Republic of Iraq
Ministry of Higher Education
and Scientific Research
University of Karbala
College of Medicine
Department of Microbiology

Association of IL-6 and TNF alpha levels

With hematological and biochemical parameters in treated COVID-19 patients

A Thesis

Submitted to the Council of the College of Medicine, University of Karbala in Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Microbiology

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2021 AD **1443 AH**

بسم الله الرحمن الرحيم

{وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّى وَمَآ أُوتِيتُم مِّن الْعِلْمِ إِلاَّ قَلِيلاً}

صدق الله العلي العظيم

سورة الاسراء (85)

Supervisors Certification

We certify that this M.Sc. thesis titled:

"Association of IL-6 and TNF alpha levels

With hematological and biochemical parameters in treated CODID-19 patients"

Was prepared under our supervision in the College of Medicine/ University of Karbala, as a partial fulfillment of the requirements for the Degree of Master of Science in Medical Microbiology.

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DEDICATION

To those who have devoted their life to provide support and wasted hours of their rest time in order allow me to complete my theses….. For my family I dedicate this effort.

And I do not forget….. My Supervisors that gave all the support and gave the fruit of their effort to me.

And to those whose memory remains etched in the White Army, to all of the above- mentioned I dedicate my theses.

Fatima Turky

ACKNOWLEDGMENTS

I would like to thank Almighty God for helping me to work in fulfilling the requirements of this research.

I would like to express my deepest gratitude and sincere thanks to my supervisors **Professor Dr. Mohaned Mohsen Ahmed** and Assistant **Professor Dr. Khalid Khalil Al-Aaraji** for their great interest, kind support and invaluable advice.

Thanks and appreciation go to the Department of Microbiology and Immunity, especially **Dr. Sawsan M. AL-Hasnawi**, Head of the Department.

Thanks and appreciation go to **Dr. Rasha Kahtan Al-Kazaly** (Immunologist) at the Immunity laboratory in Al-Hussein Medical city hospitals.

Thanks and appreciation go to the staff of Al-Hussein Medical city hospitals for helping me in the sample collection.

Thanks and appreciation go to students of the previous batch of master for their advice which is help me to complete my study.

All thanks and gratitude go to COVID-19 patients and their parents of their contribution to the study.

It is a great pleasure to thank everyone who helped me to write my thesis successfully

Summary

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

COVID-19 is characterized by a cytokine storm manifested by elevation in the levels of many inflammatory and biochemical markers and changes in the blood indices.

The aim of this study was assess the role of IL-6 and TNF-alpha levels with other hematological and biochemical parameters in disease status in COVID-19 patients who are on treatment.

For this purpose a total of 84 patients with COVID- 19- confirmed by SARS-COV-2 –specific RT-PCR of Nasopharyngeal (NP) swab specimen were enrolled in this study. The patients were attended to Al-Hussein Medical city hospitals for the period from 10-10-2020 to 29-12-2020.

Among them 54 (64.3%) were male and 30(35.7%) were female. The mean age was 56.08 years and the age range was between 25 to 85 years, these results indicate that older male patients are at more risk.

Total white blood cell counts was significantly increased in moderate and severe cases (p=0.000) compared to mild cases. Furthermore neutrophil percentages were significantly increased (p=0.000), whereas lymphocyte percentages were decreased (p=0.001) in moderate and severe patients in comparison to mild patients.

The most of the biochemical markers in this study, namely; D-dimer, serum ferritin, Lactate dehydrogenase, C-reactive protein, Alanine aminotransferase, Aspartate aminotransferase were shown to be positively

correlated with severity of the COVID-19 symptoms (r=0.259, *p*=0.018; r=0.264, *p*=0.019; r=0.441, *p*=0.000; r=0.317, *p*=0.003; r=0.225, *p*=0.049; r=0.234, *p*=0.033, respectively).

The levels of both IL-6 and TNF-α were elevated in COVID-19 patients and this raise in the levels were higher in moderate and severe patients compared to mild patients (*p*=0.20, *p*=0.07, respectively).

Within the severe COVID-19 patients, IL-6 serum levels were negatively correlated with lymphocyte percentages (r=-0.36, *p*=0.06) and positively correlated with neutrophil percentages (r=0.482, *p*= 0.009). On the other hand, within the mild COVID-19 patients, TNF-α serum levels were negatively correlated with lymphocyte percentages (r=-0.44, *p*=0.03) and with hemoglobin percentages (r=-0.415, *p*=0.031).

IL-6 serum levels were positively correlated with serum ferritin (r=0.286, *p*=0.011) and negatively correlated with Aspartate aminotransferase (r=-0.224, *p*=0.05). However, TNF-α serum levels did not show significant correlation with the studied biochemical markers in the COVID-19 patients. Nevertheless, TNF-α was positively correlated with D. dimer within the severe COVID-19 patients (r=0.406, *p*=0.039).

In conclusion, all studied biomarkers and cytokines are elevated and there were hematological changes in COVID-19 patients, however, it cannot attribute all the changes in the biochemical and hematological parameters to the elevated levels of IL-6 and TNF-α. Therefore, this study recommend studying other proinflammatory cytokines.

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List of Abbreviations

Code	Words
ACE2	Angiotensin-Converting Enzyme 2
ALP	Alkaline phosphatase
APCs	Antigen Presenting Cells
ARDS	Acute respiratory distress syndrome
CBC	Complete blood count
$CD14^+$	Clusters for differentiation 14
$CD16^+$	Clusters for differentiation 16
COVID-19	Coronavirus disease 2019
CRP	C-reactive protein
CTLs	Cytotoxic T lymphocytes
EDTA	Ethylene Di amine Tetra Acetic Acid
ELISA	Enzyme –Linked Immunosorbent Assay
ERGIC	Endoplasmic Reticulum-Golgi Intermediate Compartment
G-CSF	Granulocyte- colony stimulating factor
GM-CSF	Granulocyte Macrophage-colony stimulating factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GOT	Aspartate aminotransferase
GPT	Alanine aminotransferase
GSK3	Glycogen-Synthase-Kinase-3
HLA	Human Leukocyte Antigen

HRP	**Horse Radish Peroxidase**
IFN	**Interferon**
IL-1β	**Interlukin-1beta**
IL-6	**Interleukin-6**
LDH	**Lactate dehydrogenase**
MERS	**Middle East Respiratory Syndrome**
MHC II	**Major histocompatibility complex class II**
MHC1	**Major Histocompatibility Complex class1**
NF-κB	**Nuclear factor-κB**
NSP	**Non-structural protein**
OD	**Optical density**
PCR	**Polymerase Chain Reaction**
RBD	**Receptor-binding domain**
RNP	**Rib nucleoprotein**
SARS-CoV-2	**Severe acute respiratory syndrome coronavirus 2**
SPO2	**Saturation of Peripheral Oxygen**
SPSS	**Specific Software Statistical Package for the Social Sciences**
Th1 cell	**T-helper 1 cell**
TLRs	**Toll-like receptors**
TMB	**Tetra methyl benzidine**
TNF-α	**Tumor necrosis factor alpha**
WHO	**Word Health Organization**

Chapter One
Introduction
&
Literature
Review

1.1 Introduction:

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It causes serious respiratory illness such as pneumonia and lung failure and it was first reported in Wuhan, the capital of Hubei, China (Ahn *et al*., 2020).

In March, 2020, the World Health Organization (WHO) declared that the outbreak of coronavirus disease 2019 (COVID-19) has become a global pandemic. Currently, the virus is spreading in almost all continents (Chen *et al*., 2020).

This virus is belongs to the subfamily Coronavirinae in the family Coronaviridae and the order Nidovirales (Cong *et al*., 2017). Coronaviruses are enveloped viruses with a single-strand, positive-sense RNA genome approximately 26–32 kb in size, which is the largest known genome among all RNA viruses. The term 'coronavirus' refers to the appearance of coronavirus virions considering the protruding spike proteins on their surface that look like a crown under electron microscopy ("*corona*" means crown) (Zhao *et al*., 2020).

SARS-CoV-2 is spread primarily via respiratory droplets during close face-to-face contact. Infection can be spread by asymptomatic, pre symptomatic, and symptomatic carriers (Wiersinga *et al*., 2020).

Entry of SARS-CoV-2 depends on the binding of S proteins covering the surface of the virion to the cellular ACE2 receptor (Zhou *et al*., 2020). After entering respiratory epithelial cells, SARS-CoV-2 provokes an immune response with inflammatory cytokine production accompanied by a weak interferon (IFN) response. This is followed by the infiltration of macrophages and neutrophils into the lung tissue, which results in a cytokine storm (Hussman, 2020).

Particularly, SARS-CoV-2 can rapidly activate pathogenic Th1 cells to secrete pro-inflammatory cytokines, such as granulocyte-macrophage colony-stimulating

factor (GM-CSF) and interleukin-6 (IL-6). GM-CSF further activates $CD14^{+}CD16^{+}$ inflammatory monocytes to produce large quantities of IL-6, tumor necrosis factor-α (TNF-α), and other cytokines **(** Zhou *et al*., 2020**).**

These inflammatory mediators further damage the epithelial cells lining and reach into the blood circulation where it causes damage to other organs (Rothan & Byrareddy, 2020).

Interleukin -6 (IL – 6) is an important member of the cytokine network and plays a central inflammation (Zhang *et al*., 2020).

IL-6 can be produced by almost all stromal cells and immune system cells, including B-lymphocytes, T-lymphocytes, macrophages, monocytes, dendritic cells, mast cells, and other non-lymphocytic cells such as fibroblasts, endothelial cells, keratinocytes, glomerular mesangial cells and tumor cells(Jones & Jenkins, 2018).

More evidence suggest that critically ill patients with severe respiratory failure and SARS-CoV-2 have either immune dysregulation or macrophage-activation syndrome, both of which are characterized by pro-inflammatory cytokines. The immune dysregulation, in particular, is driven by the Interleukin-6 (IL-6) and not by Interleukin-1beta (IL-1beta)(Giamarellos-Bourboulis *et al.*, 2020).

Two key features of this immune dysregulation are: over-production of pro-inflammatory cytokines by monocytes and lymphocyte dysregulation with CD4 lymphopenia (Giamarellos-Bourboulis *et al.*, 2020).

Tumor necrosis factor (TNF)-α, an important member of the TNF superfamily of ligands, is a pleiotropic pro-inflammatory cytokine(Lai *et al*., 2016).

TNF-α in some diseases like influenza and COVID-19 are associated with lung injuries. Studies have demonstrated that high levels of TNF- α are observed in plasma and alveolar fluid lavage of patients with ARDS. Elevated levels of cytokines lead to increased endothelial permeability and decreased alveolar fluid clearance caused by down-regulation of sodium channels in the epithelium (Feldmann *et al.*, 2020).

1.1.1 Aim of the study:

This study aimed to assess the role of IL-6 and TNF-alpha levels with other hematological and biochemical parameters in disease status on response to treatment of SARS-COV-2 patients.

1.1.2 Objectives:

1. To collect blood samples from COVID-19 patients with different states of severity.
2. To perform hematological and biochemical analyses on blood samples.
3. To measure the serum levels of IL-6 and TNF-alpha levels in the COVID-19 patients.
4. To study the possible correlations between the levels of IL-6 and TNF-alpha with different hematological and biochemical markers.

1.2 Literature review:

1.2.1 COVID-19 disease:

In December 2019, Coronavirus Disease 2019 (COVID-19), a fatal zoonotic disease, occurred in Wuhan, Hubei Province, China. The disease which was caused by 2019 novel coronavirus (2019-nCoV) has rapidly spread. The pathogen could cause severe respiratory syndrome, including fever, dyspnea, and cough (Zhu *et al.*, 2020). Along with other systematic damage like acute cardiac or kidney injury(Rodriguez-Morales *et al.*, 2020).

Severe illness, of almost any etiology, is accompanied by a generalised host inflammatory response. This host immune response process is referred to as systemic inflammatory response syndrome. If this process is not controlled or is dysfunctional, it will lead to cytokine storm syndrome (Chousterman *et al.*, 2017).

Cytokine storm is one of the possible mechanisms underlying rapid disease progression(Mehta *et al*., 2020).

The COVID-19 risk is greater in older people, kids and the patients having other health problems like lung diseases, heart diseases, diabetes, and cancer (Carlos *et al*., 2020).

1.2.2 Epidemiology:

Global numbers of cases and deaths continued to decrease over the past week (14-20 June 2021) with over 2.5 million new weekly cases and over 64 000 deaths, a 6% and a 12% decrease respectively, compared to the previous week. While the number of cases reported globally now exceeds 177 million, last week saw the lowest weekly case incidence since February 2021. This week, the Americas and Western Pacific Regions reported numbers of new weekly cases similar to the previous week, while the South-East Asia and the European Regions reported a decline in the

number of new cases. The African Region recorded a marked increase in the number of weekly cases as compared to the previous week. Globally, mortality remains high with more than 9000 deaths reported each day over the past week, however, the number of new deaths reported in the past week decreased across all Regions except for the Eastern Mediterranean and the African Regions (Organization, 2021).

1.2.3 Transmission:

Many domestic and wild animals, including camels, cattle, cats, and bats, may serve as hosts for coronaviruses (Lin *et al.*, 2020).

However, there are exceptions, such as SARS and MERS, which are mainly spread though close contact with infected people via respiratory droplets from cough or sneezing. With regard to COVID-19, early patients were reported to have some link to the Hunan Seafood Market in Wuhan, China, suggesting that these early infections were due to animal-to-person transmission. However, later cases were reported among medical staff and others with no history of exposure to that market or visiting Wuhan, which was taken as an indication of human-to-human transmission (Liu *et al.*, 2020).

Respiratory viruses are transmitted in three main ways;

1. Contact transmission, where someone comes into direct contact with an infected person or touches a surface that has been contaminated.
2. Through droplet transmission of both large and small respiratory droplets that contain the virus, which would occur when near an infected person.
3. Through airborne transmission of smaller droplets and particles that are suspended in the air over longer distances and time than droplet

transmission. However, latest research suggests that this is unlikely to be a major route of transmission as although SARS-CoV-2 can persist for days on inanimate surfaces, attempts to culture the virus from these surfaces were unsuccessful. Infection control guidelines have stated that most respiratory virus transmission occurs from large infected droplets produced by coughing, sneezing, and breathing in close proximity to another person. This understanding has led to social distancing being the cornerstone of public health advice, but confusion exists as to the safe distance required between people to reduce transmission with the WHO suggesting 1 meter (Medicine, 2020).

1.2.4 Classification of coronavirus:

Coronaviruses belong to the subfamily *Coronavirinae* in the family *Coronaviridae* and the order *Nidovirales*. *Coronaviridae* are further subdivided phylogenetic ally into five genera:

1. *Alpha coronavirus*
2. *Beta coronavirus*
3. *Gamma coronavirus*
4. *Delta coronavirus*
5. *Mu coronavirus*

Alpha coronaviruses and *Beta coronaviruses* are found in mammals, whereas *Gamma coronaviruses* and *Delta corona viruses* are primarily found in birds. International Committee of Taxonomy of Viruses (ICTV) in 2018 claimed that *Beta coronavirus* lineage was reclassified into five subgenera, namely *Embecovirus*, *Sarbecovirus*, *Merbecovirus*, *Nobecovirus*, and *Hibecovirus*.HCoV-229 E in the subgenus *Duvinacovirus* and HCoV-NL63 in the

subgenus *Setracovirus* belong to the *Alpha coronavirus* genus. HCoV-HKU1 and OC43 in the subgenus *Embecovirus*, MERS-CoV in the subgenus *Merbecovirus*, SARS-CoV and SARS-CoV-2 in the subgenus *Sarbecovirus* belong to the genus *Beta coronavirus* (Cong *et al.*, 2017).

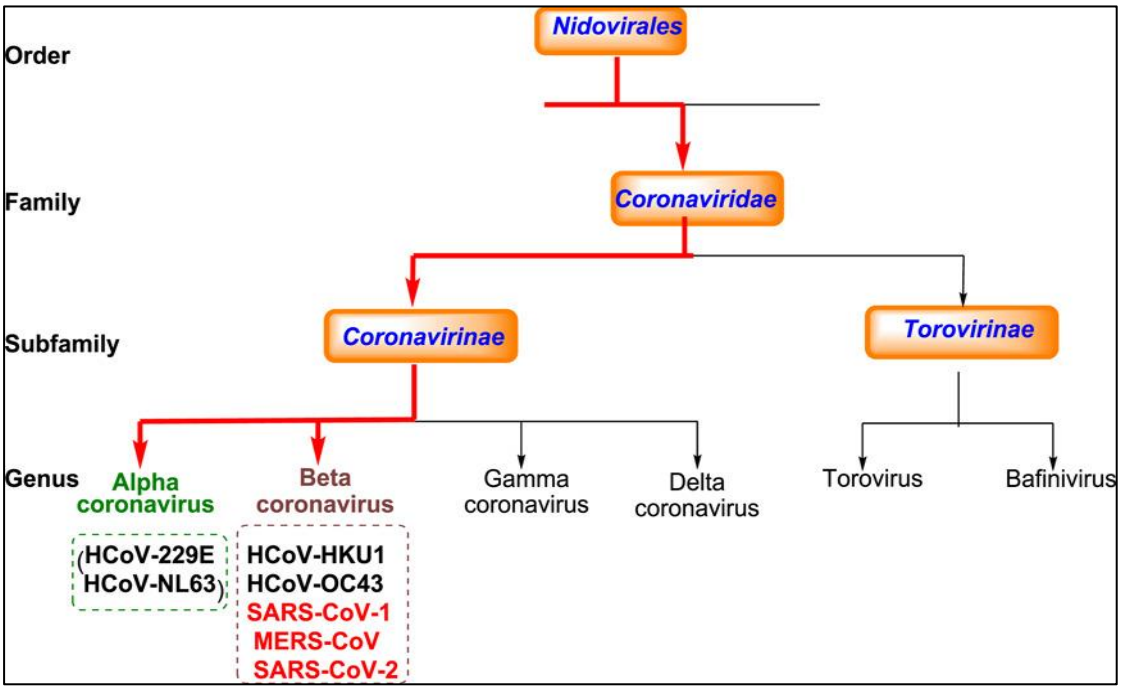

Figure 1.1: Coronaviruses classification (Pillaiyar *et al.*, 2021).

1.2.5 Coronaviruses structure:

Coronaviruses (CoVs) are enveloped viruses with a single-strand, positive-sense RNA genome approximately 26–32 kb in size, which is the largest known genome among all RNA viruses. The term 'coronavirus' refers to the appearance of CoV virions considering the protruding spike proteins on their surface that look like a crown under electron microscopy ("*corona*" means crown). The first coronavirus is an infectious bronchitis virus and was isolated from chicken embryos in 1937, along with

subsequent viral isolations in rodents, domestic animals, and humans. Coronaviruses have been identified in many mammalian animals including humans and avian species and can induce various severe diseases involving respiratory, gastrointestinal, enteric, and neurological systems (Zhao, *et al*, 2020).

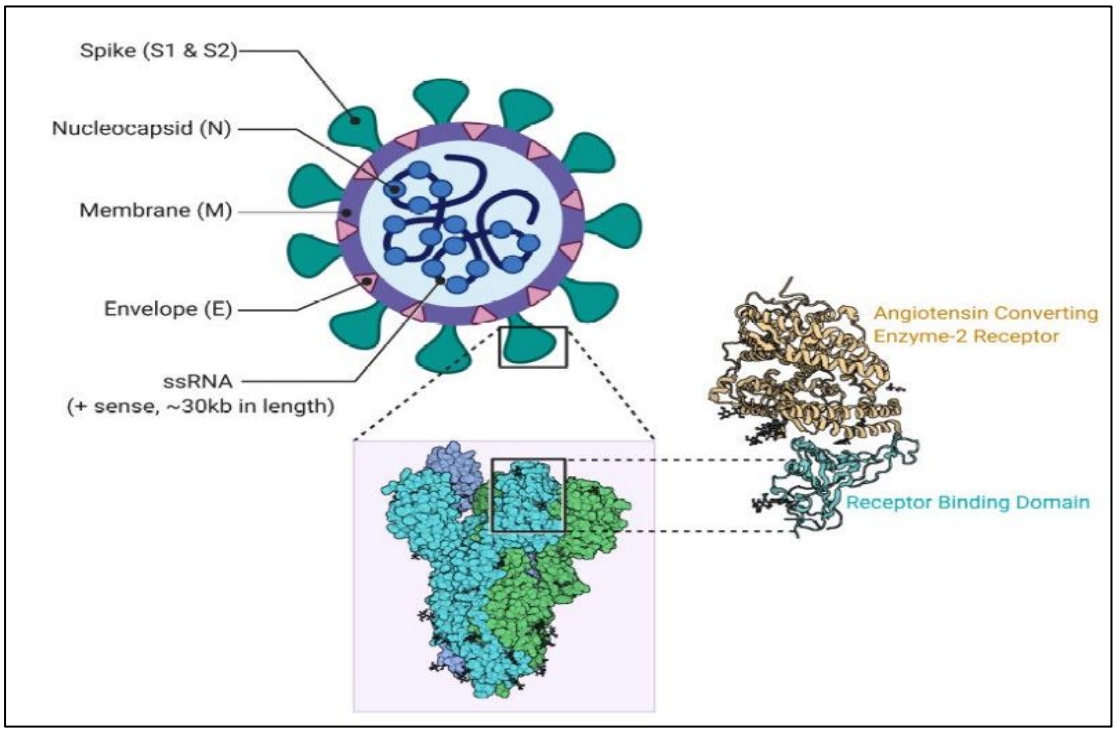

Figure 1.2: Coronavirus structure (Cascella *et al*., 2021).

1.2.5.1. Spike (S) protein:

The coronavirus spike (S) protein is a large glycosylated transmembrane protein ranging from about 1452 to 1162 amino acid residues The S protein is the most outward envelope protein of the coronaviruses. The S glycoprotein plays critical roles in mediating virus attachment to the host cell receptors and facilitating fusion between viral and host cell (Hulswit *et al*., 2016).

1.2.5.2. Membrane (M) protein:

Coronavirus M proteins represent the major protein component of the viral envelope. They play an essential role during viral assembly by interacting with all of the other structural proteins. Its length ranges from 217 to 230 amino acid residues in most coronaviruses. (Perrier *et al*.,2019).

1.2.5.3. Envelope (E) protein:

The envelope (E) protein is a small integral membrane polypeptide, ranging from 76 to 109 amino acid residues with molecular weight of 8.4–12 k Da. The E protein plays important roles in a number of aspects of the coronavirus replication cycle, such as assembly, budding, envelope formation, and pathogenesis (Schoeman & Fielding, 2019).

1.2.5.4. Nucleocapsid (N) protein:

The coronavirus nucleocapsid (N) protein is a structural phosphoprotein of 43–46 K Da, a component of the helical nucleocapsid.

The main function of the N protein is to package the viral genome into a ribonucleoprotein (RNP) particle in order to protect the genomic RNA and for its incorporation into a viable virion. The N protein is thought to bind the genomic RNA in a beads-on-a-string fashion. In addition, it also interacts with the viral membrane protein during virion assembly and plays a critical role in improving the efficiency of virus transcription and assembly (Artika *et al*., 2020).

1.2.6 Replication of coronaviruses:

1.2.6.1 Attachment and entry:

Several steps are necessary to start and complete the coronavirus infective cycle:

1. Recognition and binding to the cellular receptor(s).
2. Changes in the conformation and proteolysis of S protein.
3. Fusion to cellular membrane.
4. Entry of the virus into the host cells by endocytosis**.**

The genome of the coronavirus encodes a number of structural proteins that facilitate cellular entry and assembly of virions, of which the spike protein S appears to be critical for cellular entry. The spike protein guides the virus to attach to the host cell. The spike protein contains a receptor-binding domain (RBD), a fusion domain and a transmembrane domain. The RBD of spike protein S binds to Angiotensin Converting Enzyme 2 (ACE2) to initiate cellular entry (Pillay, 2020).

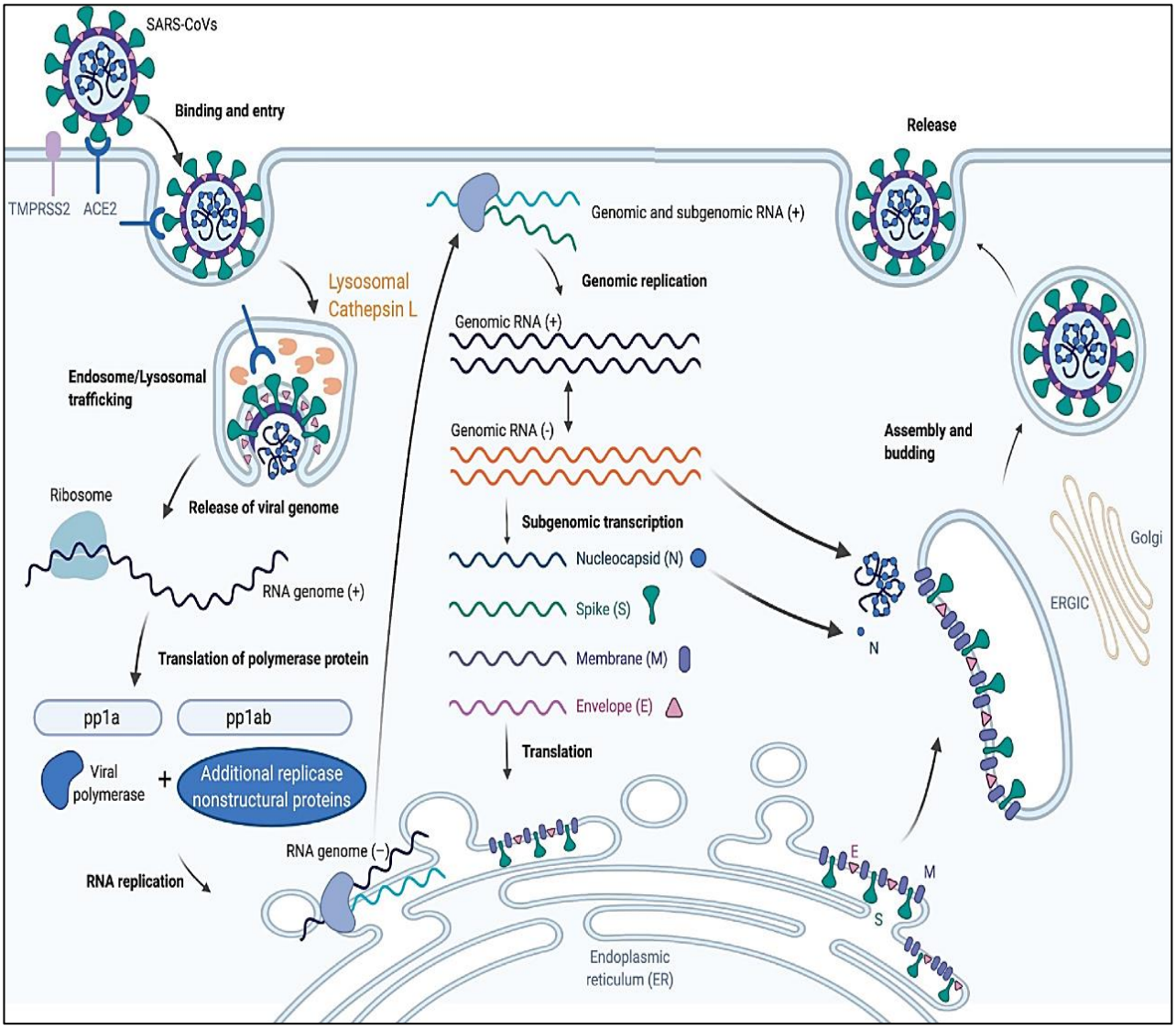

Figure 1.3: Life cycle of coronaviruses (Harrison *et al*., 2020).

1.2.6.2 Genome replication/transcription and virion assembly and release Virus:

After entry, genomic RNA (g RNA) is translated by host ribosomes in poly protein pp1a and pp1b, which are auto-cleaved to form non-structural protein (NSP). These NSPs induce a rearrangement of cellular membrane to form double-membrane vesicles where the viral replication complexes are anchored (Russo *et al*., 2020).

Using the g RNA as a template, the coronavirus replicase synthesizes full-length negative sense (−) RNA, which, in turn, serves as a template for the synthesis of new genomic (+) g RNA and a set of different sg RNA, synthetized by discontinuous transcription. These sg RNAs encode viral structural and accessory proteins (Godman, 2020)**.**

Although genome replication/transcription is mainly mediated by the viral replicase, other host factors have been involve, as an example, coronavirus N protein, known to act as an RNA chaperone to facilitate template switching, and the enzyme glycogen-synthase-kinase-3 (GSK3)(Fung & Liu, 2019).

Finally, RNA helicases (NSP13) represent the second most conserved subunit of the RNA synthesis machinery in (+) RNA coronaviruses and are involved in diverse steps of their life cycle. They utilize the energy derived from the hydrolysis of nucleoside triphosphates to unwind double-stranded RNA(Russo *et al*., 2020).

The assembly of viral particles takes place in the ER-Golgi intermediate complex under the control of M protein through homotypic interactions. In this phase, M protein acts as a scaffold for virus assembly because the interactions between S and M and M and N proteins allow the recruitment of structural proteins to the assembly site. E protein contributes in this phase interacting with M and inducing membrane curvature(Fung & Liu, 2019).

Finally, mature virions are released in smooth-walled vesicles via the secretory pathway and released by exocytosis(Russo *et al*., 2020)**.**

1.2.7 Pathogenesis of COVID-19:

1.2.7.1 Coronavirus entry and replication:

Coronavirus S protein has been reported as a significant determinant of virus entry into host cells (De Wit *et al*., 2016).

The envelope spike glycoprotein binds to its cellular receptor, ACE2 for SARS-CoV. The entry of SARS-CoV into cells was initially identified to be accomplished by direct membrane fusion between the virus and plasma membrane (Li *et al*., 2020).

This a critical photolytic cleavage event occurred at SARS-CoV S protein at position (S2′) mediated the membrane fusion and viral infectivity (Millet & Whittaker, 2014).

Besides membrane fusion, the clathrin-dependent and -independent endocytosis mediated SARS-CoV entry too. After the virus enters the cells, the viral RNA genome is released into the cytoplasm and is translated into two poly proteins and structural proteins, after which the viral genome begins to replicate (Li *et al*., 2020.)

The newly formed envelope glycoproteins are inserted into the membrane of the endoplasmic reticulum or Golgi, and the nucleocapsid is formed by the combination of genomic RNA and nucleocapsid protein. Then, viral particles germinate into the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). At last, the vesicles containing the virus particles then fuse with the plasma membrane to release the virus (De Wit *et al.*, 2016).

1.2.7.2 Antigen presentation in coronavirus infection:

While the virus enters the cells, its antigen will be presented to the antigen presentation cells (APC), which is a central part of the body's anti-viral immunity.

Antigenic peptides are presented by major histocompatibility complex (MHC); or human leukocyte antigen (HLA) in humans and then recognized by virus-specific cytotoxic T lymphocytes (CTLs).

Hence, the understanding of antigen presentation of SARS-CoV-2 will help our comprehension of COVID-19 pathogenesis. Unfortunately, there is still lack of any report about it, and we can only get some information from previous researches on SARS-CoV and MERS-CoV. The antigen presentation of SARS-CoV mainly depends on MHC I molecules, but MHC II also contributes to its presentation (Li *et al.*, 2020).

1.2.7.3 Humoral and cellular immunity:

Antigen presentation subsequently stimulates the body's humoral and cellular immunity, which are mediated by virus-specific B and T cells. Similar to common acute viral infections, the antibody profile against SARS-CoV virus has a typical pattern of IgM and IgG production. The SARS-specific IgM antibodies disappear at the end of week 12, while the IgG antibody can last for a long time, which indicates IgG antibody may mainly play a protective role (Li *et al*., 2020).

Comparing to humoral responses, there are more researches on the cellular immunity of coronavirus. The latest report shows the number of $CD4^+$ and $CD8^+$ T cells in the peripheral blood of SARS-CoV-2-infected patients significantly is reduced (Xu *et al*., 2020).

1.2.7.4 Cytokine storm in COVID-19:

Cytokine storm is a hyper-inflammatory, pathological state that results from a sudden increase in certain circulating pro-inflammatory cytokine levels, which leads to overwhelming systemic inflammation, exacerbating viral pathogenesis and causing sepsis, ARDS, and multi-organ failure(Chousterman *et al*., 2017).

The cytokine storm has also been observed in SARS, MERS, H5N1 influenza, and H7N9 influenza, and with other respiratory viruses (Thepmankorn *et al*., 2020).

In severe SARS-CoV-2 infection, the total T cell counts as well as CD4+ and CD8+ T cell counts were all significantly lower than that in more moderate cases(Chen *et al.*, 2020).

1.2.7.5 Mechanisms of cytokine storm in covid-19:

After entering respiratory epithelial cells, SARS-CoV-2 provokes an immune response with inflammatory cytokine production accompanied by a weak interferon (IFN) response, this is followed by the infiltration of macrophages and neutrophils into the lung tissue, which results in a cytokine storm (Hussman, 2020).

Particularly, SARS-CoV-2 can rapidly activate pathogenic Th1 cells to secrete pro-inflammatory cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-6 (IL-6). GM-CSF further activates $CD14^{+}CD16^{+}$ inflammatory monocytes to produce large quantities of IL-6, tumor necrosis factor-α (TNF-α), and other cytokine. Neutrophil extracellular traps, the extracellular nets released by neutrophils, may contribute to cytokine release(Zhou *et al.*, 2020).

The cytokine storm in COVID-19 is characterized by a high expression of IL-6 and TNF-α (Hirano & Murakami, 2020).

1.2.8 Bio chemical markers in COVID-19:

1.2.8.1. D. dimer:

D-dimer originate from the lysis of cross-linked fibrin with rising levels indicating the activation of coagulation and fibrinolysis **(**Zhang *et al.*, 2018**).**

D-dimer levels are commonly elevated in patients infected with SARS-CoV-2. Significantly higher levels are found in those with critical illness and may be used as a prognostic marker for in hospital mortality (Yao *et al.*, 2020).

D-dimer was associated with mortality and severe COVID-19. This finding supports the hypothesis that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection could induce the dysfunction of the hemostatic system, leading to a hypercoagulable state, a condition which we commonly encounter in sepsis (Lin *et al*., 2020).

Recent evidence of lung pathology dissection has shown occlusion and micro-thrombosis formation in pulmonary small vessels of patients critically ill with COVID-19(Luo *et al*., 2020).

1.2.8.2. S. ferritin:

Ferritin is a useful marker to predict the outcomes in COVID-19. Hyperferritinemia can activate macrophages, which increases the secretion of pro-inflammatory cytokines, and the subsequent inflammation is mainly responsible for organ damage. Although ferritin is known as a positive acute phase reactant and serum level of ferritin intracellular protein

increases during inflammation, dying cells may also release the ferritin (Gandini *et al*., 2020).

1.2.8.3. C. reactive protein (CRP):

C. reactive protein (CRP) is an acute phase inflammatory protein produced by the liver that may be elevated in several conditions, such as inflammation, cardiovascular disease, and infection (Sproston & Ashworth, 2018).

CRP has been suggested to be used as a prognostic marker, and higher levels of CRP indicating increased risk of disease progression (Diamond & Pierson, 2015**).** Increased CRP levels might be early indicators of nosocomial infections in COVID-19 patients (Feng *et al*., 2020).

1.2.8.4. Lactate dehydrogenase (LDH):

Lactate dehydrogenase (LDH) is a glycolytic cytoplasmic enzyme present in virtually every tissue. In general, its elevation suggests tissue injury. Possible subclinical tissue damage was indicated by our observation of increased LDH in the early stage of extreme COVID-19 cases (Wu *et al*., 2020).

Elevated LDH has been associated with a higher risk of ARDS, need for intensive care and mortality (Terpos *et al*., 2020).

1.2.8.5. Liver enzymes in COVID -19:

SARS-CoV-2 virus may bind to angiotensin-converting enzyme 2 (ACE2) on cholangiocytes, leading to cholangiocyte dysfunction and inducing a systemic inflammatory response leading to liver injury (Chai *et al*., 2020).

Cytokine storm caused by excessive immune response induced by the virus may also be one of the pathways of liver damage (Hu *et al*., 2020). However, ALT, AST, total bilirubin and other liver function indices were significantly increased in patients with severe COVID-19 compared to patients with mild COVID-19, and the liver function indices gradually returned to normal during recovery (Wu *et al*., 2020).

1.2.9 Immunological markers:

1.2.9.1 Interleukin -6 (IL – 6):

Interleukin -6 (IL-6) is an important member of the cytokine network and plays a central role in acute inflammation (Zhang *et al*., 202).

Is a multifunctional cytokine that plays an important role in human metabolism, autoimmune cell differentiation, disease treatment, etc. (Hunter & Jones, 2015).

IL-6 is a small polypeptide consisting of four α helices. It has a molecular weight of 19–28 K Da and comprises 184 amino acid residues, usually in monomer form, with an isoelectric point of 5.0, glycosylation sites and two disulfide bonds (Scheller *et al.,* 2014).

IL-6 can be produced by almost all stromal cells and immune system cells, including B-lymphocytes, T-lymphocytes, macrophages, monocytes, dendritic cells, mast cells, and other non-lymphocytic cells such as fibroblasts, endothelial cells, keratinocytes, glomerular mesangial cells and tumor cells (Jones & Jenkins, 2018).

The main activators of IL-6 expression are IL-1β and tumor necrosis factor-alpha (TNFα). However, there are also other ways to promote the

synthesis of IL-6, such as Toll-like receptors (TLRs), prostaglandins, adipokines, stress response and other cytokines(Hunter & Jones, 2015).

Interleukin 6 (IL-6) were found in the acute stage associated with lung lesions in SARS-CoV-1 patients. IL-6 can induce the hyper-innate inflammatory response due to the SARS-CoV-1 invasion of the respiratory tract (Magro, 2020).

This happens also with SARS-CoV-2 in COVID-19 patients: some retrospective and meta-analysis studies show how elevated IL-6 and C-reactive protein (CRP) correlate with mortality and severe disease in comparison to moderate disease (Zhou *et al*., 2020**).**

IL-6 levels in patients with severe COVID-19, and this viral load is associated with ARDS severity and lung tissue damage (Chen *et al*., 2020).

Shock and organ failure in several organs, such as the kidneys, heart, lungs, and liver, are severely damaged by cytokine storms caused by an increase in inflammatory cytokines, including IL-6, IL-1β, TNF-α, IL-8, IL-2, IL-17, G-CSF, GMCSF, in patients with COVID-19 (Farnoosh *et al*., 2020).

These cytokines can also cause extensive pulmonary damage through the accumulation of neutrophils and macrophages in lung tissue, leading to the development of hyaline membranes and diffuse thickening of the alveolar barrier and ultimately diffuse alveolar damage (Magro, 2020).

1.2.9.2 Tumor necrosis factor alpha:

Tumor necrosis factor (TNF)-α, an important member of the TNF superfamily of ligands, is a pleiotropic pro-inflammatory cytokine(Lai *et al.*, 2016).

In monocytes and macrophages, lipopolysaccharide (LPS) induces TNF-α expression by activating early growth response factor-1 (Egr-1)(Wang *et al.*, 2017), activator protein-1 (AP-1)(Shin *et al.*, 2015), and nuclear factor-κB (NF-κB)(Mehta *et al.*, 2020).

TNF-α is synthesized as a monomeric Type II protein containing 233 amino acid (27 K Da), and then it is arranged in stable homotrimers as transmembrane TNF-α. Three monomers associate around a 3-fold axis to form a compact bell-shaped trimer. This structure is typical for most members of the TNF family but comparison to known protein structures also shows structural homology to several viral coat proteins(Wang *et al.*, 2017)**.**

TNF-α plays key roles in immune responses and tissue homeostasis. Despite the role of TNF-α signaling in combating viral infections, high levels of TNF-α in some diseases like influenza and COVID-19 are associated with lung injuries. Studies have demonstrated that high levels of TNF- α are observed in plasma and alveolar fluid lavage of patients with ARDS. Elevated levels of cytokines lead to increased endothelial permeability and decreased alveolar fluid clearance caused by down-regulation of sodium channels in the epithelium (Feldmann *et al.*, 2020).

The main sources of TNF-α are activated monocytes, fibroblasts, and endothelial cells (Ma *et al.*, 2020)**.**

Chapter Two

Materials and Methods

2. Materials and Methods:

2.1. Subjects and Study Design:

2.1.1. Subjects:

This study included correlation of IL-6 and TNF alpha levels with hematological and serological parameters in COVID-19 patients at Imam AL-Hussein Medical-City in the period extending from October 2020 to December 2020.

All patients were diagnosed with COVID-19 based on Real –time PCR and CT scan according to WHO.

In this study 56 of COVID-19 patients (36 male and 20 female) had been admitted to the hospital.

Their ages were ranged between 25 to 85 years old. The groups included 28 mild patients (18male and 10 female) with the same age and sex of the patients were randomly selected from the local community.

2.1.2. Inclusion and Exclusion Criteria:

2.1.2.1. Inclusion criteria

COVID-19 patients admitted to hospitals that are diagnosed by PCR and clinically. Those include both severe and moderate cases.

2.1.2.2. Exclusion criteria

Patients on Tocilizumab (Actemra).

2.1.3. Study Design:

This is a cross sectional study which involved 84 COVID- 19 patients (severity and moderate), (54 male and 30 female). The mild had the age and sex of COVID- 19 patients.

2.2. Ethical and Scientific Approval:

Ethic's approval was obtained from Kerbala Health Directorate. In addition, verbal approval was taken from the patients and /or their parents before taking the sample. Health measures and safety were taken when sampling.

2.3. Data Collection:

The medical records of all COVID-19 patients with positive SARS-COV-2 real-time RT-PCR results were reviewed. The demographic data and laboratory parameters during hospitalization were collected. All data were checked by a team of trained physicians.

The demographic and clinical data were collected through an interview which was done with patients and /or their parents through a questionnaire**.**

2.4. Questionnaire:

The questionnaires were designed to search from the COVID -19 patients in Al- Imam AL-Hussein Medical- City, taking the international and local standards into account, for collecting data from patients with COVID-19. Socio-demographic and observed data: sex, age, clinical symptoms, hypertension and Blood glucose.

2.5. Sample Collection:

Serum will be withdrawn from each participant, the blood will be divided in to three parts:

Part one-will be put in gel tube for chemical tests (C. reactive protein, Lactate dehydrogenase, D. dimer, S. ferritin, Liver enzymes levels).

Part two-will be transferred in to EDTA tube for hematological tests Complete blood count (CBC).

Part three –in gel tube for immunological tests (IL-6 and TNF alpha).

2.6. Materials:

2.6.1. Equipment and Instruments:

In the present study, the following Equipment and Instruments were used (table 2.1 and table 2.2)

Table 2.1: Equipment and Instruments with their Manufacturing Company and country of origin

Equipment and Instruments	Manufacturing Company	Country
Autoclave	Hirayama HVE-50	Japan
ELISA Devices (washer & reader)	Bio kit ELx800	U.S.A
Freezer	Panasonic	Korea
Haematology analyser	Sysmex XN 350	Japan
Incubator	Memmert	Germany
Refrigerator	Panasonic	Korea
Water bath	GFL	Germany
Water distillatory	GFL	Germany

Table 2.2: Equipment and Instruments with their country of origin

Equipment and Instruments	Country
Cold medical box	China
Cylinders (250,500 ml)	Germany
EDTA tube	China
Eppendorf tube (0.5 ml & 1.5 ml)	China
Filter paper	China
Flasks (different size)	China
Gel and Clot Activator Tube	China
Gloves	China
Micropipettes (different size)	Japan
Tips (Yellow & Blue)	China

2.6.2. ELISA Kit:

Table 2.3: ELISA Kits used in the study

ELISA Kit	Manufacturing Company	Country
Human serum IL-6	Cayman chemical	USA
Human TNF-Alpha	Elabscience Biotechnology	USA

2.6.2.1. ELISA Kit Content of Human serum IL-6:

Table 2.4: ELISA kit for detection of human serum IL-6

Components	Format
1. Anti-IL-6(human)ELISA Strip Plate	1 plate
2. Anti-IL-6(human) Biotin Conjugate	1vial/100 dtn
3. IL-6(human)ELISA Standard	1vial/10 ng
4. IL-6(human)Streptavidin- horse radish peroxidase (HRP)	2vials/1.5ml
5. Immunoassay Buffer B Concentration(10x)	2vials/10ml
6. Polysorbate20	1vial/3 ml
7. 96-Well Cover Sheet	1cover
8. TMB(tetramethylbenzidine) Substrate Solution	1vial/12ml
9. Wash Buffer Concentrate(400x)	1vial/5ml
10. HRP Stop Solution	1vial/5ml

2.6.2.2. ELISA Kit Content of Human TNF Alpha:

Table 2.5: ELISA kit for detection Human TNF Alpha

Components	Format
1. Micro ELISA plate	12×8
2. Reference standard	2 vials
3. Concentrated Biotinylated detection Ab (100x)	120 µl
4. concentrated HRP conjugate (100x)	120 µl
5. Reference standard & sample diluent	20 ml
6. Biotinylated detection Ab diluent	14 ml
7. HRP conjugate diluent	14 ml
8. concentrated Wash Buffer(25x)	30 ml
9. Substrate Reagent	10 ml
10. Stop solution	10 ml
11. Plate Sealer	5 pieces

2.7. Methods:

2.7. 1. Measurement of human serum IL-6

Serum was analyzed to determine the human serum IL-6 concentration by BioTek ELx800 automated immunoassay analyzer (BioTek, USA) using Cayman Chemical Company ELISA kit (LOT NO.501030).

2.7. 1.1 The Principle of The Test:

This test is used the Sandwich-ELISA principle, in which the microliter wells had been pre-coated with an antibody specific to Human IL-6. Standards or samples were added to the microliter wells and combined with the specific antibody. After that a biotinylated detection antibody specific for Human IL-6 and Avidin-Horse Radish Peroxidase (HRP) conjugate were added successively to each microliter well and incubated, then free components were washed away. After that substrate solution was added to each well and incubated. Finally the enzyme-substrate reaction was terminated by the addition of stop solution and the color would turn to yellow. The IL-6 concentration was measured by means of the standard curve at a wavelength of 450 nm.

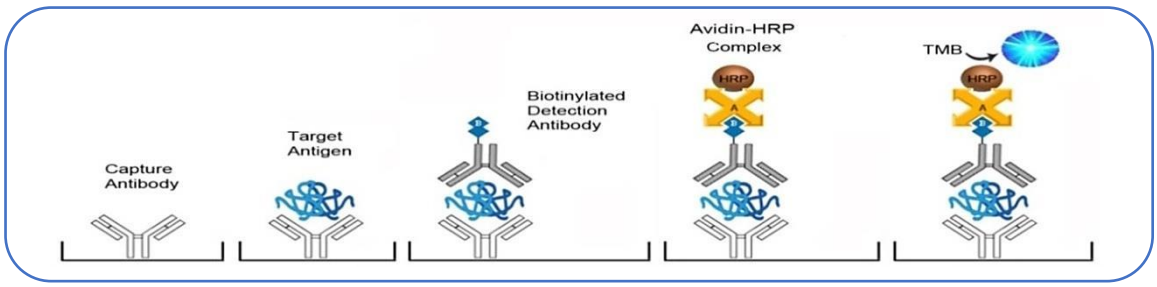

Figure 2.1: principle of sandwich ELISA

2.7.1.2. Pre-Assay Preparation:

2.7.1.2.1. Buffer Preparation:

A. Assay Buffer Preparation

Diluted the contents of each vial of Immunoassay Buffer B concentrate (10x) with 90 ml of deionized water**.**

B. Wash Buffer Preparation

5ml vial Wash Buffer diluted to total volume of 2 liters with deionized water and added 1ml of Polysorbate20**.**

2.7.1.2.2. Preparation of Assay –Specific Reagents:

A. IL-6(human) ELISA Standard

Reconstituted the lyophilized IL-6 (human) ELISA Standard with 2 ml of Assay Buffer, mix gently. The concentration of this solution is 5 ng/ml.

Prepared the standard for used in the ELISA; obtained six cleaned test tubes and labeled them (1) through (6). Aliquot 475 µl of Assay Buffer in to tube (1), and 250 µl of Assay Buffer in to tubes (2) to (6). Transferred 25 µl of freshly prepared stock standard (5 ng/ml) to tube (1). Mixed gently. Next, removed 250 µl from tube (1) and placed in to tube (2); Mixed gently. Repeated this processed for (3to5).did not added any IL-6 to tube (6).This tube is the zero-point, the lowest point on the standard curve.

B. Anti –IL-6(human) Biotin Conjugate

Reconstituted the lyophilized Anti-IL-6(human) Biotin Conjugate with 12.0 ml of Assay Buffer, Mixed gently**.**

C. IL-6 Streptavidin-HRP

This reagent is supplied as a concentrated (10x) stock solution of Streptavidin conjugated to HRP. Prepared a working solution by added1.2ml of the Streptavidin-HRP to 10.8 ml Assay Buffer (12ml total).

2.7.1.3. Procedure of the test:

1. Sample incubation**:** 100 µl of standard or sample was added to each well and incubated for 1 hour at room temperature on an orbital shaker.
2. Aspirate and washed five times with 350 µl of wash buffer.
3. Biotinylated detection Ab incubation**:** 100 µl Biotinylated detection Ab was added and incubated for 1 hour at room temperature on an orbital shaker, then aspirated and washed five times with 350 µl of wash buffer.
4. Conjugate incubation: 100 µl HRP Conjugate was added and incubated for 30 min at room temperature on an orbital shaker, then aspirated and washed five times with 350 µl of wash buffer.
5. Substrate incubation: 100 µl of substrate reagent was added and incubated for 30 min at room temperature in the dark.
6. Stopping: 100 µl stop solution was added and read at 450 nm immediately.

2.7.1.4. Interpretation of Result

1. A four-parameter logistic curve was plotted on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.
2. If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the

OD of the sample surpasses the upper limit of the standard curve, you should re-test it with appropriate dilution.

3. The actual concentration is the calculated concentration multiplied by the dilution factor.

2.7. 2. Measurement of human TNF Alpha

Serum was analyzed to determine the human TNF Alpha concentration by BioTek ELx800 automated immunoassay analyzer (BioTek, USA) using Elabscience Biotechnology ELISA kit (LOT NO.E-EL-H0109).

2.7. 2.1 The Principle of The Test:

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre- with an antibody specific to Human TNF –Alpha. Standards or samples are added to the micro plate wells and combined with the specific antibody.

Then a biotinylated detection antibody specific for Human TNF Alpha and Avidin Horsera Peroxidase (HRP) conjugate are successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human TNF Alpha. Biotinylated detection antibody and Avidin -HRP conjugate will appear blue in color.

The enzyme –substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450nm±2nm.The OD value is proportional to the concentration of Human TNF Alpha. You can calculate the concentration of Human TNF Alpha in the samples by comparing the OD of the samples to the standard curve.

2.7.2.2. Pre-Assay Preparation

A. Wash Buffer:

Diluted 30 ml of concentrated wash buffer with 720 ml of deionized water to prepared 750 ml of wash buffer**.**

B. Standard working solution

The standard was centrifuged at 10,000xg for 1 min. Added 1 ml of reference standard and sample diluent. After dissolved fully reconstitution produced a working solution of 500pg/ml. Then diluted by added 500 ml of reference standard and sample diluted to each tube. Pipetted 500 ml of the 500 pg/ml working solution to the first tube to produced 250pg/ml working solution. Pipette 500ml of the solution from the former tube in to the latter one according to these steps.

C. Biotinylated Detection Ab working solution

The concentrated Biotinylated detection was centrifuged Ab at 800xg for 1 minute. Then diluted the 100x concentrated Biotinylated detection Ab to 1x working solution**.**

D. Concentration HRP Conjugate working solution

Centrifuged the concentrated HRP conjugated at 800xg for 1 minute. Then diluted the 100x concentrated HRP conjugate to 1x working solution with HRP conjugate diluent (1:99).

2.7.2.3. Procedure of the Test:

1. Sample incubation; 100 µl of standard or sample was added to each well and incubated for 90 minute at 37C.
2. Removed the liquid; Added 100 µl Biotinylated detection Ab, and incubated 1 hour at 37C.
3. Aspirated and wash 3 times.
4. Added 100 µl HRP conjugated, then incubated 30 minute at 37C.
5. Aspirate and wash times.
6. Added 90 µl substrate reagent, then incubated15 minute at 37C.
7. Added 50 µl Stop Solution, read at 450 nm immediately.
8. Calculation of results.

2.7.2.4. Interpretation of Result

1. A four-parameter logistic curve was plotted on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.
2. If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with appropriate dilution.
3. The actual concentration is the calculated concentration multiplied by the dilution factor.

2.8. Statistical Analysis:

Data was introduced into a Specific Software Statistical Package for the Social Sciences (SPSS) version 21 for windows (GraphPad Software, San Diego, California, USA) to do statistical analysis.

The results were expressed as mean ± SD. A *p* value of <0.05 was considered to indicate the statistical significance and highly significant if *p*-value <0.001. Explore used to compare between categorical variables. In addition, the pearson correlation was used to explain the relation between IL-6 and TNF alpha levels with hematological and biochemical tests.

CHAPTER Three

Results

3. Result

3.1. Demographic data of the studied groups:

As shown in table 3.1 a total of 84 patients with COVID – 19 disease confirmed by SARS-COV-2 –specific RT-PCR on Nasopharyngeal (NP) swab specimen were enrolled in this study.

The patients were either hospitalized or attended to Al-Hussein Medical city hospitals during the period from 10-10-2020 to 29-12-2020.

According to SpO_2 percentage, COVID-19 patients were classified in to mild (≥ 95%), moderate (91-94 %) and severe (≤90%). Accordingly, the following groups of patients were selected (n=28 moderate and n=28 severe) and (n=28 mild). Among them 54 (64.3%) were males and 30 (35.7%) were females. The mean age was 56.08 years and the age range was between 25 to 85 years. The mean age of the mild patients was 44.3 years that was much less than the mean ages of moderate and severe groups (61.4 and 62.2 years, respectively).

Fifty six patients were admitted (moderate and severe patients) and 28 patients were mild who were not-admitted. Severity of the disease in this study based on SpO_2 percentage.

Table (3.1): Demographic data of the studied groups.

Total Number		
Gender		Male 54 (64.3%) Female 30 (35.7%)
Age	Range Mean ±SD	(25-85) 56.08 ± 14.25
Mean age within groups	Mild Mean ±SD	44.30±10.37
	Moderate Mean ±SD	61.36±13.68
	Severe Mean ±SD	62.18±11.71
Severity According to SpO_2	Mild	28
	Moderate	28
	Severe	28

3.2. Hematological indices in COVID-19 patients:

Table (3.2) shows the hematological parameters of included patients. Total white blood cell counts and neutrophils percentage were significantly increased in moderate and severe in comparison to mild COVID-19 cases as indicated by positive correlation (r=0.402, *p*=0.000; r=0.502, *p*=0.000 respectively). In contrast, lymphocytes were significantly reduced in moderate and severe cases as indicated by significant negative correlation (r=-0.360, *p*=0.001). This may indicate that this COVID -19 infection very specifically targeting lymphocytes.

No significant difference could be seen in hemoglobin and platelets among the three groups of patients. However, all of the three groups showed slight reduction in the hemoglobin levels (mild anemia).

Table (3.2): Hematological indices in COVID-19 patients classified according to degree of disease severity

Variables	Normal range	Mild (N=28) Mean ± SD	Moderate (N=28) Mean ± SD	Severe (N=28) Mean ± SD	
White blood cell counts ,x10^9/L	3.5-10.0	7.40 ± 2.37	13.78 ± 6.82	14.09 ± 5.89	R=0.402** *p*=0.000
Neutrophil %	40-70%	54.36 ± 13.11	77.77 ±23.15	80.32 ±16.58	R=0.502** *p*=0.000
Lymphocyte %	18-45.3 %	23.62 ± 9.86	9.97 ± 10.49	11.92± 17.03	R=-0.360** *p*=0.001
Hemoglobin g/l	13.5-17.5	13.06 ± 1.77	12.67 ±2.18	12.83 ± 2.45	R=-0.002 *p*=0.984
Platelet count x10^9/L	155-450	232.39±72.16	239.81 ±103.82	208.50 ± 83.70	R=-0.113 *p*=0.309

Data are presented as Mean ± SD (standard deviation); R (Pearson correlation); *p* value indicated the comparison between mild, moderate and severe patients. COVID -19: Coronavirus disease 2019; N (Number of patients).

3.3: Biochemical markers in COVID -19 patients:

Table 3.3 shows the most of the biochemical markers, D. dimer and serum ferritin showed several fold increase within moderate and severe groups in comparison to mild cases. And the differences in the levels of D. dimer and S. ferritin between the mild and the other groups were statistically significant (r=0.259, *p*=0.018; r=0.264, *p*=0.019, respectively).

Lactate dehydrogenase (LDH) level was slightly elevated in the mild cases, however, it was almost two times increased in the moderate and severe cases. The difference in LDH levels is positively correlated with degree of severity (r=0.441, *p*=0.000).

C. reactive protein (CRP) showed the most prominent elevation in the mild cases, where it was increased more than three times in comparison to the reference values. This result make it the most reliable indicator for COVID-19 biomarker especially for mild cases. In addition, CRP was positively correlated with the degree of severity (r=0.137, *p*=0.003).

In mild patients, the liver enzymes were in the normal range, and no increased could be seen in their mean levels. In moderate patients, only Alanine aminotransferase (GPT) levels were elevated. Whereas, in severe patients, both Alanine aminotransferase (GPT) and Aspartate aminotransferase (GOT) were elevated in comparison to mild patients. The increase in the levels of GPT and GOT were statistically significant (r=0.225, *p*=0.049; r=0.234, *p*=0.033, respectively).

Table (3.3): Biochemical markers in COVID -19 patients classified according to degree of disease severity

Variable	Normal Range	Mild (N=28) Mean ± SD	Moderate (N=28) Mean ± SD	Severe (N=28) Mean ± SD	
D. dimer ng/ml	0-500	336.95 ±202.34	3008.99±5284.97	2241.58 ±1537.04	R=0.259* *p*=0.018
S. ferritin ng/ml	13-350	330.93±182.27	1616.43±1964.77	1232.89±866.72	R=0.264* *p*=0.019
Lactate dehydrogenase U/L	109-245	260.27±60.82	513.51±202.05	488.36±251.89	R=0.441** *p*=0.000
C-reactive protein levels mg/l	0 – 6	18.03±30.44	54.34±68.21	68.65±70.78	R=0.317** *p*=0.003
Alanine aminotransferase (GPT) U/L	5-40	35.49±17.21	54.37±37.95	56.89±55.91	R=0.225* *p*=0.049
Aspartate aminotransferase (GOT) U/L	8-40	32.48±12.22	39.81±20.62	44.35±25.41	R=0.234* *p*=0.033
Alkaline phosphatase (ALP) U/L	40-150	77.33±22.96	85.95±39.96	86.39±63.45	R=0.087 *p*=0.449

3.4. IL-6 and TNF-α levels in COVID -19 patients:

Table 3.4 shows the serum levels of IL-6 and TNF-α among the COVID-19 patients. In mild patients, both cytokines were elevated, nevertheless, the increase in the cytokine levels were much more in moderate and severe patients. The increase in both cytokines were positively correlated with degree of severity for (IL-6; r=0.14, *p*=0.20, and for TNF-α; r=0.199, *p*=0.07). It is worth mention that cytokines elevation in the mild cases makes them more reliable indicators than biomarkers like D. dimer, S. ferritin and LDH.

Table (3.4): IL-6 and TNF-α levels in COVID -19 patients classified according to degree of disease severity.

Variable	Normal Range	Mild (N=28) Mean ±SD	Moderate (N=28) Mean± SD	Severe (N=28) Mean± SD	
IL-6 pg/ml	0.0-7.0	16.17 ±28.08	25.03 ±27.13	27.56±42.87	R=0.14 *p*=0.20
TNF-α pg/ml	0.0- 8.1	37.7± 54.58	95.28±112.83	81.60± 88.18	R=0.199 *p* =0.07

IL-6; Interleukin -6 TNF-α; Tumor necrosis factor alpha

3.5. Correlation of serum levels of IL6 and TNF alpha with the hematological parameters:

In table (3.5) correlation analyses were done between each of IL-6 and TNF-α serum levels with hematological markers in COVID-19 patients irrespective to the classification of patients according to severity of symptoms.

Generally, high levels of IL-6 were associated with decreased lymphocyte percentage (r=-0.265, *p*=0.016) and increased neutrophils percentages (r=0.220, *p*=0.046). However, TNF-α did not show statistically significant association with either of them.

Table (3.5.) Correlation of serum levels of IL6 and TNF alpha with the hematological parameters.

Variables	IL-6			TNF alpha		
	Low (0-7 pg/ml)	High (≥7pg/ml)	Correlation	Low (0-8.1pg/ml)	High (≥8.1pg/ml)	Correlation
WBC count $X10^9/L$	10.84 ± 5.90	12.27 ± 6.70	R=0.110 *p*=0.323	11.29 ± 6.93	11.83 ± 6.28	R=0.36 *p*=0.75
Lymphocytes %	19.45 ± 17.11	12.03 ± 10.29	R=-0.265* *p*=0.016	19.07 ± 13.32	13.77 ±13.83	R=-0.162 *p*=0.142
Neutrophils %	64.97 ± 22.29	74.52 ± 20.05	R=0.220* *p*=0.046	67.10 ±21.90	71.81 ± 21.25	R=0.093 *p*=0.402
Haemoglobin g/l	12.48 ± 2.7	12.9 ±2.0	R=-0.134 *p*=0.227	12.69 ±2.46	12.75 ± 2.23	R=-0.205 *p*=0.063
Platelet $X10^9/L$	236.48 ± 75.09	220.32 ± 94.70	R=-0.091 *p*=0.412	259.32 ± 85.61	217.08 ± 86.10	R=-0.204 *p*=0.064

3.6. Correlation of serum levels of IL6 and TNF alpha with the hematological parameters within the severity groups:

Table (3.6) shows the correlation of serum levels of IL-6 and TNF alpha with the hematological parameters after classification of patients in to severity groups. Important correlation could be seen between levels of either IL-6 or TNF-α with hematological parameters within mild, moderate and severe cases.

IL-6 was not correlated with total WBC count within the severity groups. However, high levels of IL-6 in severe groups showed trends to have low lymphocyte percentage, this did not reach the statistical significance (r=0.36, *p*=0.06). In contrast, high levels of IL-6 in severe groups were associated with trends of increased both neutrophils and hemoglobin, these correlations were also statistically significant for neutrophils (r=0.482, *p*=0.009) but insignificant with hemoglobin (r=0.324, *p*=0.07).

TNF-α was not correlated with total WBC count within the severity groups. Interestingly, TNF-α showed negative correlation with lymphocyte percentage with mild groups (r= -0.44, *p*=0.03) but not in the moderate and severe groups. This result may indicate differential effect of TNF-α on lymphocyte count in COVID -19. In addition,

a significant negative correlation was seen between TNF-α levels and hemoglobin within the moderate groups (r=-0.415, *p*=0.03).

Table (3.6) Correlation of serum levels of IL6 and TNF alpha with the hematological parameters within the severity groups

Variables		IL-6			TNF alpha		
		Low (0-7 pg/ml)	High (≥7pg/ml)	Correlation	Low (0-8.1pg/ml)	High (≥8.1pg/ml)	Correlation
Total WBC count $X10^9/L$	Mild	7.88±2.54	6.42±2.1	R=-0.133 *p*=0.500	8.54±2.85	6.64±1.95	R=-0.207 *p*=0.291
	Moderate	12.70±2.35	14.57±8.38	R=-0.085 *p*=0.674	14.08±6.42	13.95±7.31	R=-0.229 *p*=0.225
	Severe	14.90±6.92	15.34±6.02	R=0.11 *p*=0.57	15.75±4.03	15.15±6.39	R=0.056 *p*=0.782
Lymphocytes %	Mild	25.00±8.64	24.74±11.23	R=-0.300 *p*=0.121	28.00±9.97	23.10±8.93	R=-0.44* *p*=0.030
	Moderate	5.96±3.35	9.06±10.16	R=0.263 *p*=0.185	5.52±3.81	8.64±9.34	R=0.220 *p*=0.270
	Severe	11.72±9.16	7.85±4.8	R=-0.36 *p*=0.06	6.45±1.77	9.29±6.71	R=0.077 *p*=0.703
Neutrophils %	Mild	56.50±14.84	49.43±13.40	R=-0.136 *p*=0.490	62.86±16.08	48.67±10.79	R=-0.330 *p*=0.087
	Moderate	76.71±28.55	84.31±14.74	R=-0.028 *p*=0.889	68.58±37.53	84.85±13.53	R=0.090 *p*=0.225
	Severe	73.05±19.43	86.16±6.46	R=0.482* *p*=0.009	87.05±5.30	81.69±13.44	R=-0.078 *p*=0.702
Haemoglobin g/l	Mild	13.63±1.63	13.37±1.46	R=-0.029 *p*=0.882	13.84±1.36	13.36±1.66	R=0.011 *p*=0.955
	Moderate	12.91±1.80	12.60±2.33	R=-0.305 *p*=0.122	13.45±1.71	12.53±2.23	R=-0.415* *p*=0.031
	Severe	12.68±2.15	13.29±1.70	R=0.34 *p*=0.07	12.50±0.99	13.18±1.89	R=0.026 *p*=0.898
Platelet $X10^9/L$	Mild	229.42±61.75	205.14±67.70	R=-0.324 *p*=0.093	220.14±71.96	220.67±61.03	R=-0.276 *p*=0.155
	Moderate	249.57±74.66	235.53±123.175	R=-0.036 *p*=0.858	298.00±118.87	227.11±105.5	R=-0.196 *p*=0.326
	Severe	208.33±98.85	213.79±81	R=0.07 *p*=0.73	258±82.04	207.06±85.02	R=-0.141 *p*=0.484

3.7. Correlation of serum levels of IL6 and TNF alpha with the Biochemical parameters:

In this table (3.7) correlations analyses were done between each of IL-6 and TNF-α serum levels with the biochemical markers in COVID-19 patients irrespective to the classification of patients according to severity of the symptoms.

Serum levels of IL-6 showed positive correlation with serum ferritin levels (r=0.286, *p*=0.011) and negative correlation with GOT level (r= -0.224, *p*=0.05). On the other hand increased serum levels of TNF-α were only showed association with increase in LDH levels but this increase did not reach the statistical significance.

Table (3. 7) Correlation of serum levels of IL6 and TNF alpha with the Biochemical parameters.

Variables	IL-6			TNF alpha		
	Low (0-7 pg/ml)	High (≥7 pg/ml)	Correlation	Low (0-8.1 pg/ml)	High (≥8.1 pg/ml)	Correlation
D. dimer ng/ml	826.05 ± 1010.33	2476.75 ±4244.49	R=-0.182 *p*=0.099	806.72 ± 805.11	2116.01 ± 3829.79	R=-0.101 *p*=0.363
S. ferritin ng/ml	597.36±702.39	1446.89±1599.44	R=0.286* *p*=0.011	840.48 ± 911.82	1181.57± 1481.17	R=-0.088 *p*=0.443
CRP mg/l	46.63 ± 60.72	44.73 ± 55.47	R=0.043 *p*=0.695	33.21± 36.38	49.38 ± 62.18	R=-0.133 *p*=0.229
LDH U/L	314.68 ± 232.57	415.12 ± 271.02	R=0.167 *p*=0.131	257.87 ± 226.32	410.69 ± 259.81	R=0.209 *p*=0.058
GPT	46.27 ± 36.67	51.86 ± 44.12	R=0.073 *p*=0.529	41.64 ±27.70	52.08 ± 44.35	R=0.125 *p*=0.280
GOT	35.21 ± 18.45	40.13 ± 21.47	R=-0.224* *p*=0.05	34.22 ± 16.12	39.35 ± 21.43	R=0.129 *p*=0.265
ALP	80.01 ± 25.67	87.62 ±54.37	R=-0.068 *p*=0.555	79.93 ± 20.23	85.95 ± 50.21	R=0.054 *p*=0.641

3.8. Correlation of serum levels of IL6 and TNF alpha with the Biochemical parameters within the severity groups:

In table (3.8) a correlation analysis was shown between levels of IL-6, TNF-α with biochemical parameters after classifying the patients according to severity.

IL-6 increase in levels associated with the increase in s .ferritin levels within severe group, however, this increase did not reach the statistical significance (r=0.348, *p*=0.081).

Nevertheless correlation analysis of IL-6 with liver enzyme, showed negative correlation between IL-6 levels and GOT within the severe group (r=-0.451, *p*=0.031).

The other liver enzymes, in this study were not correlated with the levels of IL-6.

TNF-α serum levels showed significant positive correlation with D. dimer levels (r=0.406, *p*=0.039) within the severe COVID -19 groups, nevertheless, it did not show correlation within other groups.

On the other hands, CRP, LDH levels were not correlated with either IL-6 or TNF-α levels.

Table (3.8) Correlation of serum levels of IL6 and TNF alpha with the Biochemical parameters within the severity groups

Variables		IL-6			TNF alpha		
		Low (0-7 pg/ml)	High (≥7 pg/ml)	Correlation	Low (0-8.1 pg/ml)	High (≥8.1 pg/ml)	Correlation
D. dimer ng/ml	Mild	343.17±197.85	343.71±235.09	R=-0.105 *p*=0.595	398.71±222.03	311.08±198.29	R=-0.286 *p*=0.139
	Moderate	942.24±884.72	3895.96±6584.19	R=0.253 *p*=0.204	1128.23±1140.19	3362.34±6102.18	R=0.176 *p*=0.380
	Severe	1873.60±1642.89	2252.78±1618.69	R=-0.082 *p*=0.677	2205.20±501.62	2131.67±1678.58	R=0.406* *p*=0.039
S. ferritin ng/ml	Mild	295.96±183.83	422.57±151.01	R=0.224 *p*=0.260	278.37±206.02	380.08±159.26	R=0.161 *p*=0.421
	Moderate	1041.45±932.43	2035.06±2373.26	R=0.200 *p*=0.327	1825.58±866.64	1695.21±2255.35	R=-0.035 *p*=0.866
	Severe	826.45±1014.37	1559.33±767.64	R=0.348 *p*=0.081	1757.15±1437.90	1293.05±861.11	R=-0.205 *p*=0.325
CRP mg/l	Mild	14.88±16.76	24.58±48.35	R=-0.49 *p*=0.804	13.2±16.67	21.51±37.61	R=-0.089 *p*=0.651
	Moderate	73.2±77.43	33.51±31.71	R=-0.149 *p*=0.448	30.08±40.38	49.71±54.99	R=0.174 *p*=0.376
	Severe	82.09±83.49	68.90±74.04	R=0.076 *p*=0.702	91.47±5.28	70.77±78.98	R=-0.012 *p*=0.953
LDH U/L	Mild	200.73±66.73	202.67±58.82	R=-0.020 *p*=0.932	204.70±74.91	199.54±57.18	R=-0.061 *p*=0.794
	Moderate	561.02±217.07	515.38±206.87	R=-0.183 *p*=0.366	538.99±170.46	521.22±216.54	R=-0.150 *p*=0.446
	Severe	464.99±118.76	519.64±302.58	R=0.216 *p*=0.276	337.47±114.37	521.67±265.58	R=0.254 *p*=0.216
GPT	Mild	35.82±20.44	36.27±12.35	R=-0.023 *p*=0.907	29.97±19.72	40.16±14.66	R=0.263 *p*=0.177
	Moderate	69.72±53.33	47.03±29.35	R=-0.190 *p*=0.353	78.14±21.44	48.94±40.16	R=-0.19 *p*=0.353
	Severe	46.76±41.78	52.20±41.08	R=0.179 *p*=0.413	32.87±4.90	52.54±42.06	R=0.227 *p*=0.310
GOT	Mild	33.47±12.11	32.37±12.68	R=-0.095 *p*=0.632	29.26±13.88	35.60±10.42	R=0.216 *p*=0.270
	Moderate	44.90±28.46	35.62±18.03	R=-0.163 *p*=0.425	50.89±13.08	35.83±22.45	R=-0.234 *p*=0.251
	Severe	28.54±17.30	47.30±23.96	R=-0.451* *p*=0.031	28.15±16.28	43.17±23.97	R=0.282 *p*=0.203
ALP	Mild	79.14±22.97	76.20±24.46	R=-0.092 *p*=0.640	79.80±22.17	76.67±24.45	R=-0.088 *p*=0.656
	Moderate	70.57±22.08	96.48±47.64	R=0.021 *p*=0.290	83.07±19.19	89.39±46.44	R=0.013 *p*=0.948
	Severe	93.35±34.36	88.82±78.50	R=-0.004 *p*=0.555	74.40±20.65	91.93±70.76	R=0.138 *p*=0.542

CHAPTER FOUR

Discussion, Conclusion& Recommendations

4. Discussion:

4.1. Demographic data of the studied groups:

In this study, males were more than females (64.3% vs. 35.7%) which reflects that disease affect males more than females. These finding were also reported others studies (Jin *et al*., 2020 ; Lakbar *et al*., 2020).

The mean age of the patients in this study (56.08 years) indicate that COVID-19 is affecting older age groups. In addition, the mean age of the mild patients (44.3 years) was much less than mean age of the moderate and severe patients (61.4 and 62.2 years, respectively). This results indicate that younger patients are more likely to develop mild symptoms, whereas older age groups are more likely to develop more severe symptoms. These results are similar to the finding in other studies (Bonanad *et al*., 2020; Kim & Crimmins, 2020).

4.2. Haematological indices in COVID-19 patients:

In this study total WBC counts and neutrophil percentage were increased in moderate and severe COVID -19 patients, whereas in mild cases, the WBC counts were within the normal references values. The differences in WBC counts and neutrophil percentage between the mild cases and other types of severity were statistically significant (p=0.000).

These result are similar to (Chen *et al*., 2020), who found that WBC and neutrophil counts were significantly higher in severe cases than moderate cases were statistically significant (p=0.003; p=0.002, respectively). Also these result are similar to (Anurag *et al*., 2020).

In the current study lymphocytes were significantly decreased (p=0.001) in moderate and severe cases compared to the mild cases. These result are consistent with other studies (Chen *et al*., 2020; Dawood *et al*., 2020), who showed that the severity of COVID19 was associated with

lymphopenia. Lymphopenia associated with COVID-19 may be caused by the direct effect of SARS—COV2 virus on bone marrow.

Angiotensin converting enzymes 2, is expressed on the membrane e of hematopoietic stem cells (HSCs), it has been postulated that SARS-COV-2 may directly infect the pool of HSCs and pyroptosis in these cells (Ratajczak & Kucia, 2020). Therefore, the possible infection of bone marrow precursor cell by SARS-COV-2 can contribute to the reduction of lymphocyte production (Jafarzadeh *et al.*, 2021).

This study found no significant difference could be seen in hemoglobin and platelets among the three groups of patients (p=0.984; p= 0.309, respectively). These results are similar to that reported by the authors (Taj *et al*., 2021), who found that parameter like hemoglobin, platelet counts did not showed statistically significant association with severity of disease (p=0.648; p=0.673, respectively). And these result are compatible with (Song *et al*., 2020).

4.3. Biochemical markers in COVID -19 patients:

In this study, D. dimer and S. ferritin showed in several fold increase within moderate and severe groups in comparison to mild cases, and the differences in the levels of D. dimer and S. ferritin between the mild and the other group was statistically significant (r=0.259, p=0.018; r=0.264, p=0.019, respectively). These result are consistent with (Taj, *et al*. 2021; Afrin *et al*., 2021).

There is a correlation between D-dimer and the progression of severe COVID-19 infection. D-dimer is the principal breakdown fragment of fibrin and is used as a biomarker of fibrin formation and degradation (Adam *et al*., 2009).

D-dimer levels are also elevated in conditions of chronic inflammation, such as active malignancy, rheumatoid arthritis, sickle cell disease, and asthma (Naik *et al*., 2016).

D-dimer is directly connected with the activation of the pro inflammatory cytokine cascade, it is an important marker of coagulopathy, and elevated level is a predictor of COVID-19 progression (Magro 2020).

Ferritin is a key mediator of immune deregulation, especially under extreme hyperferritinemia, via direct immune-suppressive and pro-inflammatory effects, contributing to the cytokine storm (Abbaspour *et al.*, 2014). Serum ferritin levels during hospitalization may be important to recognize high risk individuals with COVID-19(Lin *et al*., 2020).

A significant increase in ferritin levels was demonstrated in patients with moderate and severe disease, compared to patients with mild disease (Dahan *et al*., 2020).

Lactate dehydrogenase (LDH) level was slightly elevated in the mild cases, however, it was almost two times increased in the moderate and severe cases. The difference in LDH levels is positively correlated with degree of severity (r=0.441, *p*=0.000). These result are similar to (Li *et al*., 2020).

It has been reported that elevated serum LDH levels are associated with poor prognosis in various diseases, especially in tumors and inflammation. To date, studies have shown that patients with severity COVID -19 have elevated serum LDH levels(Li *et al.*, 2020).

C. reactive protein (CRP) showed the most prominent elevation in the mild cases, where it was increased more than three time in comparison to the reference values. This result make it the most reliable indicator for COVID-19 biomarkers. In addition, CRP was positively correlated with the

degree of severity (r=0.317, *p*=0.003). These result are consistent with (Xu *et al*. 2020).

CRP is an acute phase inflammatory protein produced by the liver that may be elevated in several conditions, such as inflammation, cardiovascular disease, and infection (Sproston & Ashworth, 2018).

In CoVID-19 patients, LDH and CRP might represent an expression of lung damage and might reflect the respiratory distress consequent to the abnormal inflammation status (Poggiali *et al*., 2020).

This study showed that in mild patients, the liver enzymes were in the normal range, and no increase could be seen in their mean levels. This result agrees with the previous study (Saini *et al*., 2020).

In moderate patients, only Alanine aminotransferase (GPT) levels were elevated. Whereas, in severe patients, both Alanine aminotransferase (GPT) and Aspartate aminotransferase(GOT) were elevated in comparison to mild patients. The increase in the levels of GPT and GOT were statistically significant (*p*=0.049 and *p*=0.033, respectively). These results are consistent with (Chen *et al.*, 2020), who said that Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were significantly higher in severe cases than moderate cases (*p*=0.000, *p*=0.014, respectively). This result also agrees with (Xu et al., 2021).

SARS-CoV-2 virus bind to angiotensin-converting enzyme 2 (ACE2) on cholangiocytes, leading to cholangiocyte dysfunction and inducing a systemic inflammatory response leading to liver injury (Chai *et al*., 2020). Patients with abnormal liver tests were at increased risk of progressing to severe disease (Cai *et al*., 2020).

4.4. IL-6 and TNF-α levels in COVID -19 patients:

In this study the serum levels of IL-6 and TNF-α in mild cases were elevated, nevertheless, the increase in the cytokine levels were much more in moderate and severe patients, (for IL-6; r=0.14, *p*=0.20 and for TNF-α; r=0.199, *p*=0.07). These result are consistent with **(**Liu *et al*., 2020; Udomsinprasert *et al.*, 2020), they found the IL-6 and TNF-α levels increased with severity of disease.

It is worth mention that their elevation in the mild cases makes them more reliable indicators than biomarkers like D. dimer, S. ferritin and LDH.

Recent studies have reported an increase in serum cytokine levels in COVID-19 patients, especially in severe patients, and suggest that cytokine storm is associated with disease severity (Liu *et al*., 2020).

4.5. Correlation of serum levels of IL6 and TNF alpha with the hematological parameters:

The current study showed high levels of IL-6 were associated with decreased lymphocyte percentage (r=-0.265, *p*=0.016). This result agrees with the previous study (Khan *et al*., 2021), who found that Lymphocyte count negatively correlated with IL-6 levels. This study showed increased neutrophils percentages with IL-6 levels (r=0.220, *p*=0.046). This result agrees with the previous study (Chen *et al*., 2020).

Because the cytokine storm plays a crucial role in the pathogenesis of COVID-19, cytokines including IL-10, IL-6, and tumor necrosis factor-α may trigger neutrophil activation and proliferation as well as lymphocyte apoptosis and destruction of lymphatic tissue (Fouad *et al*., 2021).

4.6. Correlation of serum levels of IL6 and TNF alpha with the hematological parameters within the severity groups:

In this study the severe COVID -19 groups a significant positive correlation were found between IL-6 levels and the percentage of neutrophils (r=0.482, *p*=0.009). These results are consistent with (Sayah *et al*., 2021), they found positive correlations were found between IL-6 levels and neutrophil counts (r = 0.483, $p < 0.0001$).

In the current study shows a negative correlations were reported in mild cases between TNF-α levels and percentages of lymphocytes (r=0.44, *p*=0.030). These result are compatible with (Jafarzadeh, *et al*. 2021).

Lymphopenia has been related to higher levels of cytokines, especially IL-6 and TNF alpha (Diao *et al*., 2020).

In SARS-CoV-2, the underlying mechanisms of reduced lymphocyte counts have not yet been delineated. It was recently suggested that the cause of lymphopenia can be the SARS-CoV-2-induced activation of apoptosis in lymphocytes (Xiong *et al*., 2020). Alternatively, due to the cytokine storm, lymphocytes may be recruited to the lungs and other affected organs leading to their depletion. That might explain why lymphocyte counts are markedly reduced in patients with severe COVID-19 (Zheng *et al*., 2020).

This study found that negative correlation was found between TNF-α levels and hemoglobin levels within the moderate group (r=-0.415, *p*=0.031). This result is consistent with (Ke *et al*., 2020).

The significant change in hemoglobin may be explained by the fact that the virus adheres to the surface of hematopoietic cells through the angiotensin-converting enzyme (ACE) 2 receptor and enters the hematopoietic system. The substances released by the virus, viremia, and

endotoxins jointly influence the release of immune factors and immune regulatory function, affect hematopoietic stem/progenitor cells, and lead to an abnormal hematopoietic microenvironment; thereby, the hematopoietic function of bone marrow is inhibited. This ultimately affects the compensatory production of hemoglobin, causing a continuous decrease in hemoglobin and even hematopoietic failure or aplastic anemia (Zhang *et al*., 2020).

4.7. Correlation of serum levels of IL6 and TNF alpha with the Biochemical parameters:

The current study showed positive correlation between IL-6 and serum ferritin levels (r=0.286, *p*=0.011). This results agree with (Ponti *et al*., 2020).

This study showed negative correlation with AST level (r= -0.224, *p*=0.05).This result disagrees with (Effenberger *et al*., 2021).

4.8. Correlation of serum levels of IL6 and TNF alpha with the Biochemical parameters within the severity groups:

In the current study only AST was noted to show negative correlation with IL-6 levels within the severe group (r=-0.451, *p*=0.031). This result disagrees with previous study (Effenberger *et al*., 2021), they found IL-6 positively correlated with AST in all patients (r=0.481, *p*<0.001).

In this study D. dimer showed significant positive correlation with TNF-α levels (r=-0.406, *p*=0.039 within the severe COVID -19 group. These result are contrast with the study of (Liu *et al*., 2020).

Prognosis of patients with COVID significantly increased in D-dimer level(Yin *et al* .2020), another reported suggested a relationship between IL6, IL8, TNF alpha, INF alpha and activation of the coagulation cascade, involved D-dimer in critical cases(Pettilä *et al*., 2002).

4.2. Conclusions:

1. The mean age of the mild COVID-19 patients is lower than the mean age of the moderate and severe cases.
2. Lymphopenia and neutrophilia in addition to increased total blood cell counts appears in the most severe cases (moderate to severe) but not in the mild cases.
3. Only C.reative protein is shown to be elevated in mild cases in addition to other severity groups. Whereas other biomarkers such as D. dimer, S. ferritin were elevated in the more severe forms of the COVID-19 disease.
4. The levels of both of IL-6 and TNF-α were elevated in all severity groups, however, moderate and severe forms characterized by higher levels of the cytokines.
5. There was a positive correlation between IL-6 and neutrophils indicating that neutrophils production is affected by this proinflammatory cytokine.
6. A negative correlation between TNF-α and lymphocytes may indicate that this cytokine possibly has a direct effect on lymphocyte production in bone marrow.

4.3 Recommendations:

1. This study recommend the use IL-6 and TNF-α serum levels as routine evaluation for every COVID-19 cases because their Levels are well correlated with severity of the disease.
2. Further study to correlate IL-6 and TNF-α serum levels with mortality and COVID -19 complications.
3. Clinical study to evaluate the efficacy of drugs targeting TNF-α or TNF-α receptor.

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الخلاصة

مرض فيروس كورونا 2019 هومرض معد بسبب الالتهاب الرئوي الحاد الوخيم الفيروسي 2. يسبب فيروس كورونا مرضًا تنفسيًا خطيرًا مثل الالتهاب الرئوي وفشل الرئة ، وقد تم الإبلاغ عنه لأول مرة في ووهان ، عاصمة هوبي ، وسرعان ما انتشر في كل الدول تقريباً مما تسبب في جائحة. يتميز فايروس كورونا بعاصفة خلوية تتجلى في ارتفاع مستويات العديد من العلامات الالتهابية والكيميائية الحيوية والتغيرات في مؤشرات الدم.

كان الهدف من هذه الدراسة هو تقييم دور مستويات انترلوكين 6 و TNF-alpha مع المعلمات الدموية والمصلية الأخرى في حالة المرض لدى مرضى كوفيد 19الذين يخضعون للعلاج.

لهذا الغرض تم تسجيل ما مجموعه 84 مريضًا مصابًا بـ فايروس كورونا، حيث تم تأكيد الاصابة عن طريق SARS-COV-2 RT-PCR لعينات مسحة البلعوم الأنفي . تم علاج المرضى في مستشفى مدينة الحسين الطبية للفترة من 10-10-2020 حتى 29-12-2020.حيث وجد ان المرضى كان بينهم 54 (64.3٪) ذكور و 30 (35.7٪) إناث. كان متوسط العمر 56.08 سنة والمدى العمري ما بين 25 إلى 85 سنة. هده النتائج تشير الى ان المرضى الذكور الاكبر سناً هم الاكثر عرضة للخطر.

زاد العدد الكلي لخلايا الدم البيضاء زيادة معنوية في الحالات المتوسطة والشديدة مقارنة بالحالات الخفيفة(p=0.000). علاوة على ذلك زادت نسبة خلايا نتروفيل معنوياً(p=0.000)، بينما انخفضت نسب الخلايا الليمفاوية (p=0.001) في مرضى المعتدل والشديد مقارنة بالمرضى الخفيف.

اكثر العلامات البايوكيميائية في هده الدراسة هي :دي دايمر، فيريتين مصل الدم ، لاكتات ديهيدروجينيز، بروتين سي التفاعلي ، ألانين أمينو ترانسفيراز، أسبارتات أمينوترانسفيراز أضهرارتباطاً ايجابياً مع شدة اعراض فايروس كورونا (r=0.259, p=0.018; r=0.264, p=0.019; r=0.441, p=0.000; r=0.317, p=0.003; r=0.225, p=0.049; r=0.234, p=0.033)

ومع ذلك ، كان الفوسفاتيز القلوي هوالعلامة الحيوية الوحيدة الدي تمت دراستها والتي لا ترتبط بشدة اعراض مرض كورونا (r=0.087, p=0.449).

مستويات كل من IL-6 وTNF-α كانت مرتفعة في مرضى كورونا وهذا الارتفاع في المستويات كان عالي في المرضى المتوسطة والشديدة مقارنة بمرضى الخفيفة (p=0.20, p=0.07).

ضمن مرضى كوفيد 19 الحادين، ارتبطت مستويات انترلوكين 6 في المصل سلباً بنسب الخلايا الليمفاوية(r=-0.36, p=0.06) و ارتبطت ايجاباً بنسب النتروفيل (r=0.482, p=0.009). من ناحية اخرى، في مرضى كوفيد 19 الخفيف، كانت مستويات مصل TNF-α مرتبطة سلباً بنسب الخلايا اليمفاوية (r=-0.44, p=0.03) ومع نسب الهيموغلوبين (r=-0.415, p=0.031).

ارتبطت مستويات انترلوكين 6 في الدم بشكل ايجابي مع فيرتين المصل (r=0.286, p=0.011) وارتبطت سلباً مع الاسبارتات امينوترانسفيراز(r=-0.224, p=0.05). ومع ذلك ، لم تظهر مستويات مصل TNF-alphaارتباطاً كبيراً بالعلامات البيوكيميائية المدروسة في مرضى كوفيد 19. ومع ذلك، كان TNF-alpha مرتبطاً بشكل ايجابي مع دي-دايمر في مرضى كوفيد 19 الحاد(r=0.406, p=0.039).

في الختام، جميع المؤشرات الحيوية والسايتوكينات المدروسة مرتفعة وكانت هناك تغيرات دمية في مرضى كوفيد 19، ومع ذلك ،لايمكن إرجاع جميع التغييرات في المعلمات البيوكيميائية والدمية إلى المستويات المرتفعة من انترلوكين 6 وTNF-alpha . لذلك ، توصي هذه الدراسة بدراسة السايتوكينات الأخرى المسببة للالتهابات.

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء / كلية الطب

فرع الاحياء المجهرية

ارتباط مستويات IL-6 و TNF alpha بالمعايير الدموية والمصلية في مرضى جائحة COVID-19 الخاضعين للعلاج

رسالة مقدمة الى

مجلس كلية الطب وهي جزء من متطلبات نيل شهادة الماجستير في الاحياء المجهرية

من قبل الطالبة

فاطمة تركي خليف

بكالوريوس علوم 2006

بإشراف

الاستاذ الدكتور

مهند محسن العتبي

الاستاذ الدكتور

خالد خليل الاعرجي

2021

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