinic affirmations were related with rs7948108, which was seen with a low (0.97%) rate in our companion.

Conclusions and Recommendations

1. Conclusions:-

1. This outcome may indicate that the disease's activity caused various modifications in a number of indicators, particularly in diabetic patients. The cytokine and particular antibody, were utilized to track the viral diagnostic response (IgM and IgG)**.** In the demographic factor analysis, age, gender, educational status, ethnicity, marital status, residential type, and occupation were significantly associated with COVID-19 severity.

2. SARS-CoV-2 could employ similar pathways to increase morbidity and mortality by causing a cortisol synthesis insufficiency linked to a serious disease.

3. IFNs were initially described as molecules that interfere with viral replication and functionally inhibited, because certain diabetic patients might be have low immunity and late response to infectious against such Sars-Cov2 virus.

4. Increasing blood sugar level and HBA1C, have no effect on antibody production against Sars-Cov2 virus as well as there is an increased in IgG level during infection, and slightly reduced in association with increased cortisol level.

5. The weaker male immune system leaves men more susceptible to a range of infectious diseases.

6. The female has low hemoglobin level than male , so the glycosylated hemoglobin (HBA1C) well be reduced accordingly in association with Covid-19 infection.

7. Increased blood sugar and HBA1C lead to increased consumption of cortisol hormone and lead to decreased it level and revealed negative correlation.

8. The 305 base-pair PCR findings of the *IFITM3* center advertiser region surrounding record start site (rs6598045), (rs7478728), (rs71452596), and four single-nucleotide polymorphisms (SNPs) were genotyped (rs7479267). Each of these SNPs was in Hardy-Weinberg equilibrium (P 0.05) in both the Covid -19 and control populations. Among the three SNPs rs7478728, rs71452596, and rs7479267, was discovered.

2. Recommendations:-

1. Study the local immune reactivity by estimation of mucosal IgA level in acute and chronic infection.

2.Study other risk factors associated with covid-19 infections.

3.Molecular study for some immunological parameters related to the infections.

4. The HbA1c and cortisol have a risk factor to Covid - 19 complication. Further study was recommended to assessment each level at different population.

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الخلاصة:

أجريت هذه الدراسة في محافظة بابل خلال الفترة من تشرين الاول ٢٠٢١ إلى كانون الثاني ٢٠٢٢ على مرضى مصابين بفيروس SARS-CoV2. كمرضى موجودين في الردهات الوبائية في مستشفيات محافظة بابل. تم إجراء الدراسة من خلال جمع عينات دم من ٥٤ مريضًا مصابًا بفيروس COVID-19 ومن ٤١ شخصًا سليمًا كمجموعة ضابطة لفئات عمرية مختلفة (ذكور وإناث).

أظهرت نتائج الدراسة عن زيادة معنوية في مستويات بروتين سي التفاعلي CRP و D. Dimer و RBS و γ- Interferon و HBA1c و AntiSars -Cov2- IgM و IgG - AntiSars -Cov2 مع عدم وجود اختلاف في مستويات Interleukin- 6. ان الفئة العمرية للبالغين (٥٠-٥٩) سنة لديها مستوى مرتفع من بروتين سي التفاعلي مقارنة مع الفئات العمرية الأخرى وكذلك المجموعات الضابطة. جميع الفئات العمرية لديها مستويات أعلى من سكر الدم العشوائي مقارنةً بالضوابط ، باستثناء الفئة العمرية للبالغين (٦٠ عامًا) ، والتي لديها أعلى مستوى من السكر العشوائي في الدم بسبب الإجهاد والتمثيل الغذائي والتغيرات الهرمونية. ان الفئة العمرية للبالغين (من ٥٠ إلى ٥٩ عامًا) كان لديها مستوى أعلى من D-Dimer بدلاً من الفئات العمرية الأخرى على الرغم من أنها زادت في جميع الفئات العمرية ، إلا أنها كانت تشير إلى حدوث مضاعفات الجلطة في المرضى المصابين. ان الفئة العمرية الصغيرة (٣٠-٣٩ سنة ، تليها ٤٠-٤٩ سنة) لديها مستوى مرتفع من هرمون الكورتيزول مقارنة بالفئات العمرية الأخرى والضابطة. وأن الفئة العمرية الصغيرة (٢٠-٢٩ سنة) لديها مستوى أعلى من HBA1c مقارنة مع الفئات العمرية الأخرى وكذلك المجموعات الضابطة. حيث ان الزيادة في نسبة HBA1c في بعض المرضى المصابين بعدوى SARS-CoV2 حدثت بسبب ظروف الإجهاد التي تحصل أثناء الإصابة.

أظهرت النتائج وجود مستوى مرتفع من الأجسام المضادة لـ Anti SARS-CoV2-IgGمقارنة بالفئات العمرية الأخرى والمجموعة الضابطة ، في الفئة العمرية (٥٠-٥٩ عامًا ، تليها ٦٠ عامًا). في حين أن الاجسام المضادة لـ Anti SARS-CoV2-IgM في الفئة العمرية (٤٠-٤٩ سنة) قد زادت مقارنة بالفئات العمرية الأخرى والمجموعة الضابطة.

أظهرت النتائج وجود نسبة عالية من haemoglobin A1C في الذكور أكثر من الاناث، وقد زادت النتيجة الاجمالية لمجموعات المرضى مقارنة بالمجموعة الضابطة، وقد تكون هذه النتيجة بسبب انخفاض مستوى الهيموجلوبين في الاناث اكثر من الذكور.

أظهرت النتائج وجود علاقة أرتباط بين نسب HBA1c ومستوى الكورتيزول ، وان زيادة نسبة السكر في الدم و HBA1c يزيد من أستهلاك هرمون الكورتيزول ويؤدي إلى أنخفاض في مستواه.

بينت نتائج الدراسة الحالية وجود زيادة في مستوى الإنترفيرون أكثر من الكورتيزول في مرضى السكري أثناء الإصابة بـ Sars Cov-2. زاد IL-6 أكثر من الكورتيزول في مرضى السكري Sars Cov-2. قد تؤدي زيادة IL-6 إلى تنشيط جهاز المناعة عن طريق تحريض إنتاج بروتين المرحلة الحادة ، وقد تمت زيادته إما في البداية الأولية للعدوى أو أثناء الإصابة المتأخرة بتوقعات سيئة أو معقدة.

أظهرت النتائج وجود زيادة في مستوى IL-6 فوق INF -γ نتيجة لعدوى Cov -2 Sars ، وهناك علاقة مباشرة بينهما ، ارتفع مستوى IL-6 بسرعة بدلاً من INF-γ. قد تكون هذه النتيجة بسبب كون الإنترلوكين ٦ أكثر حساسية وزيادة الإنتاج أثناء هذه العدوى.

أظهرت نتائج التسلسل الجزئي للجين *IFITM3* وجود 4 طفرات في المواقع < 155G A و158G > T و161G > A و168A > G. أظهرت هذه الطفرات تواترً ا عاليًا يشير إلى تعدد أشكال النيوكليوتايد. بعد الكشف عن صحة هذه الطفرات ، أظهرت النتائج أن الطفرات في الموقع 155 كان rs7479267 G > A وفي الموقع 158 كان rs71452590 G > T ، وفي الموقع161 كان rs7478728 G > A وفي الموقع 168 كان < rs6598045 A.

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دراسة لبعض المعايير المناعية والجزيئية لمرضى كوفايد - 19

رسالة مقدمه الى مجلس كلية العلوم للبنات جامعة بابل وهي جزء من متطلبات نيل درجة الماجستير في علوم الحياة

من قبل

حسين رضا محمد الشمري

بإشراف

الاستاذ الدكتور
عبير فوزي مراد الربيعي

الدكتور
رحيم طعمه المعموري

٢٠٢٢ ميلادي

١٤٤٤هجري