Ilam University

Faculty of Basic Sciences

M.Sc. Thesis

Cytokines storm syndrome in covid-19 patients associated with copy number of coronavirus by real times PCR technique

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2021

بسم الله الرحمن الرحيم

وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا (٨٥).

سورة الاسراء اية (٨٥)

Dedication

I dedicate this work to my father and mother… I will never forget you in all my life… I love you

My family….. You are my other half. Thank you for your encouragement, patience and support…. Thank you

My dear wife and children…… Thank you all for your support and patience…… I love you

To Dr. Zahrah Adnan Al-Shammari ,,, I am so grateful, it is really hard to find words to express my gratitude,,, Thank you, with all due respect

To everyone who wishes me the best…thanks

Acknowledgment

I would like to express my sincere thanks and gratitude to my supervisor, Prof. Dr. Arman Rostamzad.. I would also like to thank my co-supervisor, Dr. Zahrah Adnan Al-Shammari, for her scientific guidance, kind advice, encouragement and assistance, and providing information and support throughout the research period.

Special thanks to all the staff of the Department of Biology, College of Science and College of Nursing, University of Maysan for their encouragement and generous cooperation.

I would like to acknowledge and appreciate the kind assistance of Mr. Ehsan Judeh and Mr. Jassem Owaid during the practical work trip of my thesis, and I am also grateful to all the other staff who helped me in Al Sadr Teaching Hospital and Al Majar Grand Hospital… Thank you for the kind welcome, help and support.

I would like to deeply thank Dr. Alaa Shamikh Al-Saadi and Ms. Mudhafar Raad for helping me facilitate sample collection for this study.

I would like to thank the patients for their cooperation in obtaining study samples, and without their assistance this work would not have been possible.

To my beloved family members.....Thank you for everything you've done for me...

Abstract

Background: The Covid-19 pandemic is a persistent infectious agent. The immunological characteristics of the interaction between the host and the virus. It stimulates high levels of cytokines such as IL-2, IL-6, IL-10 and other immune cells in infected individuals that play an important role in physiology.

Materials and Methods: Samples (blood and swabs) were collected for (100) samples from February to June 2021 who were isolated from patients infected with or infected with Coronavirus for Al-Sadr Teaching Hospitals and Al-Majar Al-Kabeer Hospital.

Results: Total (100) samples (Swabs and blood) between infected and non-infected with Covid-19. It was found that the highest percentage of age groups (50-59) was (22.6%) 14 patients, and the percentage of males was higher than females (53.2%) in 33 patients. Also the highest percentage of pressure diseases appeared (46.8%). The study showed the symptoms associated with infection at different levels where the highest temperature was (44.1%), while laboratory tests for blood serum showed that cytokines in acute and acute patients at different levels i.e. IL-2 Mean ±Std. deviation (214.33±169.08),T.Test (9.98) , IL-6 (537.24±196.78), (21.49) while IL-10 had very low levels . The study also showed analyzes of immune cell , WBC Mean ±Std. deviation (9.71±4.19) T.Test (18.25) , neutrophil (80.85±12.98) , (51.81) , Monocytes (4.67±2.54) , (14.467) and lymphocyte (10.35±7.37), (11.03). Had serological analyzes of ferritin Mean ±Std. deviation (488.32±903.22) T.Test (14.156) , D.dimer (1.24±1.44), (6.781), LDH (173.39±22.67), (22.67), CRP (35.26±43.45), (9.70), platelet (103.66±219.50), (16.674) and HGB (2.71±13.154), (38.14).

Discussion: study showed cytokine storming of COVID-19 patients, with different levels for patients with severe and acute infection, our study confirmed IL-2, IL-6 with high levels, while IL-10 with low levels due to inflammatory respiratory distress. COVID-19.

Keywords: COVID-19, IL-2, IL-6, IL-10, RT-PCR, Cytokine storm syndrome.

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List of abbreviations and acronyms

Abbrev.	Meaning
Covid-19	Corona virus 19
SARS	Severe acute respiratory syndrome
MERS	Middle East respiratory syndrome
WHO	World Health Organization
IL (2-6-10)	Interleukin (2-6-10)
CSS	Cytokine Storm syndrome
PAMPs	Pathogen - Associated Molecular Patterns
DAMPs	Damage - Associated Molecular Patterns
NK	Natural killer cells
Shlh	secondary hemophagocytic lymphohistiocytosis
MAS	macrophage activation syndrome
MCP-1	monocyte chemotaxis protein1
GCSF	A granulocyte Colony Stimulating Factor
IP-10	Interferon Stimulating Protein10
MIP-1α	macrophage inflammatory protein 1-α
BALF	Bronchial lavage fluid
ARDS	acute respiratory distress syndrome
CD4	Cluster of aifferentiation 4
CD8	Cluster of aifferentiation 8
WBC	White Blood Cell
RT-PCR	Real-time Polymerase Chain Reaction
Elisa	Enzyme linked immunosorbent assay
LDH	Lactic dehydrogenase

Chapter One

Introduction

1.1. Introduction:-

In december 2019 ,the outbreak of a disease was recorded at a first time, Corona virus that began to appear, On February 11, 2020 in Wuhan city Hubei Province , China . The World Health Organization (WHO) named this coronavirus "Severe Acute Respiratory Syndrome Virus 2" (SARS-CoV-2). Coronaviruses (CoVs) have been associated with the rapid spread of diseases in East Asia and the Middle East. Severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) began to appear in 2002 and 2012, respectively[1].

In late 2019, Corona virus posed a clear health risk across the world as a result of the ongoing outbreak of the epidemic in many countries of the world [1-2]. Corona virus 19 is a clinical syndrome caused by a mutated Beta RNA virus. This epidemic has spread around the world and is accepted by the world as a pandemic by the world health organization (WHO) . On May 25, 2020 , SARS-CoV-2 was affected in many continents and more than 212 countries, with about 5,529,192 people died.

The cause of these deaths is a Cytokine Storm syndrome (CSS) [1-3]. The inflammatory stimulant cytokine storm is known as a final deterrent force in severe COVID-19 pandemic infection, which leads to multiple system failures in people who are immuno compromised or low immunity [4-5]. The incubation period of corona virus range between (1-5) days, and about (97.5%) peoples show signs will have it within (11)days of infection. [6-7]. An inflammatory process begins that occurs as a result of infection with this virus in some tissues affected by the infection and this process is change in count of white blood cells and plasma proteins, macrophages, and the natural killer cells (NK) dendritic cells and these are activated by superior to the harmonious molecular markers: molecular pathogen-related patterns (PAMPs) and molecular patterns linked to damage (Dampers). In covid-19 patients an inflammatory cytokines are activated by key pathway markers DAMP or PAMP and elevated plasma levels

of IL-2 and IL-6 . As well as ferritin and other inflammatory biomarkers, which are a typical indicator of secondary hemophagocytic lymphohistiocytosis (sHLH) and macrophage activation syndrome (MAS) . There are some other signs of COVID-19, which are low lymphocytes (white blood cells) with T-cell depletion, persistent fever, and depletion of the blood ferritin [8-9-10].

Cytokine storm syndrome is associated with the affected symptoms, resulting from the effects of T cell responses helper1 (Th1). The high concentrations of interleukin (IL) ,IL-2, IL-6, IL-10, A granulocyte colony stimulating factor (GCSF) , Interferon Stimulating Protein10 (IP-10) , macrophage inflammatory protein 1-α (MIP-1α), tumor necrosis factor α (TNF-α), monocyte chemotaxis protein1 (MCP-1) and IFN- can be found in patients with COVID-19 [11]. Bronchial lavage fluid (BALF) tests of cells, done by transcriptional sequencing, result from the appearance of depleted chemical releases caused by Covid-19 infection , such as CXCL10 and CCL2 (10). These imunity factors which was causes to die of COVID-19[12-13] .Patients and postmortem lung examination indicate acute respiratory distress syndrome (ARDS) and depleted T-cell activity. This phenomenon is due to an increase in the number T-helper and the high cytotoxicity of CD8 + T Cells [14]. Effectiveness of adaptive and innate immune responses by SARS- CoV-2 infection causes uncontrolled inflammation responses and eventually causes a cytostorm [15]. A cytokine storm causes apoptosis of endothelial cells and epithelial cells, and vascular leakage ultimately causes acute respiratory distress syndrome (ARDS) and other severe syndromes and leads to death [16].

1.2. Aims of study

1. Determination IL-2 , IL-6 and IL-10 of cytokines, which related to infection with Covid-19 patients.
2. Detection of immune cells (white blood cells, neutrophils , monocytes, Lymphytes) in plasma of COVID-19 patients.
3. Determination of copy number and virus concentration in each sample of covid-19 patients.

Chapter Two

Literature reviews

2.1. Background of coronaviruses

In 1965 a first human cases of Coronavirus (HCoV) appeared by two discoverers Tyrrell and Pynoe [17]. They were able to carry a virus know as B814. This virus was isolated from human fetal tracheal organ, cultures acquired from the respiratory tract of an adult with a cold. It was concluded that the presence of an infectious agent through inoculation of the medium from these cultures through the nose in human volunteers; The common cold was induced in a high proportion of people, at that time tyrrell and bynoe would not be able to grow virus in tissue cultures. Hamry and Brocknow [18]. Were able to cultivate a virus with unusual traits in tissue culture from samples collected from medical students suffering with the common cold. Both the B814 virus and the Hamry virus, which called 229E, were sensitive to the ether and therefore needed a lipid layer for infection. It was found that these two viruses were not related to any mucinovirus or Paramix. During work at report mackintosh and colleagues, Robert Chanock's lab at the national institutes of health, reproduces several strains of ether-sensitive agents in the human respiratory system using a technique similar to that of Tyrrell and Bynoe . These viruses were called (OC) to denote that they were grown in organ cultures. At the same time , Almeida and Tyrrell4 both performed electron microscopy on fluids from organ cultures infected with B814 and found infectious bronchitis virus-like particles of chickens. It was observed that the particles are of medium size (80-150 nm), with multiple shapes, enclosed in a membrane, and covered with highly diverging surface projections. The factor 229E identified by Hamre and Procknow2 and previous OC viruses identified by McIntosh et al had a similar appearance [19].

Corona respiratory virus infection occurs more in winter and spring than summer and autumn. The information found that MERS-CoV infection contributes up to (35%) of the total respiratory viral activity during epidemics. In general, it was been concluded that the proportion of adult colds caused by

corona viruses is (15%). [20] The human strains OC43 and 229E were been studied in the three decades after the discoveries in particular, due to a large proportion that they were the easiest to deal with [21]. At the time of Oujda, the OC43 strain was the most indicative of localized disease spread. As with many other respiratory viruses, infection was common [22]. Corona viruses cause respiratory and intestinal infections in humans [23] . It did not have a strong pathogenic effect on humans until it appeared in 2002 and 2003 in Guangdong Province, China, with severe acute respiratory syndrome (SARS) [24-25]. As detected and pathogenic HCoVs can evolve, work has increased in diagnosing and characterizing HCoVs. This led to the discovery of two new family members in 2004 and 2005, HCoV-NL63 and HCoV-HKU1 , respectively [26-27]This infection with HCoV-HKU1 or HCoV-NL63 causes hospitalization for elderly patients and patients with immunodeficiency [26]. HCoV-NL63 is associated with diphtheria, a common form of lower respiratory infection, with potency occurring in the first two years of life [28].

After a period, it was discovered by a rapidly spreading Coronavirus, the Middle East Respiratory Syndrome (MERSCoV), in the countries of the Middle East [29]. The SARS coronavirus (SARS-CoV) uses angiotensin converting enzyme 2 (ACE2) as a receptor and mainly infects ciliary epithelial cells and type II lung cells, [30-31]. While MERS-CoV uses peptidase dipeptide 4 (DPP4; also known as CD26) as a receptor and infects uninfected bronchial epithelial cells and lung type II cells [32-33]. In December 2019 in Wuhan, China, which was called SARS-CoV-2 and causes a disease known as COVID-19 [34]. The new virus caused severe pneumonia in China and spread with high force all over the world due to high infection rates, which led to a global pandemic. SARS-CoV-2 can cause severe and very fatal pneumonia with clinical symptoms similar to those reported for SARS-CoV and MERS-CoV1[35].

2.2. Characteristic and Morphology

Infection control guidelines are based on the assumption that the characteristics of COVID-19 are similar to those of the SARS-CoV outbreak in 2003. Initial similarities in the genetics and immune development between COVID-19 and SARS-CoV can been inferred to give insight into some of the epidemiological characteristics. The transmission of Covid-19 occurs primarily through respiratory droplets from sneezing and coughing, and through contact with contaminated surfaces . These circulating strains result in mild signs in immunocompromised people. These signs include Coronavirus, runny nose, cough, sore throat, headache and fever that can last for several days. However, in immunocompromised patients, the virus is believed to cause lower respiratory diseases such as pneumonia and bronchitis [36-37].

Coronaviruses refer as large number of viruses that share rare characteristics in the viral world. One of the most important of these forms is the morphology of the aura, which becomes clear when examined with an electron microscope. Corona virus have large environmental particles that give a shape similar to the shape of a crown, This crown-like shape is due to Spike-encapsulated glycoproteins that form petal-shaped protrusions about (20) nanometers long. The name Corona, which means "crown" or "aura." It also has the advantage of having a large RNA genome for the viral world: at 30 KB or more, this extremely long RNA genome allows a high degree of genetic plasticity and partly explains the diversity and adaptability of coronaviruses. Coronaviruses have a transcription mode: they produce a number of mRNA without the knowledge of the genome, using a 1 mechanism for changing the template during replication [38-39].

Coronavirus is an enveloped RNA virus with a positive result related to Betacoronavirus genus [40-41]. Genetic analysis showed that Covid-19 is closely related (88-89% similarity) to SARS-like coronaviruses from bats, and less similar to SARS-CoV (79% similarity) and MERS-CoV with (50%

similarity) [42- 43] . Coronavirus has a diameter of about (50 -200) nanometers. Other characteristic of coronaviruses, it has a corona virus lipid coat on the spike protein (S), a protein membrane (M), and an envelope protein (E). Protein S mediates the viral binding to the host cell membrane through interaction with the angiotensin converting enzyme (ACE2) receptor [44]. The nucleocapsid (N) protein encapsulates the viral RNA genome, and it forms the core of virion [45-46].

2.3. Structure of coronavirus

2.3.1. Protein S

The coronavirus S protein is spike – shaped protrusions on the surface of the virion and is a type I memberane fusion protein about 180 KDa . It has three main divisions, the transmembrane domain , the extracellular domain and the intracellular tail [47]. The extracellular domain of the S protein is detected upon coronavirus maturation , and partitioned by the host protease into two subunits , S1 and S2 [48]. S1 is defined and is a globular structure located near the N-terminus , and S2 is a rod- like structure near the C-terminus. The S1 subunit consists of two main domains: the N-terminal domain (NTD) and the C-terminal domain (CTD). Both NTD and CTD are binding (RBDs), which are mainly responsible for recognizing the coronavirus receptor and mediating virus binding to the receptor [49].

2.3.2. Protein M

The virus responsible for the most abundant virion is the transmembrane (M) protein. It is a key factor in the assembly process of the virion because, through its interaction with the protein Envelope (E), it forms the budding site and serves to arrange all the basic components of the emerging virions . The M protein includes from 221 to 230 amino acids. It is a triple-membrane extended protein, and its N-terminal domain is exposed to the outer segment of virion glycosylations [50]. The M protein includes a large C-terminal domain localized within the viral particle. The interacting C-terminal domains of M proteins

within the virion create an additional layer of coating and form a matrix-like platform to which the viral RNP can bind [51]. The M protein, through its interaction with the S and N proteins, is instrumental in viral formation. [52]. In addition, the M protein of SARS-CoV has been shown to induce apoptosis [53].

2.3.3. Envelope (E)

The Envelope (E) protein is one of the smallest structural proteins of the coronavirus at 9 to 12 kDa, and is relatively little abundant in viral particles. He speculates that E is a double membrane protein spanning over the protein with both N- and C-terminals located within the virion (exposed to the cytoplasm during protein trafficking). When connected to the reticulum structure, the M protein has the primary function of forming a curvature of intracellular membranes during viral budding [54]. And has also been found to help E capture the virion from the host membrane at the end of the budding process [55].

2.3.4. Nucleoprotein (N)

The nucleocapsid (N) protein is a protein with a size of 45 to 60 kDa that is structurally and functionally important and a critical component of the virion found in all coronaviruses. . It binds to viral genomic RNA [56]. and also, only the complete form is incorporated into virions. That is, protein N is the only known structural phosphoprotein of coronaviruses [57]. The N proteins of coronaviruses bind to the M proteins, facilitating the insertion of genomic RNA into the viral particle. Also, the N protein is important for efficient replication and transcription. Immediately, it was confirmed that antibodies directed against N reduce RNA polymerase synthesis of the coronavirus genome RNA by 90% [58].

2.3.5. Hemagglutinin esterase (HE)

Hemagglutinin esterase (HE) Most coronaviruses have a crown-like structure made up of shorter mutations. This second structure includes Hemagglutinin-Esterase molecules, a 65 to 70 kDa transmembrane protein of type I that is disulfide-linked dimers. The outer domain is N-linked glycosylation at 8 to 10 putative sites. [59]. The HE protein is thought to modulate infection. Immediately, it causes inhibition of the activity of Esterase, which is responsible for the cleavage of acetyl groups of 9-O-acetyl neuraminic acid, [60]. HE has another activity, Hemagglutinin, which, in the case of coronaviruses, helps the viral particle connect to 9-O-acetylated neuraminimic receptors. The combined effects of hemagglutinin and esterase activities facilitate binding and cleavage to salivary receptors present in mucus, which is essential for movement during mucus toward the target cell. It is also thought that HE may be required for virion release into the extracellular environment. Although HE protein is not essential for MERS-CoV infection, it certainly has beneficial roles at different stages of the virus life cycle, particularly in promoting infection and motility within the mucus.[61-62].

2.4. Phylogeny of Coronaviruses:

Coronaviruses date back to the first isolations in the 1960s, and are single-stranded, positive-sense RNA viruses called and related to some of the largest viral genomes. A nidovirales arrangement was created, where viruses contained a genetic regulation and replication strategy. The Internastional committee on taxonomy of viruses (ICTV) has recently developed the classification of coronaviruses and related viruses by introducing new subcategories and genera.

Coronaviruses are now classified into the new subclass Coronavirinae, which belongs to Coronaviridae family. This family of viruses includes another subfamily, the Torovirinae family Coronaviridae, together with families of Arteriviridae and Roniviridae under the order Nidovirales [63-64-65].Species belonging to the genus Alphacoronavirus and Betacoronavirus are believed to

infect mammalian hosts, while viruses of the genus Gammacoronavirus and Deltacoronavirus have been identified as mainly infecting bird species [66- 67-68].

Coronavirus genomes are known for their genomic flexibility, and thus clarifies several key factors. RNA-dependent RNA polymers (RdRp) have high mutation rates, from 1 in 1,000 to 1 in 10,000 nucleotides during replication [69-70]. Corona viruses are known to operate a template-change mechanism that helps in increasing rates of homologous RNA reconfiguration among their viral genomes [71-72]. It is been expected that the large size of the coronavirus genome can contain gene mutations [67].

The rapidly spreading human coronaviruses, severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome (MERS-CoV) all belong to the B (Sarbecovirus subgenus) and C (sex) lineage Merbecovirus) subcomponents of Betacoronavirus, respectively [73-74] , and these are the result of transmission of zoonotic to humans and the emergence of symptoms of viral pneumonia, with fever, breathing difficulties and more [75- 76].

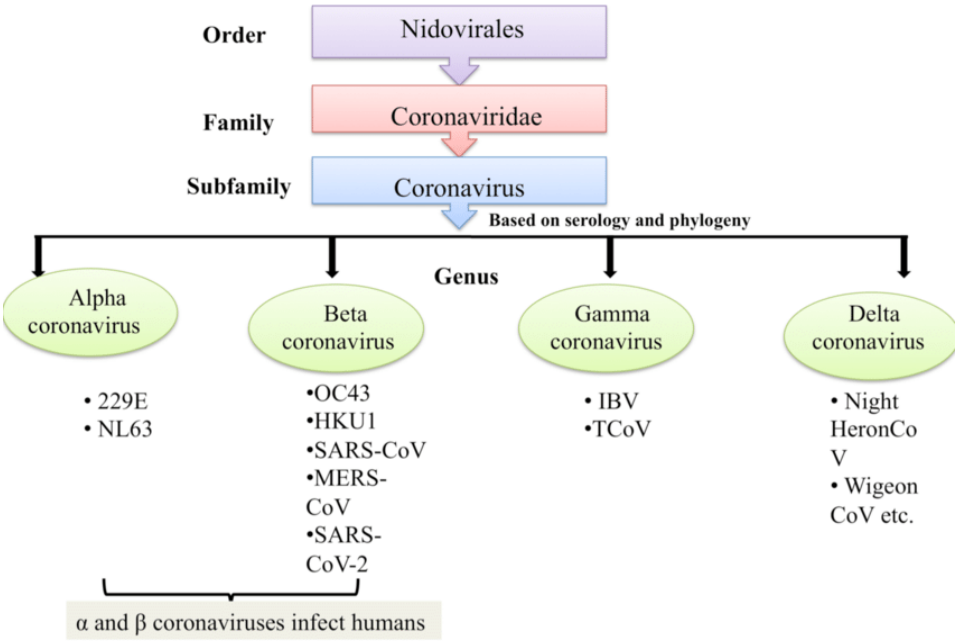

Figure (2-1): Taxonomy of corona virus include order , family ,genus [77].

2.5. Reproduction of coronavirus

The life cycle of the virus begins through the contact of the virion with the host cell by interactions between the S protein and its receptors. . [78]. This S protein receptor interaction is the key identifier of MERS-CoV infection in host species. Many coronaviruses use peptides as their cellular receptors. Which gets penetration even in the absence of enzymes for these proteins. After the process of attaching to the receptor , the virus must be able to reach the host's cytosol. Confirmation is accomplished by protein-dependent cleavage of the S protein by cathepsin , another protease , or TMPRRS2, and then cell membrane and virus fusion. The cleavage of the S protein occurs at two sites within the S2 portion of the protein [79] and fusion generally occurs within acidic endosomes. Cleavage at S2 is shown by a fusion peptide inserted into the membra involvesne , which the joining of two repeat heptagons at S2 forminga bundle of six parallel helices [80]. This bundle is able to fuse the viral and cellular membranes, which leads to the fusion and eventual release of the viral genome into the cytoplasm. The homologous gene functions by translating viral genomic RNA as well as encodes two large ORFs , repla and rep1b . Occasionally , the ribosome relaxes the pseudo-node structure and translation remains in continuity until it encounters the stop codon rep1a . Occasionally [81-82]. After the process of replication and synthesis of genomic RNA, it translates the viral structural proteins, S, M and E and places them in the endoplasmic reticulum (ER). These proteins travel along the secretory pathway in the endoplasmic reticulum - the intermediate Golgi compartment (ERGIC) [83-84].

Viral genomes are flanked by N-protein buds in ERGIC membranes that include viral structural proteins, resulting from mature viruses . It transports viruses to the cell surface in vesicles and is released by exocytosis. Protein assembly does not occur in viruses that travel to the cell surface as they mediate cell fusion between infected cells and cells of neighboring uninfected cells. This leads to the formation of multinucleated giant cells, which enables the virus to

spread within the infected organism undetected or neutralized by virus antibodies[85].

2.6. Pathogenesis of coronavirus:

The onset of coronavirus infection, coronaviruses have been speculated to result from limited, mild, and spontaneous respiratory infections in humans. Four of human coronaviruses are β-corones (HCoV-OC43 and HCoV-HKU1), and the other four are α-corones (HCoV-229E and HCoV-NL63). These viruses is the difference in susceptibility to genetic alterations [85] in which large numbers of bats have been found that reveal SARS-associated COV sequences and provide serological evidence of pre-CoV infection [86-87]. Corona virus begins to infect lung epithelial cells. It is possible that the virus invades dendritic cells and macrophages but progresses to a failed infection [88- 89]. Infection with these types of cells may be necessary to activate pro-inflammatory cytokines that can lead to disease [90]. The process of lung damage and the cause of serious infection remain undetermined in humans. The incubation period of SARS-CoV-2 from (5 -14) days, and about (80%) of patients show mild or no signs, (15%) are acute (oxygen required) and (5%) are critical [91-92]. The most common clinical manifestation was Fever, cough, short breath and fatigue [93].

2.7. Clinical Manifestation

Professor Nan Shanzhong's team demonstrated their recent study by sampling 1,099 laboratory-confirmed cases, common clinical signs include fever (88.7%), cough (67.8%), fatigue (38.1%), sputum production (33.4%), and shortness of breath. Breathing (18.6%), sore throat (13.9%), headache (13.6%) [94]. Some patients was found gastrointestinal symptoms, with diarrhea (3.8%) and vomiting (5.0%). [95]. Cough and fever were common clinical signs while upper respiratory and gastrointestinal symptoms were rare, indicating differences between tropical virus compared to SARS-CoV [96], MERSCoV.[97] . (bottom panel, Figure 1). Laboratory tests found that most patients had a low number of normal or white blood cells, and a lack of lymphocytes [94-98]. But in severe

cases, neutrophil count, D-dimer, creatinine, and blood urea Lymphocyte levels were significantly higher. In addition to the infection factors (interleukin (IL)-6, IL-10, tumor necrosis, increased factor alpha (TNF-α), which indicate the immune status of patients, studies have revealed that intensive care patients have higher plasma levels of and IL- 7, IL-2, TNF-α, granulocyte colony-stimulating factor (GCSF), 10 kDa interfering induced protein (IP-10), protein 1 (MCP-1), and macrophage protein 1-α (MIP-1α) [99].

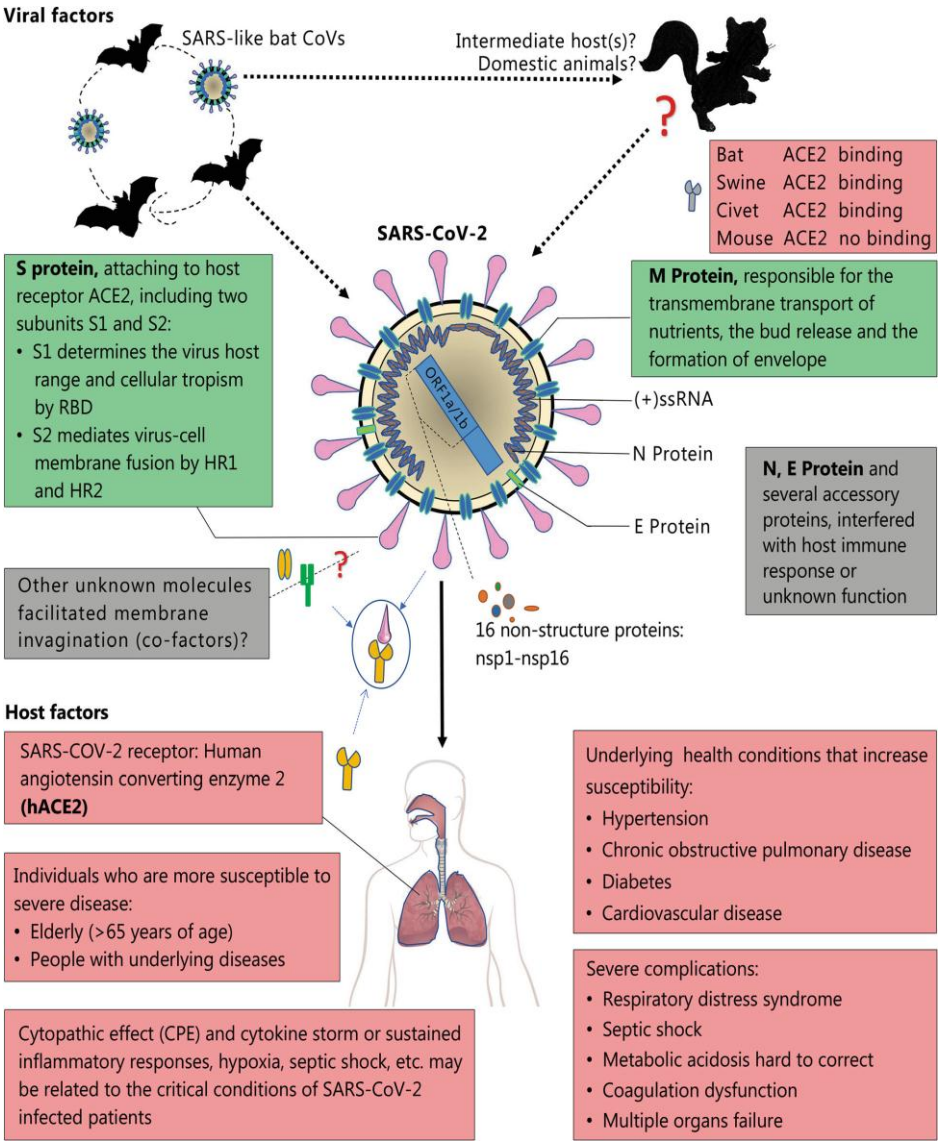

Figure (2-2): the pathogenesis of corona virus and effected on host factors with reservoirs [100].

2.8. Epidemiology

In December 2019 of Chinese city of Wuhan , became the outbreak of novel coronavirus covid-19 diseases [101-102]. Under way to understand more about the mechanism of transmission, the severity, and other features associated with COVID-19. An infected animal was been believed to have transmitted the virus to humans [103-104]. After a secondary source of infection was been revealed which is human-to-human transmission of covid-19 virus [105]. New studies have found populations with poor immune function such as diabetes, vascular disease, heart disease, chronic respiratory disease, renal impairment, cancer, and liver disease are more likely to infected with severe covid-19 than children was less sensitive [106]. Coronavirus 2019 has spread very quickly throughout China and has been exported to a large number of countries, some of which have been observed to be transmitted continuously. According to the World Health Organization (WHO). On May 2, 2020, the World Health Organization announced more than three million confirmed cases of covid-19. The growing global statistic includes increases in Italy, Korea, Iran, Spain, Germany and France. It also spread in African countries, including Nigeria, Algeria, Senegal, South Africa, Burkina Faso, Cameroon, Ivory Coast and Morocco[107].

2.9. Cytokines storm syndrome

Covid-CSS concept believe that "a cytokine storm has no definition, "and there is no relationship that COVID patients develop a cytokine storm [108-109]. In fact, experts define cytokine storm syndrome as a clinical phenotype of a disorder in the regulation of immunity characterized by the continuous activation of lymphocytes and macrophages. Then Increased secretions of cytokines. It causes overwhelming systemic inflammation and multiorgan failure with high mortality [110-111].

The term CSS is first used to detect hyperalgesia in allogeneic stem transplantation for graft-versus-host disease [112]. In the later periods, CAR T-cell CRS cells, in which IL-6, IL-2, are significantly elevated in serum at the

time of admission and predict poor outcome, were seen to respond to IL-6, IL-2, as were used Blockade in the general term CSS [113]. The strength of COVID-19 is closely related to the level of inflammatory cytokines such as interleukins (IL-6 and IL-2). In patients who complain of the strength of the disease, revealed a significant decrease in the number of lymphocytes [114-115-116].

Flow cytometry analysis of acute COVID-19 patients found significantly lower natural killer (NK) cells and T lymphocytes (CD8+ and CD4), as well as higher expression of natural killer group 2A (NKG2A) and T-cell-associated immunoglobulin (Timothys-3). It is mediated by the overexpression of T lymphocytes in early-infected cases [117-115].

2.10. Diagnosis of cytokine storm syndrome in covid-19:

Cytokine storm syndrome in covid-19 is refer to elevate IL-2, interferon-γ ,monocyte, chemoattractant protein-1 , macrophage inflammation , protein1α , and tumor necrosis factors-α .In china ,Wuhan was recorded (150) patients who was increase of ferritin ,IL-6 and it refers to predictors of fatality and sever diseases or hyperinflation [118].Cytokine storm syndrome is similar to secondary haemophogocytics lymphohistiocytosis a less recolonized entry commonly triggered by viral infection [119]. Secondary haemophogocytics lymphohistiocytosis has similar to cytokine syndrome in clinical and patholobiological profile of hyperinflamation and biochemical marker of hyper-inflammation include increase of ferritin ,LDH and other markers [120].

2.11. Prevention and control covid-19:

Lack specific sign, symptoms, and diagnosis of corona virus outbreak and mix with seasonal flu lead to make difficult Covid-19 to diagnosis [121]. In absences of treatment to overcome virus lead to know a ways of prevention of infection with covid-19 and control the source of infection [122]. Prevention from covid-19 follow by individual prevention and social prevention. Individual prevention include personal protective equipment such as masks and cloves, washing and disinfecting hands after touching surface in public places , present

the spread of respiratory droplets when coughing , sneezing with mask and other protective equipment .Void touching eyes, nose and mouth , avoid attending in crowded places and close contact with people. Stay at home and follow government guideline [123].Wash your personal items with soap and water [124]. In social prevention of covid-19 as restriction on travels as international travel. Screen of people in officers when enter and controlling from sign [125]. Observances of social distances of at least 2m to reduce exposure and cut transmission chain as early identification of infected peoples , identify and reduces transmission from animals reservoir ,school closures and use N95 – mask and protective clothing [121].

2.12. Covid-19 treatment

2.12.1. Current treatments

Due to the lack of an effective treatment against COVID-19, the current treatments have generally been shown to support respiratory and symptomatic support according to the Diagnosis and Treatment of COVID-19 Pneumonia announced by the National Health Commission of the Republic of China [126]. Most patients agreed to oxygen therapy, and the World Health Organization required extracorporeal membrane oxygenation (ECMO) for patients who complain of hypoxemia [127]. Rescue treatment with convalescent plasma and IgG is provided to the most critical cases according to their circumstances [128].

2.12.2. Antiviral treatments

The epidemiological control of SARS-CoV and MERS-CoV, information has been clarified for some treatments against MERS-CoV [129]. Remdesivir was been used successfully in the first US case of covid-19 [130]. Chloroquine is been used again with a powerful ability to treat Covid-19. Chloroquine has been used to treat malaria for several years [131] and chloroquine was found to inhibit the pH-dependent phases of replication of many viruses [132], with great efficacy in SARS virus infection and spread [133]. Chloroquine has immunomodulatory activities, which suppresses the production/release of (TNF-

α) and (IL-6). It acts as an inhibitor of autophagy [134], which associated with viral infection and replication. Some studies have shown that chloroquine interferes with the glycosylation of cellular receptors for SARS-CoV [133] and acts in both entry and post-entry phases of COVID-19 infection in Vero E6 cells [135]. They showed that a combination of chloroquine and Remdesivir mainly inhibited SARS-CoV-2 that was been recently shown in the laboratory. [136].

2.13. Vaccination of covid-19

Modern studies of the vaccine and the reduction of the Corona Virus 2019 epidemic have begun to be a global necessity and having the vaccines available for distribution and approved on a large field before the end of 2020 or even 2021 will be unprecedented. New manufacturing platforms, structure-based antigen formation, protein engineering, computational biology, and genetic synthesis have now equipped important tools for making vaccines quickly and accurately. It was been classified into two main parts. Gene-dependent vaccines develop genetic sequences that encode protein antigens produced by host cells. They are represented by live virus vaccines , DNA vaccines, or recombinant vaccine vectors. Protein-based vaccines include fully inactivated virus, individual viral proteins or grouped viral proteins as particles or subdomains, all of which are been synthesized in vitro. DNA vaccines and recombinant vaccine vectors are best for speed because they can be more easily adapted to platform manufacturing technologies in which the upstream supply chains and downstream processes are the same for each product [137].

2.14. Copy number of coronavirus:

Copy number is a phenomenon in that expresion of gene are been repeated and the number of repeated in genome variables between individual .it refer to structure variation[138]. Copy number variation can be generally categories in two groups short repeats and long repeats . The diagnostic confirmation of COVID-19 is based on the molecular detection of the viral genome (RNA detection by PCR) or its proteins (antigens) [138-139]. In addition tothe

dynamics of the infection and viral secretion in different fluids is still under study, to date it has been possible to establish that the virus can be detected from at least 48 hours before the onset of symptoms (pre-symptomatic cases) and up to(12-14) days (at least 6-7 days) after, in samples from the upper respiratory tract (oropharyngeal swabs / naso) and up to (20) days in samples from the lower respiratory tract including sputum, tracheal aspirate , alavage and bronchioalveolar [140].

Chapter Three

Materials& Methods

3.1. Materials

3.1.1. Equipment and Instruments

Equipment and Instruments were used in this study of enrolled in the table (3-1) with their companies and countries.

Table (3-1): General equipment and instruments used in this study.

Equipment & Instrument	Company	Country and Origin
Thermal cycler pcr devise	BioRad cfx 96	Sinqapor
Hood	GEIO TECH	Germany
Autoclave	GEIO TECH	South Korea
Vortex	IKA	Germany
Microfuge	Eppendrof	Germany
Incubator	Memmert	Germany
Deep Frezee	ALS	Italy
ELIZA	Paramedical	Italy
Centrifugal force	ROWA (GMBH)	Germany
Mini vidas	Biomeriux	France
CBC 5diffrential	Genex	China
Fully Biochemistery	Dongji 240	China
Getein1100	Getein Biotech,Inc	China

Table (3-2): Manufactured companies and countries of origin.

Chemical substances	Company	Country of Origin
Gal tubes(5ml)	ALS laboratory Supplies	Iraq
Viral Transport Medum(VTM) Tabes(5ML)	Zybio	China
Automated adjustable micropipettes	Slamed	Germany
Plain tubes (5ml)	Bio Plus Bware	Canada
Sterile pipette tips	Bio Plus Bware	Canada
Sterile PCR Eppendorf tubes (0.2 µl) & Eppendorf tubes (1.5 µl)	Bio Plus Bware	Canada

3.1.2. Blood Samples

Blood samples (swab sample, blood) were taken for (100) samples (62 positive) on February to June 2021 st who were isolated from patients suffering or infected with Corona virus for Al-Sadr Teaching Hospitals and Al-Majar Al-Kabeer hospitals , with age, gender and laboratory analyzes, in addition to identifying clinical symptoms.

3.1.3. Questionnaires

All Patients also filled out questionnaires by providing answers to important information (Appendix 1). The questionnaires were been used in assessing the risk factor associated upper respiratory tract infection. Questions included in the questionnaires included; Gender, age, and clinical symptoms such as fever, cough, shortness of breath, and others.

3.1.4. Swabs Sample collection:

Swabs were taken from the patient's nose and kept in viral transport medium tubes at a temperature of (2-8°C) and transferred to the molecular biology laboratory for RT-PCR assay.

Table (3-3) Composition of Sample extraction operation

Composition	50 test / Kit
Adsorption Column	50 x 1 Pack
Collection Tube	50 x 1 Pack
Lysis Solution	11 ml x 1 Bottle
Wash Buffer I	14 ml x 1 Bottle(add 18ml absolute ethanol before the first use)
Wash Buffer II	8 ml x 1 Bottle(add 24ml absolute ethanol before the first use)
Elution Solution	3ml x 1 Bottle
Instructions	1 serving

Table (3-4) Components of SARS-CoV-2 Nucleic Acid Detection Kit.

Components	Main Constituents	Volume (32 – 96 T/Kit)
SARS-Cov-2 PCR Reaction Solution	Tris-Hcl Buffer , dNTPs, Mg , primers and probes	256 µl -768 µl
SARS-Cov-2 Enzyme Solution	Reverse Transcriptase, Taq DNA polymerase, Uracil N-glycosylase (UNG)	64 µl -192 µl
SARS-Cov-2 Negative Control	0.9% (W/V) NaCl	800 µl-1.6 µl
SARS-Cov-2 Positive Control	Armored virus contained SARS-Cov-2 target fragment sequence	800 µl-1.6 µl
SARS-Cov-2 Intermal Control	Armored virus contained internal control fragment sequence	32 µl-96 µl

Table (3-5): Components of Interleukins (2 , 6 ,10) Kit.

Content	Quantity
Avidin-HRP	5.5 ml
Human 2,6,10 Detection AS	5.5 ml
Substrate	5.5 ml
Washing Buffer 10X	35 ml
Human ELISA Interleukin plate	96well
Human Interleukin Standard	0pg/ml
Human Interleukin Standard	50pg/ml
Human Interleukin Standard	100pg/ml
Human interleukin Standard	200pg/ml

3.2. Methods

3.2.1. Qualitatively detects of covid-19 RNA from nasal swabs

Specimens detection process mainly consist of two steps:

1- gene was isolated manually and all specimens samples was received biopsy training .Throat swabs or nasal was collected in biosafety cabinet of BSL-2 laboratory after samples.

2- Revers transcription and PCR amplification: The applicable instrument is used for detection covid-19 RNA was prepared in step (1) is reverse transpiration to generated complementary DNA (cDNA) in RT-PCR reaction system . Specimens primers and probes are designed according to conserved sequencing of ORE lab and N- gene for PCR amplification .Qualitative reaction of Covid-19 is detected by monitors the change of fluorescent signal intensity during RT-PCR amplification and after extraction covid-19 covid-19 and preparation of samples volum of 200 µ/L of protease k nucleic acid extraction in proportion with 1 µl test mix through before extraction gene.

3.2.2. Sample extraction operation

First, All samples were taken from nasopharyngeal from patient have clinical manifestation cough , headache, and short breathing and RNA extraction was according to (BIO FluX Kite).

1- 10 µl from protease k in to 1.5 ml micorcentrifuge tube.
2- 200µl from samples with phosphate buffer solution in to micorcentrifuge.
3- 200µl was added to buffer then votexed for 30 sec and incubated mixture at room temperature.
4- 250 µl was added of ethanol (96-100%) to the sample then vortex for 15 sec .
5- The mixture was transferred in to spin column then centrifuged at 10,000rpm for 1 min and discard superratant.

6- 500µl from wash buffer I was added into spin column then centrifuged at 10,000rpm for 1 min and discard superratant.
7- 500µl from wash buffer II was added into spin column then centrifuged at 10,000rpm for 1min and discard it and repeat step 3
8- All samples were centrifuged at 10,000 rpm.
9- All samples were placed in all spin column in a clean 1.5 ml collection tube then then centrifuge at 10,000 g for 2 min to dry it .
10-All samples were placed in all spin column in a clean 1.5 ml collection tube then then was been added (50-100) µl of Elution buffer or RNase – free water (pH>7).
11-All samples were centrifuged at 12,000rpm for 1 min and removed spin basket and discard it
12-All micorcentrifuge tubes which were contain RNA and kept preciptate for use.

3.2.3. Covid-19 nucleic acid detection:

Second , all extracted RNA samples were taken according to (Zybio Kite).

1- The PCR reaction solution, enzyme and internal control were mixed in a tube to form a master mix and then shaken with a shaker.
2- 10 microliters Master Mixes were added to each the pallet.
3- 10 microliters RNA extract were added to each the pellet as well.
4- - The pallet was well covered from the top.
5- The pellets were placed in the real PCR machine and after an hour the results were read on the computer screen of the device as shown in Figure (3-1).

Table (3-6) : The genetic sequence of the Corona virus 19.

Name	Sequence (5’ → 3’)	Referenc
Probe	FAM-TCCTCACTGCCGTCTTGTTGACCA-BHQ1	[173]
Probe	ROX-ATGCATCTCAAGGTCTAGTG-MGB	[174]

Figure (3-1): Thermal cycler PCR device determines the result of a positive or negative nasal swab for Covid-19 patients.

3.2.4. Diagnosing the plasma of COVID-19 patients by ELISA:

Plasma (100) samples of COVID-19 patients were examined using the indirect method to detect levels of cytokines in the blood plasma.

3.2.4.1. IL-2 , IL-6 and IL-10

1- All plate were taken out of the desired package and bring it to room temperature in a dry environment. Then add 50 microliters from standard 4 (CH: KPG-HI2S4) to 1 in vials A1 to D1.
2- 50 microliters were added to the rest of the vials of the desired sample and incubate on the shaker for 1 hour (at least at 200 rpm).
3- All plates were rines for 3 times. Note that the washing solution is prepared in a concentration of 10 times. Therefore, before washing, dilute the washing solution with distilled water twice 10 times. Incubation the plates at room temperature for approximately 1 minute after adding the washing solution and then drain.
4- 50 microliters were added to conjugate antibody to all the cavities and incubate on a shaker (at least at 200 rpm) for 1 hour.

5- All plates washed 3 times using the washing solution. Plates were Incubated at room temperature for approximately 1 minute after adding the washing solution and then drain. Add 50 µl of HRP-Avidin solution to all cavities and incubate on a shaker (at least at RPM 200) for half an hour.

6- All plate were washed 5 times with washing solution. plates were incubated at room temperature for approximately 1 minute after adding the washing solution and then drain.

7- 50 microliters was added of substrate to all the holes and incubate for 15 minutes.

8- 25 microliters were added of the stopper solution to all the holes and measure the absorption of the samples in the ELISA reader with a wavelength of (450) nm.

3.2.5. CRP Latex test:

1. Put one drop (50 µl) of plasma from COVID-19 patients into one of the circles on the card.
2. added one drop of CRP-Latex reagent to the sample chamber to be detected.
3. Mixing the contents of the circle of a special stirrer can be disposed of during distribution over the entire area enclosed in the ring. Use separate stirrers for each mixture.
4. Leave the shaker device on the sliding device for mechanical rotation (100 rpm) for two minutes.
5. The card is observed immediately under a suitable light source to see the extent of a degree of agglutination. The reading in the case of the degree of agglutination is clear with the naked eye, the result is positive, while the result is negative with a soft suspension without visible agglutination.
6. The positive sample was diluted into several dilutions with distilled water, where 50 distilled water was taken and added to the cardstock circles. 50

microliters were added, mixing well, then 50 of this mixture was taken and the circles were added sequentially and this addition was repeated several times.

7. The result was read from the first circle and in succession and the result is in the form of numbers in the case of circles positive (6, 12, 24, 48,...)

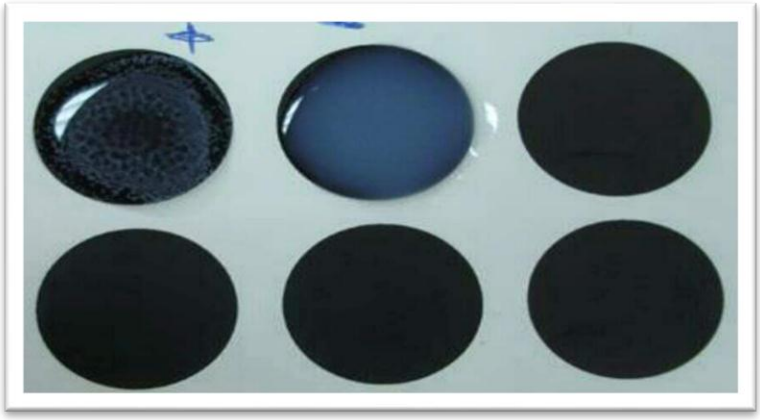

Figure (3-2): C-reaction protein latex card positive and negative result for Covid-19 patients.

3.2.6. D.dimer Test:-

The serum sample of Covid-19 patients was diagnosed to detect D.dimer according to the method of work of the kit (D.dimer fast test kit) from (GP Getein Biotech, Inc) using the Getein 1100 device as in figure (3-3).

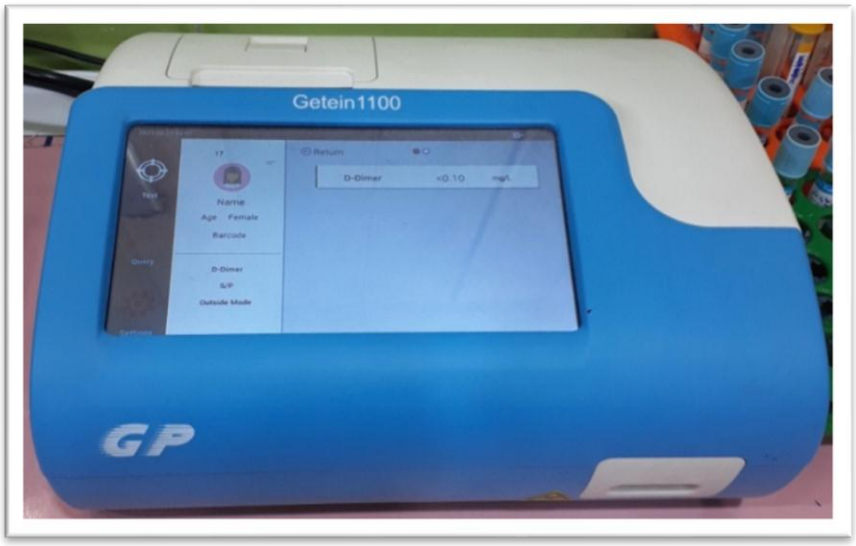

Figure (3-3): Getein1100 a devince that measure D.dimer for serum of Covid-19patients

3.2.6. Ferritin

Ferritin was diagnosed using mini vidas as in figure (3-4). one of the most advanced and modern devices that are characterized by accurate results and speed of performance. The VIDAS FER KIT method was used to diagnose the serum of COVID-19 patients. From his partner (bioMeriux).

Figure (3-4): Minividas device works to read ferritin in the serum of patients with Covid-19

3.2.7. LDH

Lactate Dehydrogenase was diagnosed using Fully Biochemistery as in figure (3-5). one of the most advanced and modern devices that are characterized by accurate results and fast performance. The Lactate Dehydrogenase Assay Reagent Kit was used to diagnose the serum of COVID-19 patients. From his partner (Dongji 240).

Figure (3-5): Fully Biochemistry reads LDH in serum of patients with Covid-19.

Chapter Four

Results

4.1. Age group of Covid-19 patients:-

Age is first factors, which associated with Covid-19 infection. In different age groups the results showed that the highest rate of Covid-19 infection in the range (50-59) years 14 (22.6)%, while the lowest percentage of infection appeared in the range (10-19) years 2 (3.2%) as in Table (1-4) and Figure (4-1) shows the high infection in the different age groups of the participants.

Table (4-1): Age group of Covid-19 patients.

Age group	Frequency	Percentage%	Control	Mean	S.D
10-19	2	3.2			
20-29	6	9.7			
30-39	8	12.9			
40-49	9	14.5			
50-59	14	22.6	38	51.47	17.243
60-69	12	19.4			
70-79	8	12.9			
80 ≥	3	4.8			
Total	62	100%			

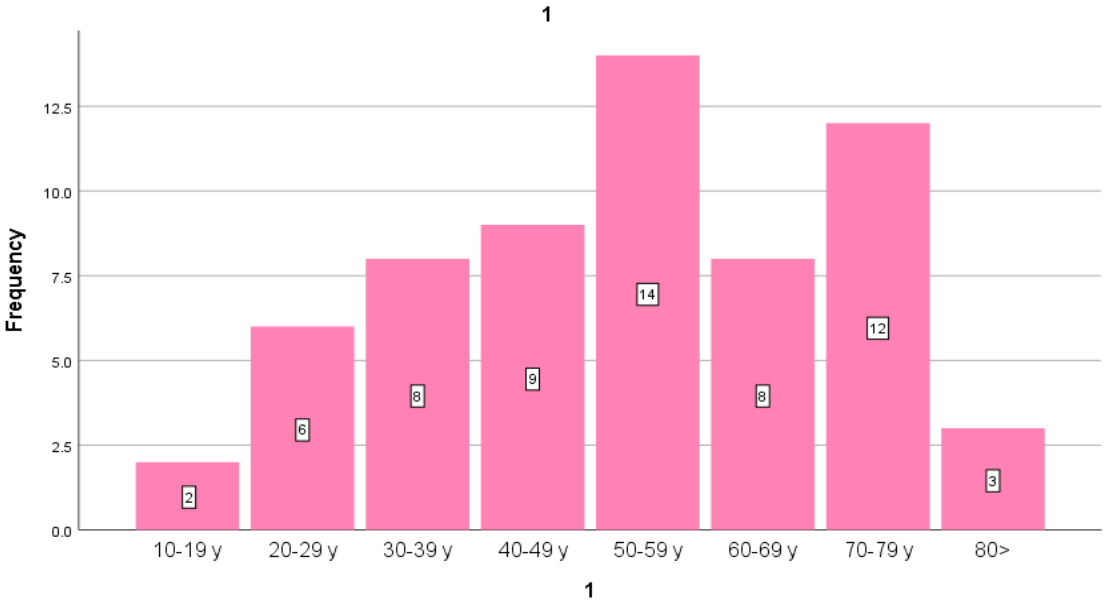

Figure (4-1): Age group of Covid-19 patients

4.2. Sex distribution of Covid-19 patients:

Sex is a second factor which effected on COVID-19 as Male was recorded a high rate 33 (53.2%), and the lower rate in female was 29 (46.8%), as shown in the table (4-2) and figure (4-2) .

Table (4-2): Sex distribution of Covid-19 patients.

Sex	Frequency	Percentage %	Control	Mean	S.D
Male	33	53.2			
Female	29	46.8	38	1.47	0.503
Total	62	100%			

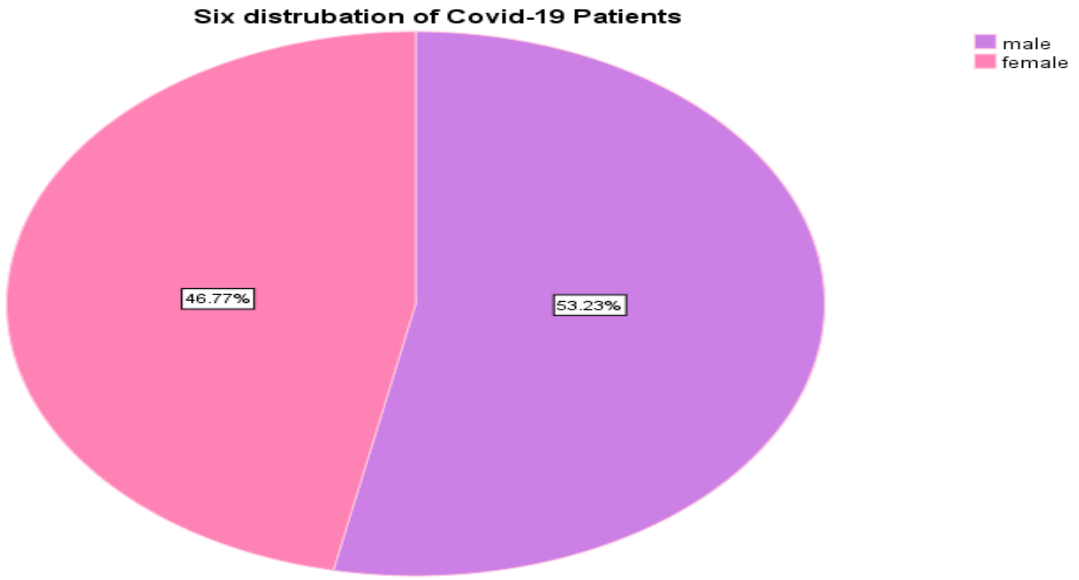

Figure (4-2): Sex distribution with covid-19 patients

4.3. Disease associated with Covid-19 patients

The disease associated with covid-19 patients in a careful study showed that patients who suffer from some diseases of high blood pressure diabetes and other diseases. The incidence was high 29 (46.8%) with hypertension, followed by non-diseases with an incidence rate. out of 18 (29%). Diabetics, the incidence was 15 (24.2%) as in the table (4-3) and Figure (4-3) below that shows these results.

Table (4-3): Disease associated with Covid-19 patients

Diseases	Frequency	Percentage%	Control	Mean	S D
Diabetics	15	24.2	38	1.65	1.332
Hypertension	29	46.8			
Without Disease	18	29			
Total	62	100%			

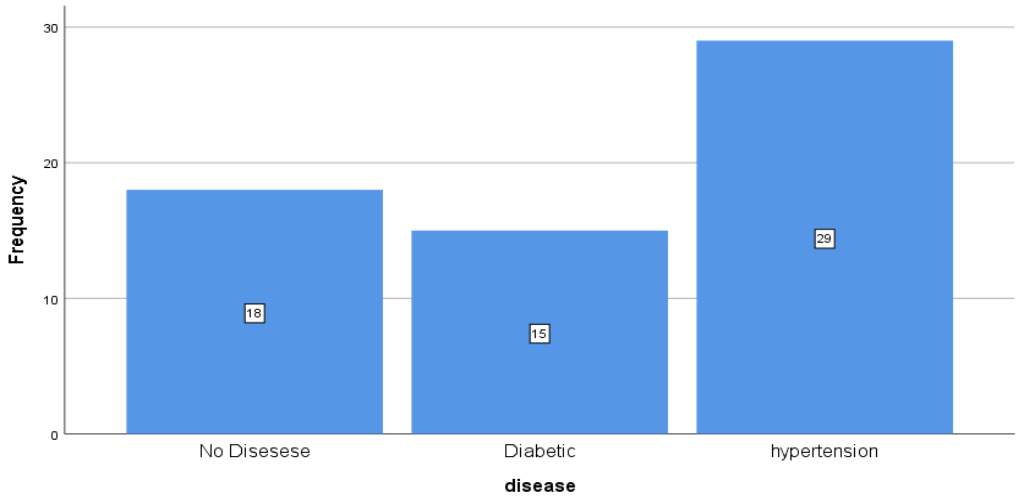

Figure (4-3): Disease associated with Covid-19 patients

4.4. Sign and Symptoms associated Covd-19 disease

Signs and symptoms of Covid-19 disease is one of the important clinical factors, and the study showed that the proportion of symptoms and signs among people who were exposed for the first time to infection with Covid-19 in different proportions, where the highest percentage of people suffering from fever (44.1%) in 26 cases, then cough by (32.2% (%) in 19 cases, and Pneumonic patients (22%) in 13 cases, and the lowest percentage in patients. Short breath (1.7%) in 1 cases, while people who have been exposed to more than one infection, the highest percentage in people who suffer from cough by (66.7) and the lowest (33.3) in 1 case, and the table (4-4) and figure (4-4) below illustrates this.

Table (4-4): Sign and Symptoms associated Covd-19 disease

Number of infection	Fever	Short breath	Cough	Pneumonic	Total	Control	P-vale
Once	26(44.1%)	1(1.7%)	19(32.2%)	13(22%)	59(100%)	38	0.004
More that once	0	1(33.3%)	2(66.7%)	0	3(100%)		

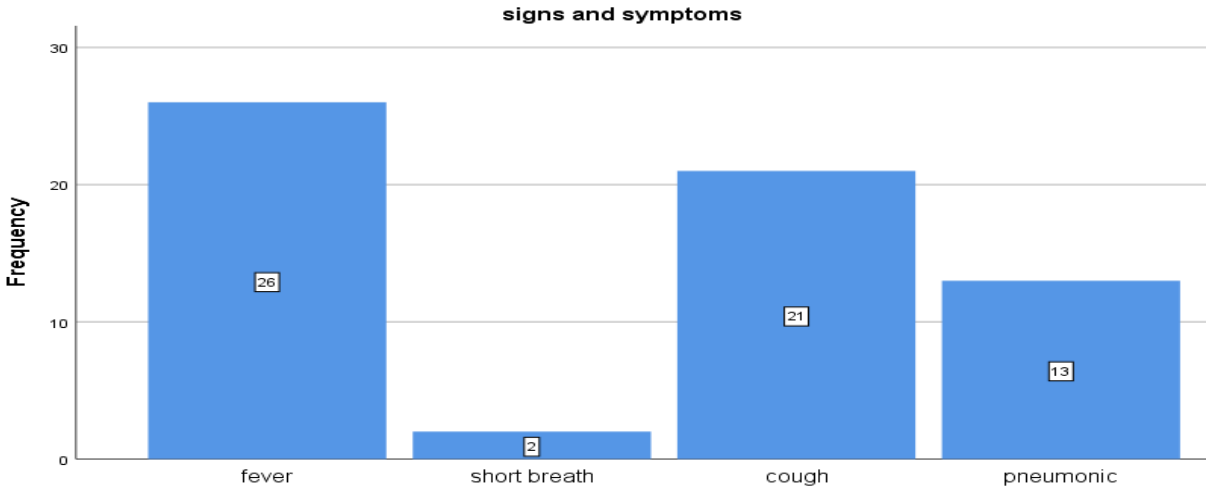

Figure (4-4): Sign and Symptoms associated Covd-19 disease

4.5. WBC count in serum Covd-19 disease:-

WBC is one of the important immunological factors for Covid-19 patients, as the results of accurate studies showed that the mean was (9.71), Std.deviation (4.19), T.test (18.25) and Statistical significance (p-vale 0.00). As shown in figure (4-5) and table (4-5) .

Table (4-5): Immunity cell associated with Covid-19 patients.

Immunity cell	Mean	Std.deviation	T.Test	Control	P-vale
WBC	9.71	4.19	18.25	38	0.00
Neutrophil	80.85	12.98	51.81		
Lymphocyte	10.35	7.37	11.03		
Monocyte	4.67	2.54	14.467		

(P-vale<0.05)

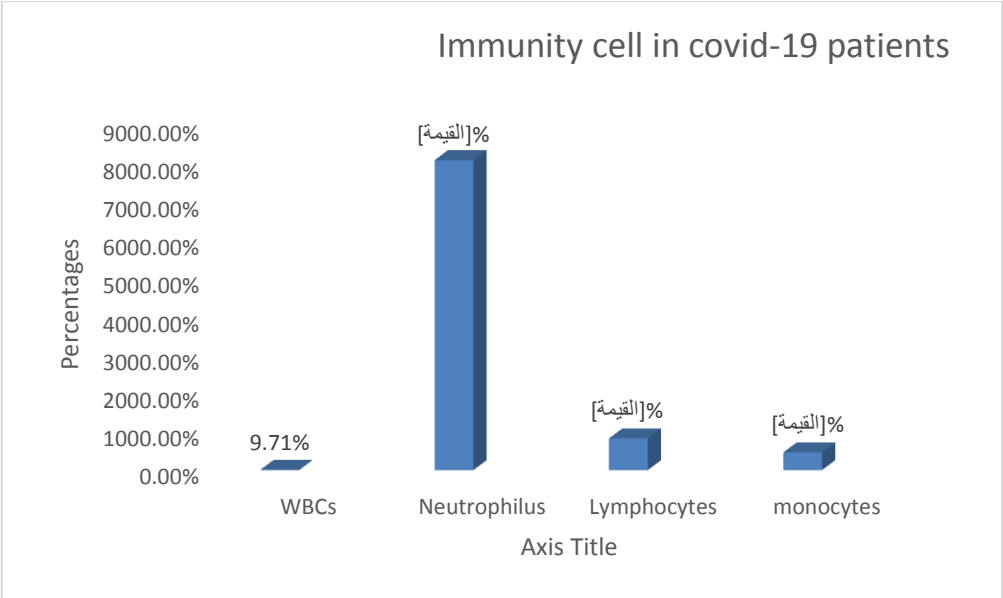

Figure (4-5): Immunity cell associated with Covid-19 patients

4.6. Neutrophil in serum Covid-19 disease:-

Neutrophils are the second immune factor for Covid-19 patients. The results of the study showed accurate that the mean was (80.85), Std.deviation (12.98), T.test (51.81) and Statistical significance (p-vale 0.00). As shown in table (4-5) and figure (4-5).

4.7. Lymphocytes associated with Covid-19 patients

Lymphocytes are the other important immune factor for COVID-19 patients, which accurate results found that the mean was (10.35), Std.deviation (7.37), T.test (11.03) and Statistical significance (p-vale 0.00). As shown in table (4-5) and figure (4-5).

4.8. Monocyte Count associated with Covid-19 patients

Monocytes are also an important immune factor for patients with COVID-19, the results of the careful study appeared that the mean was (4.67), Std. deviation (2.54), T.test (14.467) and Statistical significance (p-vale 0.00). As shown in table (4-5) and figure (4-5).

4.9. Ferritin analysis in serum with Covid-19 patients

Ferritin is one of the main and important factors for Covid-19 patients, as the results of the careful study showed that the mean was (903.22), Std. deviation (488.32), T.test (14.156) and Statistical significance (p-vale 0.00). As shown in table (4-6) and Figure (4-6).

4.10. LDH Count in serum with Covid-19 patients

Lactic dehydrogenase (LDH) is one of the main and important factors for Covid-19 patients, as the results of the careful study showed that the mean was (22.67), Std. deviation (173.39), T.test (22.67) and Statistical significance (p-vale 0.00). As shown in table (4-6) and figure (4-6).

4.11. D.dimer in serum with Covid-19 patients

D.dimer is one of the main and important factors for Covid-19 patients, as the accurate results showed that the mean was (1.24), Std. deviation (1.44), T.test (6.781) and Statistical significance (p-vale 0.00). As shown in table (4-6) and figure (4-6).

4.12. CRP in serum with Covid-19 patients

CRP is one of the main and important factors for Covid-19 patients, as the accurate results showed that the mean was (43.45), Std. deviation (35.26), T.test (9.70) and Statistical significance (p-vale 0.00). As shown in table (4-5) and figure (4-6).

4.13. Platelets in serum with Covid-19 patients:

Platelets in the serum of COVID-19 patients, where the exact results showed that the mean was (219.50), Std. deviation (103.66), T.test (16.674) and Statistical significance (p-vale 0.00). As shown in table (4-6) and figure (4-6).

4.14. Hemoglobin Count in serum with Covid-19 patients

Hemoglobin Count in serum with Covid-19 patients, where the exact results showed that the mean was (13.154), Std. deviation (2.71), T.test (38.14) and Statistical significance (p-vale 0.00). As shown in table (4-6) and figure (4-6).

Table (4-6) : Biomarker variables associated with Covid-19 patients.

Biomarker variables	Mean ±Std. deviation	T.Test	Control	P-vale
Ferritin	488.32±903.22	14.156	38	0.00
LDH	173.39±22.67	22.67		
CRP	35.26±43.45	9.70		
D-dimer	1.24±1.44	6.781		
PLT	103.66±219.50	16.674		
HGB	2.71±13.154	38.14		

(P-vale<0.05)*

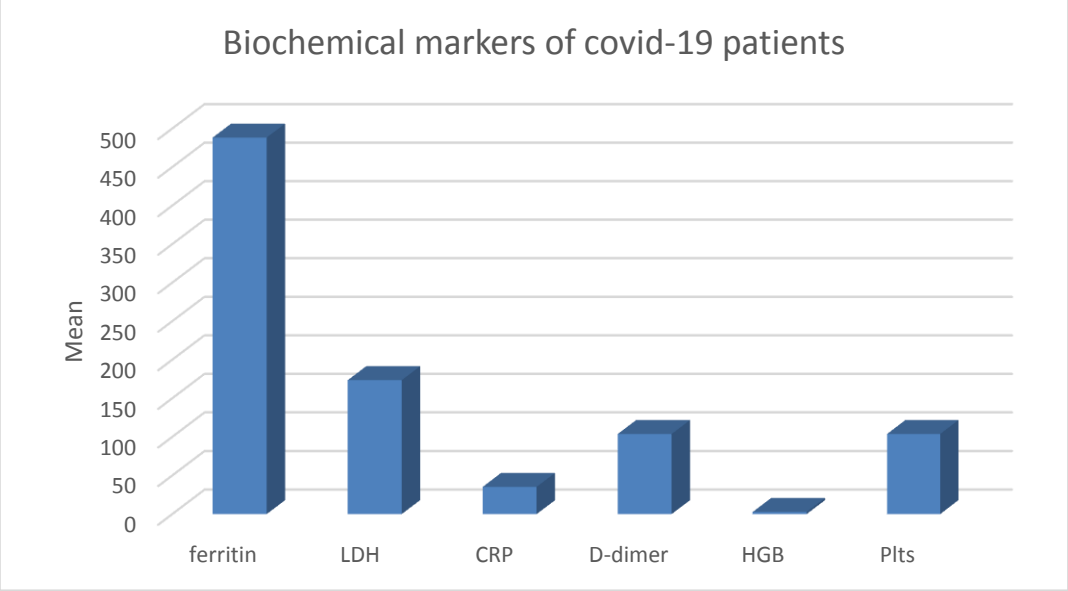

Figure (4-6): Biomarker variables associated with Covid-19 patients.

4.15. Interleukin 2 with serum Covid-19 patients:

Interleukin 2 is one of the immune factors associated with Covid-19 patients, as the results of the careful study in the ELISA device showed that the mean was (214.33), Std. deviation (169.08), T.test (9.98) and Statistical significance (p-vale 0.00). and was used been a control cases (62) As shown in table (4-7) and Figure (4-7).

4.16. Interleukin 6 with serum Covid-19 patients:

Interleukin 6 is one of the immune factors associated with Covid-19 patients, where the results of the careful study in the ELISA device showed that mean was (537.24), Std. deviation (196.78), T.test (21.49) and Statistical significance (p-vale 0.00). As shown in table (4-7) and figure (4-7).

4.17. Interleukin 10 with serum Covid-19 patients:

Interleukin 10 is one of the immune factors associated with Covid-19 patients, where the results of the careful study in the ELISA device showed that mean was (0), Std. deviation (0), T.test (0) and Statistical significance (p-vale 0.00). As shown in table (4-7) and figure (4-7).

Table (4-7): Interleukin associated with Covid-19 patients.

Interleukin	Mean	Std. deviation	T. Test	P-vale
IL-2	214.33	169.08	9.98	0.00
IL-6	537.24	196.78	21.49	
IL-10	0	0	0	
Control = 38				

(P value<0.05)*

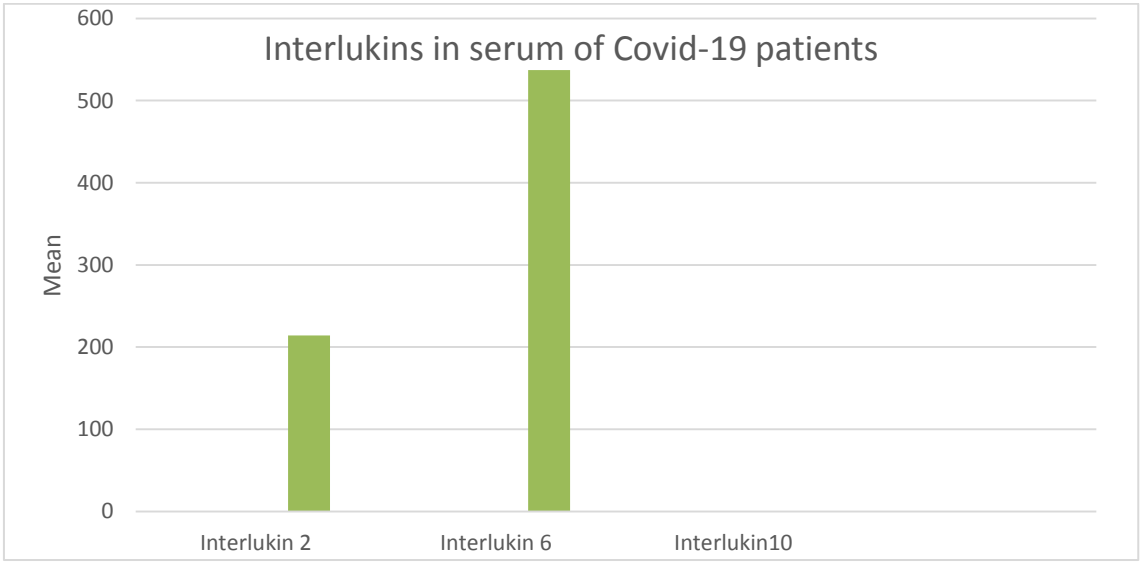

Figure (4-7): Interleukin associated with Covid-19 patients.

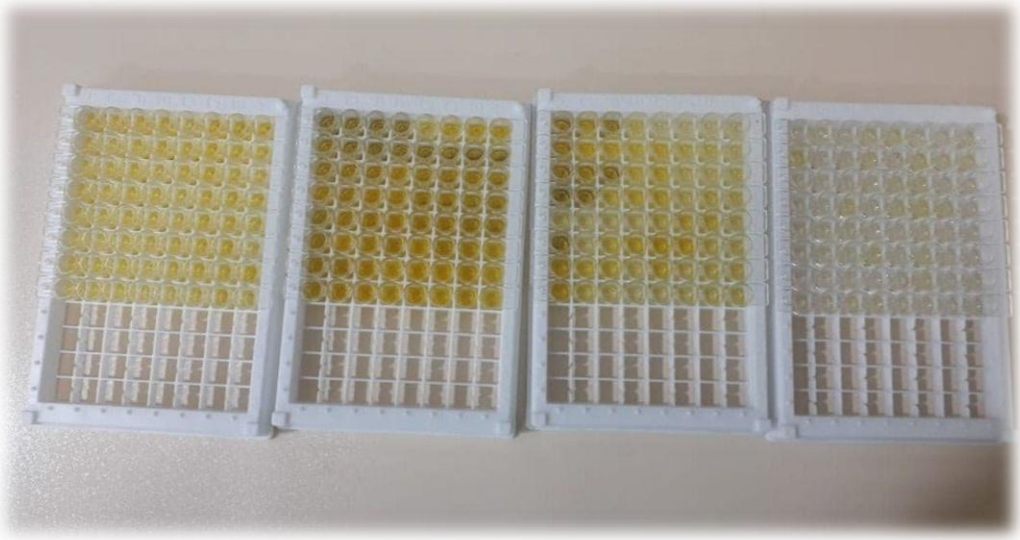

Figure (4-8): Interleukins Il-2,IL-10 and IL-6 in covid-19 patients

4.18. ROX and FAM in real time PCR

In table (4-8) show Rox and Fam curve in real time PCR for detection positive results for amplification RNA of covid-19 virus figure (4-2) show curve of positive results in nasal swab patients.

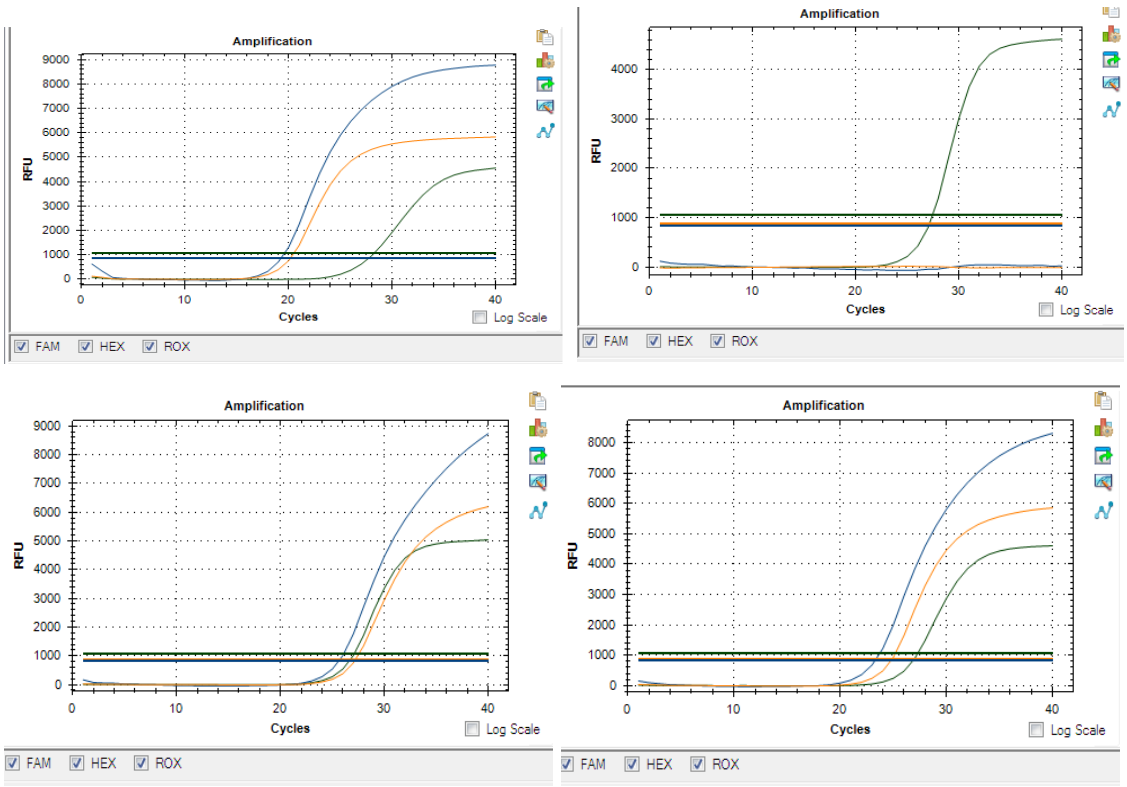

Figure (4-9): Curves of positive Amplification of RNA virus corona with ROX and FAM.

Table (4-8): ROX , FAM in real time PCR for positive results

Sample number	ROX	FAM
Sample one	15.91	13.01
Sample two	32.25	30.89
Sample three	27.99	26.33
Sample four	30.20	26.75
Sample five	26.11	23.70
Sample six	40.69	37.16
Sample seven	28.33	25.52
Sample eight	22.55	21.55
Sample nine	18.04	20.09
Sample ten	26.93	25.01
Sample eleven	29.45	28.02
Sample twelve	20.19	18.41
Sample thirteen	10.34	36.57
Sample fourteen	40.69	37.16
Sample fifteen	28.33	25.52
Sample sixteen	18.50	17.27
Sample eighteen	30.20	26.75
Sample nineteen	26.11	23.70
Sample twenty	15.91	13.01

Chapter Five

Discussion

5.1. Age group, Sex and Diseases of Covid-19 patients:

Data currently was been studied for patients with Covid-19 infection indicates the effect of age on the incidence rate. These findings are been found to have important clinical implications such as clinical management of Covid-19 patients and specific preventive measures, and since the beginning of the epidemic, age has been identified as a primary indicator for COVID-19 patients. Based on early statistical data for China [141]. The incidence rate increased significantly, reaching 62/14(22.6%) in people over (50-59) years old as shown in Table (4-1). The results of this study are consistent with the high susceptibility to infection and severe manifestations seen in the elderly. Selene G *et al* .,2020[142]. study age group of covid-19 patients was recorded a high rate in age (45-49)years was 52/108(4.55%) male more than female 50/276 (4.39%) .

Regarding to sex ,the highest infection rate was in males 62/33(53.2%) and lowest in women 62/29(46.8%), as shown in Table (4-2) and Figure (4-1). Chaolin H etal ., 2020[143]. was reported man was a high percentage 30(73%) and female 11(27%) and these similar to this results. Other diseases associated with covid-19 were recorded, the highest rate of hypertension 62/29 (46.8%), followed by diabetes 62/15(24.2%), as shown in Table (3-4), this study agreement with the previous study by Chen *et al*.,2020 [144]. In terms of age and gender, medical data were been assigned to 99 patients as of January 25, 2020. The patients' ages ranged from 21 to 82 years, with a mean age of (55.%) about (32%) of patients were female and (68%)were male. From this sample of patients, scientist Chen *et al* .,2020[145]. Differs in pathology, having studied most patients with other underlying cardiovascular disease. It was been found that elderly people who complain of weak immune systems are more likely to get infections. The percentage of sick women is also low as noted in this regard for a number of patients, and the protection of the natural female immune system from X chromosomes and sex hormones [144]. It believed to play a role.

A key role in fungi and adaptive immunity [146]. And as a result they are less susceptible to infection. with various infections. Hongdon ., 2020 [147]. Show associated between covid-19 patients and other diseases as hypertension and severity and morality and about 5260/1315 from covid-19 patients suffering from hypertension as risk fator for covid-10 and this agree with accurate study (Hongdon) . However, more observations and studies needed to confirm this relationship. While the study disagree in terms of age with the previous study of Tegan K.*et al*.,2020 where it found that the highest percentage of infected persons aged between (20-29) and (19.5%) [148]. Chaolin *etal* ., 2020: was study some diseases as diabetics 8(20%) and this disagree with this study[143].

5.2. Sign and symptoms associated Covid-19 disease:-

The results of the study showed for patients with Covid-19 through the clinical information of the infected, where the highest percentage of symptoms was found: fever (44.1%), followed by cough (32.2%), pneumonic (22%), and the lowest percentage of short breath (1.7%) as in table (4-4) .This is consistent with the study by Chen *et al*.,2020 [144], about symptoms Patients also have various clinical symptoms such as fever (83%) , cough (82%) , shortness of breath (31%) this study agree with accurate data . It was noted that (90%) of patients had more than one symptom, while (15%) had fever, cough and shortness of breath. In the same study, laboratory tests found a general decrease in lymphocytes, and this indicates that SARS-CoV-2 may primarily target cells, and in particular respiratory T lymphocytes, through diffusion through the mucosa, and these results from immune responses and negative changes in the respiratory system. Immunity. Thus, this potentially leads to acute respiratory distress syndrome (ARDS) and septic shock in some patients, eventually resulting in organ failure [143].Chaolin H *et al.*, 2020 was documented sign and symptoms as fever and a high rate 40(98%) and this agree with this study[143].

5.3. WBCs, Neutrophils, Monocytes , Platelets Lymphocytes and Hemoglobin with Covid-19 patients:-

The results of the study showed that the different blood cells of COVID-19 patients are at different levels. That is, WBC Mean ±Std. deviation (9.71±4.19) T.Test (18.25) , neutrophil (80.85±12.98), (51.81),Monocytes(4.67±2.54), (14.467) and lymphocyte (10.35±7.37), (11.03) all Statistical significance (p-vale 0.00) as in table (4-5) platelet (103.66±219.50), (16.674) and HGB (2.71±13.154),(38.14) all Statistical significance (p-vale 0.00) as in table (4-6). The study with Qin C *et al*.,2021[149]. Whose results showed that WBCs and Neutrophils had a significant upward curve, while Lymphocytes showed a declining trend with the exacerbation of the disease. This leads to a significant increase in the relevant parameters, NLR, NMR and PLR, and a significant decrease in LMR. She also confirmed that follow-up of NLR and lymphocyte subsets was useful in early critical Covid-19 screening, which is consistent with the findings of this study[150]. In early infection, B lymphocytes secrete antibodies that bind directly to the virus and thus destroy the virus. T lymphocytes can engulf the infected cells and thus eliminate the virus. In this process, a large number of lymphocytes are been onsumed. Aditya A *etal*., 2020[151] was study some immunity cells associated with covid-19 Neutrophils 64.92± 4284.27 lymphocytes 24.96± 17.14 ,and monocytes 6.86± 3.77 and these total counts of this immunity is increased with severity covid-19 and this is similar with yuan *etal* .,2020 reported agree and finding in sever cobva-19 patients[152]. Dodji Kossi Djakpo *et al*.;2020 Our analysis found a decreased HGB (124.84 + 15,877) P = 0.004. This study is consistent with our study[153].

5.4. LDH,CRP, D.dimer and Ferritin levels:

Found the cohort-based study of Covid-19 patients that specifically assessed different levels of age and mortality-related factors during the period of our

study of Covid-19 patients. It found that serum levels of D-dimers, CRP, LDH and Ferritin, and the absence of anticoagulants were factors independently associated with mortality in those with Covid-19. With regard to comorbidities and dependencies, mood, nutrition and mobility, ferritin Mean ±Std. deviation (488.32±903.22), T.Test (14.156), D. dimer (1.24±1.44), (6.781), LDH (173.39±22.67), (22.67) and CRP (35.26±43.45), (9.70),(38.14) all Statistical significance (p-vale 0.00) as shown in tabe (4-6).were found, and the study of these enigmatic factors was the basis. To confirm the conclusions, whereby by comparing laboratory results, it was found that there were significant differences in the levels of WBC, lymphocytes, neutrophils , CRP , D-dimer and LDH between the non-severe and severe groups. These indicators were very similar to those reported by Huang et al., 2019 [154]. LDH showed a strong association with other indicators by Pearson's correlation analysis, adding that LDH was a major factor associated with the risk of COVID-19 patients. When the body suffers from acute inflammation or hypoxia, the level of LDH in the serum rises dramatically. COVID-19, caused by SARS-Cov-2 infection, infects the lungs as well as other tissues and organs [155-156], and this leads to hypoxia, formation of inflammation, thromboembolism, and organ injury. In theory, an elevated serum LDH level confirms an important laboratory indicator for assessing COVID-19 [157]. Previous studies in chronic obstructive pulmonary disease (COPD) and community-acquired pneumonia (CAP) patients have also indicated that the D-dimer level is elevated in severe cases. Acute and potentially a prognostic biomarker [158-159-160], of excessive thromboembolic events in Covid-19 patients, this protective effect of anticoagulants combined with high serum levels of D-dimers explains to the associated vascular impairment and a possible direct effect covid-19 on normal endothelial cells. Calibration and differentiation. Other scores have been suggested to predict the risk of progression, [161] and D-dimer >1 μg/ml is a risk factor for death among adult patients with COVID-19 [162]. Recently, a study by Lee and colleagues

reported that acute COVID-19 patients had higher levels of ferritin in infected blood[163]. A logistic regression analysis indicated that serum ferritin level on admission was an independent risk factor for disease severity in COVID-19 patients. Also, lymphocyte counts and CRP were found to be two additional independent risk factors for disease severity by a multivariate logistic regression model. It was suggested that increased levels of ferritin in the blood predicted an increased risk of disease severity in COVID-19 patients. It found that the levels of ferritin in the blood have a positive relationship with the levels of CRP, and an inverse relationship with the number of lymphocytes. The study by Wu *et al.* Clinical outcomes in patients with COVID-19 pneumonia who developed acute respiratory distress syndrome (ARDS) demonstrated that increased serum ferritin levels was a risk factor associated with the development of acute respiratory distress syndrome [164]. Some have found hyperproteinemia to have a higher incidence of severe cases and a rate of bilateral pulmonary infiltration than patients without hyper ferritinemia. Patients complaining of COVID-19 were much older [165]. Additionally, these patients had higher LDH levels, lower levels of lymphocytes, and higher levels of inflammatory markers, such as d-dimer and CRP, than patients with COVID-19. in the non-hyperferritinemia group. These signs indicate that they are warning factors for severe or critical covid-19.Fillipopo B .,2021 was recorded ferritin level in covid-19 patients 674(1284%) ng/ml and non covid-19 patients was less than 200 ng/ml and this agree with accurate study[166] .Ian H., 2019was study some associated with sever covid-19 patients as elevated of CRP 184 (1.45.2-33) an CRP $\geq$ 10mg/L has a sensitivity 51% ,88 specify and D dimer > 0.5 mg/L has a sensitivity 58% and 69% specify and a high serum ferritin was 0.90(0.64, 1.15) with p< 0.0001[167].

5.5. Interleukin 2 , IL-6 and IL-10 levels in Covid-19 :

The results of the study showed that the COVID-19 patients stormed cytokines at different levels. High levels of cytokine storming have found to pose an independent risk to those with severe or acute injuries. Where our study found an increase in the proportion of interleukin 2 and 6 in the patient's blood serum. . IL-2 Mean ±Std. deviation (214.33±169.08) T.Test (9.98) , IL-6 (537.24±196.78), (21.49) while IL-10 had very low levels all Statistical significance (p-vale 0.00) as shown in in the table (4-7). Sujan K. Dar *et al*., 2021 was studied COVID-19 patients with IL-2 (0.12) p-vale(0.281) and IL6 (0.63) pvale(0.0001) and this is in agreement with this study. Interleukin-10 also showed a (0.65) pvale (<0.0001) and this is inconsistent with accurate study [168]. Also this study disagree with the researcher Hongbo Shi *et al*.; 2020 in terms of IL-2 and IL-10, and the study is similar to IL-6 and confirmed in his study IL-2 has a low concentration in plasma, and thus causes a significant decrease in lymphocytes and CD8 + T cells in acute patients with COVID-19 pneumonia, who found in his study that the total number of natural killer cells, T cells and B cells decreased significantly in severely injured patients. Attributed to the latest teachings of the new Covid-19 pneumonia, a gradual decrease in the number of lymphocytes in the peripheral blood is one of the clinical early warning signs of critically ill elderly patients. On the decision of previous studies [169], secretion of cytokines has an important role in the development and differentiation of immune cells. Several studies have shown that cytokine storms may lead to multiple organ failure and death in critically ill patients with COVID-19 pneumonia [170-171]. Also, his study showed elevated inflammatory cytokines such as IL-6 and IL-10 in critical patients with COVID-19 pneumonia. Differently, this study likened that IL-2 was elevated in acute patients but decreased in critical patients with COVID-19 pneumonia Liang C etal .,2021 show the lower levels of IL-10 or decreased in serum covid -19 patients and recorded 0.985 with p-value(0.0173) this study agree with accurate

study (Liang) . Therefore interleukins 2,6,10 as a predictive for higher level of cytokine storm syndrome and severity of covid-19. Liang cheng *et al*.,2021.show the lower levels of IL-10 in covid-19 patients as IL-10 was recaedel 0.9859 with pvale 0.0173.This study agree with accrnet study[172].

Conclusion and Recommendation

Conclusion

1- The current study is a first study in Misan governorate well as registration of biomarker and immune marker for indication cytokine storm syndrome.

2- Age group was more infected with covid-19 range from (50-59) year was reported 14(22.6%) and male more infected with covid-19 than female 33(53.2%).

3- Hypertension patients was more infected with covid-19 patients and enter cytokine storm syndrome 29(46.8%) .

4-All biomarker parameter as LDH, ferritin, D-dimer ,Hemoglobin ,Interleukins which was elevated in all cases of covid-19 patients with cytokine storm syndrome.

5- Concentration of virus associated with severe infection of covid-19 .

Recommendation

1-Further steps can applied on studied other interleukins as IL-8,Il-2,IL-13 IL-17 and related with IL-10 in covid-19 patients .

2- Determine a gene, which responsible on cytokine storm syndrome.

3- Study co-infection and bacterial and fungi secondary infection in covid-19 patients.

4- Study corona virus as molecular levels and determine transgenic type in Iraq.

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Appendix

Appendix (1)

Name				
SIX				
Age				
Disease	Diabetic	Heart	Cancer	

Sing and symptom							
Fever		**Headache**		**Short breath**		**Cough**	
Pneumonic		**Lost teeth**		**Lost of hear**		**Lost weight**	
Other							

Number of infection with COVID-19			
Once		**More that one**	

Treatment							
Azithromycin		**Zinc**		**Vit D**	**Vit C**		**Other**	

Serum:- cytokine	
Interleukin-2(IL-2)	
Interleukin-6(IL-6)	
Interleukin-10(IL-10)	

Nasal swab Rt-pcr concentration of virus	
Postive test	
Negative test	

Time patient infection							
One week		Two week		Three week		Four week	

خلاصه :

سابقه و هدف: بیماری همه گیر COVID-19 یک عامل عفونی مداوم است. ویژگیهای ایمونولوژیکی تعامل بین میزبان و ویروس. سطح بالایی از سایتوکاین ها مانندIL-2 ، IL-6 ، IL-10 و سایر سلول های ایمنی را در افراد آلوده که نقش مهمی در فیزیولوژی دارند ، تحریک می کند.

مواد و روش ها: نمونه ها (خون و سواب) برای (۱۰۰) نمونه از فوریه تا ژوئن ۲۰۲۱ جمع آوری شد که از بیماران آلوده یا مبتلا به کروناویروس در بیمارستان های آموزشی السدر و بیمارستان المجر الکبیر جدا شده بودند.

نتایج: تعداد (۱۰۰) نمونه (سواب و خون) بین افراد آلوده و غیر آلوده به کووید -۱۹. مشخص شد که بیشترین درصد گروه های سنی (۵۹-۵۰) (۱۴.۶٪) ۱۴ بیمار و درصد مردان بیشتر از زنان (۵۳.۲٪) در ۳۳ بیمار بود. همچنین بیشترین درصد بیماریهای فشاری (۴۶.۸٪) ظاهر شد. این مطالعه علائم مربوط به عفونت را در سطوح مختلف نشان داد که بالاترین درجه حرارت (۴۴.۱٪) بود ، در حالی که آزمایشات آزمایشگاهی برای سرم خون نشان داد که سایتوکاین ها در بیماران حاد و حاد در سطوح مختلف یعنی .IL-2 Mean ± Std انحراف (۲۱۴.۳۳ ± ۱۶۹.۰۸ (، T.Test (9.98)، IL-6 (53.7.24 ± 196.78)، (21.49) در حالی که IL-10 سطوح بسیار پایینی داشت. این مطالعه همچنین تجزیه و تحلیل سلول ایمنی ، WBC Mean ± Stdرا نشان داد. انحراف (۹.۷۱ ± ۴.۱۹ T.Test (18.25) (، نوتروفیل (۸۰.۸۵ ± ۱۲.۹۸) ، (۵۱.۸۱) ، مونوسیت ها (۴.۶۷ ± ۴.۶۷) ، (۱۴.۴۶۷) و لنفوسیت ها (۷.۳۵ ۱۰. ۱۰.۳۵) ، (۱۱.۰۳). تجزیه و تحلیل سرولوژی فریتین میانگین .Std ± انحراف (۴۸۳.۳۲ ± ۹۰۳.۲۲ T.Test (14.156) (، D.dimer (1.24 ± 1.44)، (6.781)، LDH (17.3.39 ± 22.67)، (22.67)، CRP (35.26 ± 43.45)، (9.70)، پلاکت (۱۰۳.۶۶ ± ۲۱۹.۵۰) ، (۱۶.۶۷۴) و HGB (2.71 ± 13.154) ، .(38.14)

بحث: مطالعه نشان داد طوفان سیتوکین در بیماران COVID-19 ، با سطوح مختلف برای بیماران مبتلا به عفونت شدید و حاد ، مطالعه ماIL-2 ، IL-6را با سطوح بالا ، در حالی که IL-10 با سطوح پایین به دلیل ناراحتی تنفسی التهابی تأیید کرد. کووید -۱۹.

کلیدواژه ها: COVID-19 ، IL-2 ، IL-6 ، IL-10 ، RT-PCR، سندرم طوفان سایتوکاین.