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

Association of serum chemokine receptor 1and chemokine receptor 5 with COVID-19 severity

A THESS
Submitted to the council of the College of Medicine/University of Kerbala, for the fulfillment of the requirement for the degree Master of Science in Medical Microbiology.
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2021 AD **1443 A.H**

﴿ يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴾

صدق الله العلي العظيم

[سورة المجادلة: (١١)]

“Supervisors Certification”

We certify this thesis was prepared under our supervision at the College of Medicine, University of Kerbala, as a partial fulfillment of the requirement for the Degree of Master of Science in Medical Microbiology

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Dedication

To my father and mother

To my family

To everyone I love…

Saja-2021

Acknowledgment

First, I would like to thank “God” for his care and support throughout my life.

I would like to express my deepest thanks and sincere gratitude to my supervisor, Dr. Sawsan M. Jabbar. Al-Hasnawi for her valuable scientific guidance, understanding, encouragement and support that enabled me to complete this study.

I express my gratitude to the Deanship and Department of Microbiology at the College of Medicine / University of Karbala for providing all facilities and guidance.

Sincere thanks to all the consultants and other staff at Al-Zahra Hospital, Department of Internal Medicine in Imam Al-Hussein Educational City, Dr. Qassem Al-Azzawi, and I would also like to thank the laboratory workers, especially the blood draw section

Sincere and many thanks to the patients and for the health control and mercy for those who died in the central care unit and everyone who contributes to raising the level of my work...

Saja-2021

Summary

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), is now pandemic with nearly 219 million cases reported to date. Although the majority of COVID-19 patients experience only mild or moderate symptoms, a subset will progress to severe disease with pneumonia and acute respiratory distress syndrome (ARDS) requiring mechanical ventilation. Emerging results indicate a dysregulated immune response characterized by runaway inflammation, including cytokine release syndrome (CRS). Critically ill COVID-19 patients experiencing chemokine storm are believed to have a worse prognosis and increased fatality rate.

In SARS-CoV-2 infected patients, chemokine storm appears important to the pathogenesis of several severe manifestations of COVID-19 as acute respiratory distress syndrome. The chemokine receptor seems to be a key player since it is involved in the development and progression of the most dangerous and potentially life-threatening COVID-19. Understanding the pathogenesis of chemokine storm will help unravel not only risk factors for the condition but also therapeutic strategies to modulate the immune response and deliver improved outcomes in COVID-19 patients at high risk for severe disease. So, the aim of the current study is to reveal the relation between chemokine receptors (CCR1, CCR5) and severity of COVID-19 disease with its role in pathogenesis and future possible treatment. Ninety blood samples were taken from patients who were according clinical examine ,PCR result and CT scan diagnosed with coronavirus. Samples were taken from both sexes (50) males and (40) females, their ages ranged between (20-85) and were divided into moderate and sever-critical cases.

All included patients were from the Internal Medicine Department and the Intensive Care Unit of Al-Husseini Teaching Hospital in Karbala / Iraq during the period from December 2020 until February 2021. Analysis of blood samples was done using ELISA kits for chemokine receptors CCR1 and CCR5. The mean CCR1 & CCR-5 levels were significantly lower in patients with severe-critical cases than those with moderate COVID-19 with P*<0.001*.

Receiver Operating Characteristics (ROC) curve analysis was performed and revealed that CCR1 & CCR-5 were good predictor of severe form of disease with the area under the ROC curve (AUC) = 0.937, 0.852 respectively with high sensitivity and specificity. This study shown that lower serum level of CCR1 and CCR5 associated with more severe disease status and both receptors could be regarded as good biomarkers for severity in COVID-19 infection.

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List of Abbreviations

Abbreviations	Meaning
SARS	Severe acute respiratory syndrome
MERS	Middle East Respiratory Syndrome
COVID-19	corona virus disease of 2019
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
HCoV-NL63	human coronavirus NL63
TGEV	transmissible gastroenteritis coronavirus
PEDV	Porcine epidemic diarrhea virus
PRCV	porcine respiratory coronavirus
MHV	mouse hepatitis coronavirus
BCoV	bovine coronavirus
IBV	infectious bronchitis coronavirus
PdCV	porcine deltacoronavirus

CoVs	coronavirus
ssRNA	single stranded RNA
FCoV	Feline Coronavirus
TM	transmembrane
SDS-PAGE	sodium dodecyl sulphate–polyacrylamide gel electrophoresis
S protein	Spike glycoprotein
M protein	Membrane Protein
ER	endoplasmic reticulum
E Protein	Envelope Protein
HE protein	Hemagglutinin-esterase
ACE2	engage angiotensin-converting enzyme 2
HS	heparan sulfate
RBD	receptor binding domain
IL-6	interleukin 6
CRS	cytokine release syndrome
AT2	type II alveolar cells
TMPRSS2	Transmembrane Protease, Serine stands
ARDS	acute respiratory distress syndrome
GI	gastrointestinal
GCSF	Granulocyte colony-stimulating factor
MCP-1	Monocyte chemoattractant protein-1
IP-10	Interferon gamma-induced protein 10
MIP-1A	Macrophage Inflammatory Proteins -1 alpha
TNF-α	tumor necrosis factor alpha
NK	natural killer
PD-1	Programmed death-1
Tim-3	T-cell immunoglobulin mucin-3
HLA-E	Human Leukocyte Antigen –E
IFNγ	Interferon gamma
Th1	T helper 1 cells
PRRs	pattern recognition receptors

PAMPs	pathogen associated molecular patterns
nuclear factor kB	nuclear factor kappa-light-chain-enhancer of activated B cells
ICU	intensive care unit
WHO	World Health Organization
RT	respiratory tract
CK	Creatine Kinase
LPV/RTV	Lopinavir/Ritonavir
USFDA	US Food and Drug Administration
HCQS	Hydroxychloroquine Sulfate
CCR1	CC chemokine receptors -1
CCR5	CC chemokine receptors -5
CCL27	C-C Motif Chemokine Ligand 27
CCL11	C-C Motif Chemokine Ligand 11
CXCL12	C-X-C Motif Chemokine Ligand12
CXCL1	C-X-C Motif Chemokine Ligand1
GPCR	G protein-coupled receptor

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Chapter One

Introduction and Literature Review

1.1. Introduction

A novel coronavirus (CoV) named ‘2019-nCoV’ or ‘2019 novel coronavirus’ or ‘COVID-19’ by the World Health Organization (WHO) is in charge of the current outbreak of pneumonia that began at the beginning of December 2019 near in Wuhan City, Hubei Province, China (Hui *et al* ,.2019) &(Fong *et al* ,. 2020). The virus is an RNA virus, and this genetic composition allows it to replicate quickly within cells and spread rapidly among what threatens to cause a global epidemic. Because of the permanent change in the composition of his genetic tape is what makes any treatment for the virus difficult. (Mousavizadeh & Ghasemi, , 2020) & (Felsenstein, *et al* 2020).

inflammatory mediators include TNF-α, interleukins such as IL-1β and IL-6 as well as chemokines. Clinical studies have identified a cytokine storm in the third stage of disease progression in critical ill patients with coronavirus disease 2019 (COVID-19). Hence, effectively suppressing the uncontrolled immune response of the host towards the invaded viruses in a cytokine storm is a critical step to prevent the deterioration of patient conditions and decrease the rate of mortality. Therapeutic monoclonal antibodies (mAbs) are found to be effective for the management of acute respiratory distress syndrome in patients with COVID-19. Clinical trial data indicated that therapeutic monoclonal antibodies targeting interleukins (IL-6, IL-1ra, IL-8, IL-1β, IL-17A, IL-33), interferon-gamma, tumor necrosis factor-alpha, P-selectin, connective tissue growth factor,(Patel., Saxena., & Mehta, (2021)).

The chemokine receptors CCR1, CCR2, and CCR5 were shown to be protective in a mouse model infected with MA15-SARS-CoV, and in human DCs infected with SARS-CoV. The deficiencies of these receptors were associated with severe disease and mortality due to the reduction in the recruitment of immune cells into the lungs(Kawabata,., Hagio,., & Matsuoka,. (2002)) .

Increase in the transcription of CCR5 and reflected the activation of their signaling pathways. The deficiency of these receptors in mice infected with mouse-adapted SARS-CoV virus aggravated the disease and increased the mortality due to defect in directing immune cells into the sites of viral infection (Xiong, *et al* ., 2020). a recent epidemiological analysis suggested that the CCR5 delta32 mutation is associated with increased susceptibility to SARS-CoV-2 infection and fatal COVID-19 outcome (Panda et al.,2020). CCR5 is known to play a decisive role as a chemotactic receptor abundantly expressed on monocytes, macrophages and T-cells and its heterozygous mutation-related deficiency implies impaired memory CD4 + T-cell response (Martín-Leal, et al .,2020). Thus, in regard to the clinical course of COVID-19, the roles of CCR5 (Ellwanger,et al .,2020) within the oral–pharyngeal immune system as the first line of antiviral immune defense and (Kindberg, et al .,2008) as a critical receptor governing adaptively induced T-cell memory provide a compelling immune-mechanistic rationale for the putative association of the delta32 near-loss-of-function mutation of CCR5 with altered susceptibility to SARS-CoV-2 infection and COVID-19 morbidity (Rijkers,et al 2020, Zheng et al .,2020, Pauza et al .,2015,and Ellinghaus,et al .,2020).

Aim of study :

The aim of the study is to reveal the relation between chemokine receptors (CCR1, CCR5) and severity of COVID-19 disease with its role in pathogenesis through the following**:**

1. ELISA Detection of serum chemokine receptor1 (CCR1) & serum chemokine receptor5 (CCR5) in moderate and sever –critical patients.
2. Correlate serum levels of Chemokine receptors with COVID-19 severity.
3. Determination of c- reactive protein in serum of patient by agglutination reaction .

4. Determination of ferritin in serum of patient by enzyme linked fluorescent assay.
5. Determination of D.dimer in serum of moderate and sever –critical patients by chemilumination immune assay analyzer
6. Determination of CBC in blood of patients by swelab alfa complete count analyzer

Literature Review

1.2 Coronaviruses

Coronaviruses are a group of related RNA viruses that cause diseases in mammals and birds. In humans, these viruses cause respiratory tract infections that can range from mild to lethal. Mild illnesses include some cases of the common cold (which is similar to common cold caused by other viruses, predominantly rhinoviruses), while more lethal varieties can cause SARS(Severe acute respiratory syndrome), The viral genome is(26–32) kilobases in length. The particles are typically decorated with large (~20 nm), club- or petal-shaped surface projections (the "peplomers" or "spikes"), which in electron micrographs of spherical particles create an image reminiscent of the solar corona (Rajeev Shah *et al* .,2020) .

MERS(Middle East Respiratory Syndrome), and COVID-19(corona virus disease of 2019) SARS-CoV-2(Severe acute respiratory syndrome coronavirus 2) is the causative agent for the pandemic COVID-19 outbreak, was first found in Wuhan, China, and initial analysis of viral RNA obtained from patients hospitalized in late 2019 revealed it was 96% identical at the whole-genome level to a bat SARS-like coronavirus (Bergmann *et al*., 2020).

COVID-19 is a novel coronavirus with an outbreak of unusual viral pneumonia and then pandemic. Based on its phylogenetic relationships and genomic structures the COVID-19 belongs to genera Beta-coronavirus. Human Beta-coronaviruses

(SARS-CoV- 2, SARS-CoV, and MERS-CoV) have many similarities, but also have differences in their genomic and phenotypic structure that can influence their pathogenesis. COVID-19 is an enveloped virus containing single stranded (positive-sense) RNA associated with a nucleoprotein within a capsid comprised of matrix protein. (Mousavizadeh ., & Ghasemi, 2020).

1.3 Coronavirus history

SARS-CoV emerged first in southern China and rapidly spread around the globe in 2002–2003. In November 2002, an unusual epidemic of atypical pneumonia with a high rate of nosocomial transmission to health-care workers occurred in Foshan, Guangdong, China. In March 2003, a novel CoV was confirmed to be the causative agent for SARS, and was thus named SARS-CoV . A 64-year-old nephrologist who travelled from southern China to Hong Kong on 21 February 2003 became the index case of subsequent large community and health-care-associated outbreaks of SARS in Hong Kong and other regions. The high infectivity of SARS was highlighted by the super-spreading event at a major teaching hospital in Hong Kong in which 138 people, including many previously healthy health-care workers, were infected within(2)weeks of exposure to an index patient who was being managed in a general medical ward for community-acquired pneumonia. Through international air travel, SARS-CoV was spread to(29) countries and regions with a total of (8,098) cases and (774) fatalities (9.6% of cases) by the end of the epidemic in July 2003, (Zumla *et al*., 2016) .

Horseshoe bats (genus Rhinolophus) as the natural reservoirs of SARS-related CoVs and the likely origin of SARS-CoV. In 2016, Swine Acute Diarrhea Syndrome (SADS)-CoV caused the death of over (25,000) pigs in farms within Guangdong province. This virus appears to have originated within Rhinolophus spp. bats, and

belongs to the HKU2-CoV clade previously detected in bats in the region. In 2019, a novel CoV (SARS-CoV-2) causing respiratory illness (COVID-19) was first reported in Wuhan, Hubei province, China. This emerging human virus is closely related to SARS-CoV, and also appears to have originated in horseshoe bats with its full genome 96% similar to a viral sequence reported from Rhinolophus affinis. Closely related sequences were also identified in Malayan pangolins(Latinne, *et al* .,2020). Since then, numerous studies have described novel bat CoVs, including close relatives of the newly emerging Middle East respiratory syndrome (MERS) CoV , (Drexler *et al*., 2014) .

The Middle East Respiratory Syndrome coronavirus (MERS-CoV) is a positive-sense RNA virus of the coronaviridae family that was first reported in Saudi Arabia in 2012. Information about its transmission pattern is still scant. Although MERS-CoV has been shown to have a fatality rate as high as 40% , no medication or vaccine with proven efficacy has yet been developed. Although the first case of MERS-CoV was reported in Saudi Arabia in October 2012, nosocomial infection with MERS-CoV was reported in Jordan as early as March 2012 . Thereafter, MERS-CoV has caused outbreaks of various scales in hospitals, while community-acquired infections have remained extremely rare. Although most patients with MERS-CoV have been reported in the Middle East, both before and after the outbreak in the Republic of Korea (hereafter Korea) in May 2015, the outbreak in Korea was the largest in any country other than Saudi Arabia. This outbreak made MERS-CoV an important international health issue . After the first outbreak in Pyeongtaek St. Mary's Hospital, where the patient zero was hospitalized, the outbreak spread when infected patients moved to other hospitals, resulting in an unprecedentedly large outbreak in Korea.(Kim *et al* ., 2015).

The first human cases of COVID-19, the disease caused by the novel coronavirus causing COVID-19, subsequently named SARS-CoV-2 were first

reported by officials in Wuhan City, China, in December 2019. Retrospective investigations by Chinese authorities have identified human cases with onset of symptoms in early December 2019. While some of the earliest known cases had a link to a wholesale food market in Wuhan, some did not. Many of the initial patients were either stall owners, market employees, or regular visitors to this market. Environmental samples taken from this market in December 2019 tested positive for SARS-CoV-2, further suggesting that the market in Wuhan City was the source of this outbreak or played a role in the initial amplification of the outbreak. The market was closed on 1 January 2020, It was documented as a public health emergency of international concern and a pandemic on 30 January and 11 March 2020, respectively, making SARS-CoV-2 the first hCoV to cause a pandemic (Zhu *et al.*,2020).

On 31 December 2019, the Wuhan Municipal Health Commission in Wuhan City, Hubei province, China, reported a cluster of (27) cases of pneumonia which were said to be linked to a wholesale fish and live animal market in the city. The first recorded cases of what would become known as coronavirus disease (COVID-19) and the virus that causes it (the severe acute respiratory syndrome coronavirus 2 - SARS-CoV-21) was confirmed in China in early January. The genetic sequence of the virus was shared publicly on 11–12 January shortly after the first death had been recorded in China that of a 61-year-old man with underlying health conditions. By 13 January Thailand had recorded its first case the first outside of China and by 20 January human-to-human transmission of the disease was confirmed by the Lancet medical journal .The first cases of COVID-19 in Europe were recorded in France and Germany on 24 and 28 January 2020, respectively. (Colfer and Barry., 2020). On March 11, 2020, the World Health Organization declared Coronavirus Disease 2019 (COVID-19) a global pandemic (Delgado *et al.*, 2020).

1.4Classification of Coronaviridae

The coronaviruses (CoVs) belong to the genus Coronavirus, the family Coronaviridae, and the order Nidovirales ,(Paules,et al.,(2020). They are enveloped and have a non-segmented, single-stranded, positive-sense ribonucleic acid (ssRNA+) as their nuclear material The RNA group of viruses is classified into three orders that include the order Nidovirales, which is further classified into four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus . (Fehr, A. & Perlman,. (2015). Among them, alpha- and betacoronaviruses infect mammals, gammacoronaviruses infect avian species, and deltacoronaviruses infect both mammalian and avian species. Representative alphacoronaviruses include human coronavirus NL63 (HCoV-NL63), porcine transmissible gastroenteritis coronavirus (TGEV), Porcine epidemic diarrhea virus (PEDV), and porcine respiratory coronavirus (PRCV). Betacoronaviruses include SARS-CoV, MERS-CoV, bat coronavirus HKU4, mouse hepatitis coronavirus (MHV), bovine coronavirus (BCoV), and human coronavirus OC43. Gamma and delta coronaviruses include avian infectious bronchitis coronavirus (IBV) and porcine deltacoronavirus (PdCV), respectively. (Li, 2016). coronavirus (CoVs)have been identified as human-susceptible virus,among which α-CoVs HCoV-229E and HCoV-NL63, and β-CoVs HCoV-HKU1 and HCoV-OC43 with low pathogenicity, cause mild respiratory symptoms similar to a common cold, respectively. The other two known β-CoVs, SARS-CoV and MERS-CoV lead to severe and potentially fatal respiratory tract infections . It was found that the genome sequence of SARS-CoV-2 is(96.2%) identical to a bat CoV RaTG13, whereas it shares(79.5%)identity to SARS-CoV. Based on virus genome sequencing results and evolutionary analysis, bat has been suspected as natural host of virus origin, and SARSCoV-2 might be

transmitted from bats via unknown intermediate hosts to infect humans. It is clear now that SARS-CoV-2 could use angiotensin-converting enzyme 2 (ACE2), the same receptor as SARS-CoV , to infect humans (Guo, 2020) .

1.5 Virus structure

Coronaviruses form enveloped and spherical particles of(100–160 nm) in diameter. They contain a positive- sense, single stranded RNA (ssRNA) genome of (27–32 kb) in size , (Cui,*et al*., 2019).The coronavirus nucleocapsid (N) is a structural protein that forms complexes with genomic RNA, interacts with the viral membrane protein during virion assembly and plays a critical role in enhancing the efficiency of virus transcription and assembly, some studies have confirmed that N is a multifunctional protein , (McBride *et al* .,2014). The membranes of all coronaviruses contain at least three viral proteins. These are spike (S), the type I glycoprotein that forms the peplomers on the virion surface giving the virus its corona- or crown-like morphology in the electron microscope, the membrane (M) protein, a protein that spans the membrane three times and has a short N-terminal ectodomain and a cytoplasmic tail; and small membrane protein (E) .(Weiss., & Navas-Martin.,2005).

1.5.1 Spike glycoprotein ‘S’

Coronavirus S protein is a large multifunctional class I viral transmembrane protein. The size of this abundant S protein varies from (1160amino acids) Infectious Bronchitis Virus (IBV) in poultry to (1400 amino acids) Feline Coronavirus (FCoV) , It lies as a trimer on the virion surface, giving the virion a (corona) or crown-like appearance, Functionally it is required for the entry of the infectious virion particles

inside the cell through interaction with various host cellular receptors, (Kuldeep Dhama *et al* .,2020).

With a size of (180–200 kDa) the S protein consists of an extracellular N-terminus, a transmembrane (TM) domain anchored in the viral membrane and a short intracellular C-terminal segment , S protein normally exists in a metastable, prefusion conformation once the virus interacts with the host cell extensive structural rearrangement of the S protein occurs, allowing the virus to fuse with the host cell membrane. The spikes are coated with polysaccharide molecules to camouflage them, evading surveillance of the host immune system during entry (Yu *et al* ,.2017). Because they possess an envelope coronaviruses entry into host target cells requires the successful completion of two critical steps. The first is binding to the cell surface by means of attachment to a host cell receptor. The second is fusion of the viral envelope with cellular membranes allowing release of the virus genome into the host cell's cytoplasm, enabling viral replication to ensue. Both steps are controlled by the S envelope protein, (Millet and Whittaker ., 2014).

1.5.2 Membrane Protein (M)

The M glycoprotein (formerly called E1) is the most abundant constituent of coronaviruses and gives the virion envelope its shape. The preglycosylated M polypeptide ranges in size from 25 to 30 kDa (221–262 amino acids), but multiple higher-molecular-mass glycosylated forms are often observed by SDS-PAGE(sodium dodecyl sulphate–polyacrylamide gel electrophoresis) . The M protein of mouse hepatitis virus (MHV) has also been noted to multimerize under standard conditions of SDS-PAGE .M protein is a multispanning membrane protein with a small, aminoterminal domain located on the exterior of the virion, or intracellularly, in the lumen of the ER ,(Masters.,2006).

The M protein of coronavirus plays a central role in virus assembly, turning cellular membranes into workshops where virus and host factors come together to make new virus particles. M structure and organization is related to virus shape and size using cryo-electron microscopy, tomography and statistical analysis. Present evidence that suggests M protein can adopt two conformations and that membrane curvature is regulated by one M conformer. Elongated M protein is associated with rigidity, clusters of spikes and a relatively narrow range of membrane curvature. In contrast compact M protein is associated with flexibility and low spike density. Analysis of several types of virus-like particles and virions revealed that S protein, N protein and genomic RNA each help to regulate virion size and variation, presumably through interactions with M protein. These findings provide insight into how M protein functions to promote virus assembly,(Neuman *et al.,* 2011).

1.5.3 .Envelope Protein (E)

The CoV envelope (E) protein is a small, integral membrane protein involved in several aspects of the virus' life cycle, such as assembly, budding, envelope formation, and pathogenesis. Recent studies have expanded on its structural motifs and topology, its functions as an ion-channelling viroporin, and its interactions with both other CoV proteins and host cell proteins.(Schoeman *et al* .,2019). accessory proteins, such as HE protein(Hemagglutinin-esterase), 3a/b protein, and 4a/b protein . These mature proteins are responsible for several important functions in genome maintenance and virus replication.(Leila and Sorayya,.2020)

1.6 .Life cycle of SARS CoV-2

SARS-CoV-2 can hijack the cell in two ways, either via endosomes or via plasma membrane fusion. (In both ways) Spike proteins (S1, S2) of SARS-CoV-2 mediate attachment to the membrane of a host cell and engage angiotensin-converting enzyme 2 (ACE2) as the entry receptor(Hoffmann *et al* ,.2020).

Inhibitors like Griffithsin (Inhibitor III) bind to the spike glycoprotein, thus preventing viral entry. Cell surface vimentin (VIM) acts as a critical co-receptor and is essential for successful ACE-2 binding.(Henderson,*et al* ,.2020). Binding of heparan sulfate (HS) to the receptor binding domain (RBD) enhances binding to ACE2 as well. Viral adhesion may be inhibited by exogenous heparin. Heparin competes with HS for binding of the SARS-CoV-2 S protein (Shirato, *et al* ,.2018). When virions are taken up into endosomes, cathepsin L activates the spike protein. the pH dependent cysteine protease can be blocked by lysosomotropic agents, like bafilomycin A1 or ammonium chloride (Inhibitor Classes IV,V) Alternatively, the spike protein can cleaved between the S1 and S2 domains by the cellular serine protease TMPRSS2 in close proximity to the ACE2 receptor, which initiates fusion of the viral membrane with the plasma membrane (Inhibitor II: Camostat). (Hoffmann, *et al* ,.2020).

The plasma membrane fusion entry is less likely to trigger host cell antiviral immunity and therefore more efficient for viral replication.(Zhavoronkov *et al* .,2020). After the viral RNA is released into the host cell, polyproteins are translated. The coronavirus genomic RNA encodes nonstructural proteins (NSPs) that have a critical role in viral RNA synthesis, and structural proteins which are important for virion assembly. First, polyproteins pp1a and pp1ab, are translated which are cleaved by the Papain-like protease (Pl^{pro}) and 3C-like protease($3CL^{pro}$) (Inhibitor VIII) to

form functional NSPs as Helicase or the RNA replicase–transcriptase complex (RdRp), (Shin *et al* 2018) .

RdRp is responsible for replication of structural protein RNA. Structural proteins S1, S2, Envelope (E), Membrane (M) are translated by ribosomes that are bound to the endoplasmic reticulum (ER) and presented on its surface as preparation of viron assembly. The nucleocapsids (N) remain in cytoplasm and are assembled from genomic RNA. They fuse with the virion precursor which is then transported from the ER through the Golgi Apparatus to the cell surface via small vesicles. Virions are then released from the infected cell through exocytosis and search an another host cell. Oseltamivir inhibits cleavage of sialic acids by neuroamidase from the cell receptors thus preventing release of newly formed (influenza) virions from the cell surface,(McKimm-Breschkin, (2013).Infection of human cells by pathogens, including SARS-CoV-2, typically proceeds by cell surface binding to a crucial receptor. In the case of SARS-CoV-2, angiotensin-converting enzyme 2 (ACE2) has been identified as a necessary receptor, but not all ACE2-expressing cells are equally infected, suggesting that other extracellular factors are involved in host cell invasion by SARS-CoV-2. Vimentin is an intermediate filament protein that is increasingly recognized as being present on the extracellular surface of a subset of cell types, where it can bind to and facilitate pathogens' cellular uptake. That extracellular vimentin might act as a critical component of the SARS-CoV-2 spike protein-ACE2 complex in mediating SARS-CoV-2 cell entry. Direct binding between vimentin and SARS-CoV-2 pseudovirus coated with the SARS-CoV-2 spike protein and show that antibodies against vimentin block in vitro SARS-CoV-2 pseudovirus infection of ACE2-expressing cells. (Suprewicz, et al .,(2021)

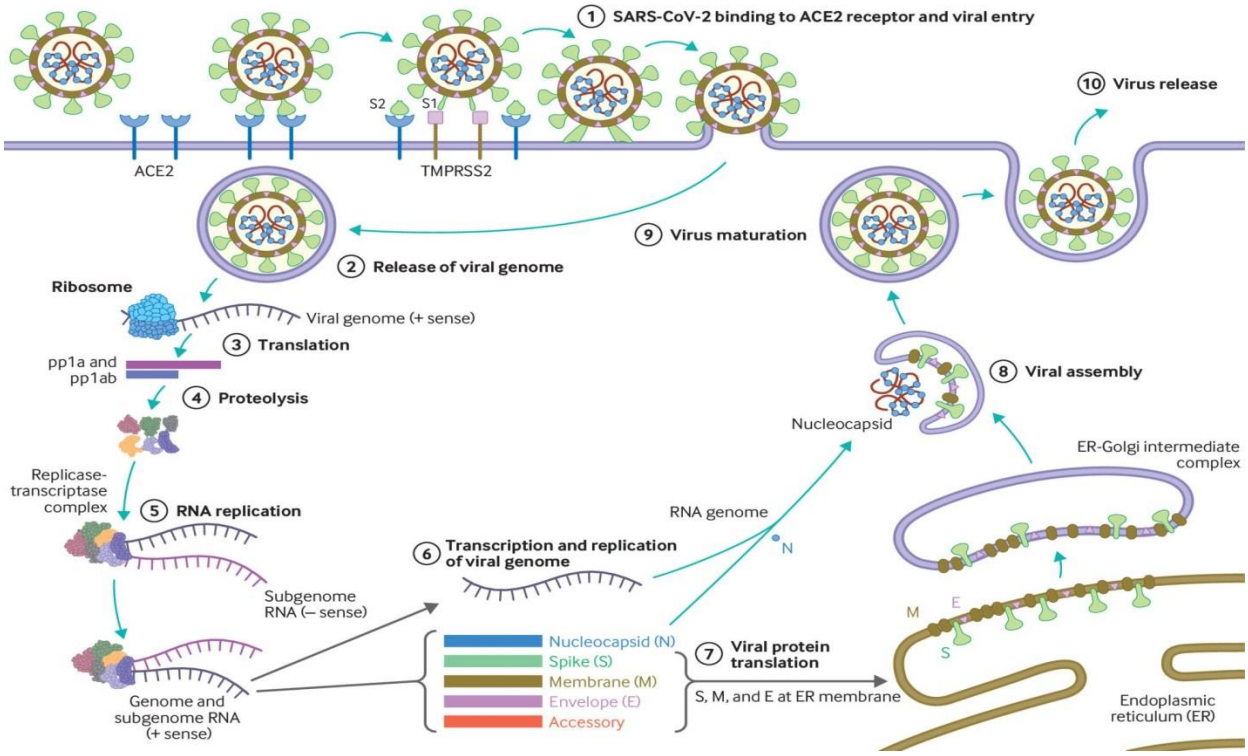


Fig 1.1 : (1) The virus binds to ACE 2 as the host target cell receptor in synergy with the host's transmembrane serine protease 2 (cell surface protein), which is principally expressed in the airway epithelial cells and vascular endothelial cells. This leads to membrane fusion and releases the viral genome into the host cytoplasm (2). Stages (3-7) show the remaining steps of viral replication, leading to viral assembly, maturation, and virus release (Cevik et al ,.2020).

1.7 Transmission

Unprecedented containment and mitigation policies have been implemented in an effort to limit the spread ofCOVID-19, including travel restrictions, screening and testing of travelers, isolation and quarantine, and school closures. A key goal of such policies is to decrease the encounters between infected individuals and susceptible individuals and decelerate the rate of transmission. Although such social distancing strategies are critical in the current time of pandemic, it may seem surprising that the current understanding of the routes of host-to-host transmission in respiratory

infectious diseases are predicated on a model of disease transmission developed in the 1930s that, by modern standards, seems overly simplified. Implementing public health recommendations based on these older models may limit the effectiveness of the proposed interventions.(Bourouiba,. (2020)).

Respiratory viruses are transmitted between individuals when the virus is released from the respiratory tract of an infected person and is transferred through the environment, leading to infection of the respiratory tract of an exposed and susceptible person. There are a number of different routes (or modes) through which transmission could occur, can be transmitted via respiratory secretions over multiple routes independently and simultaneously. Traditionally, it is believed that respiratory viruses are transmitted directly via physical contact between an infected individual (infector) and a susceptible individual (infectes), indirectly via contact with contaminated surfaces or objects (fomites) or directly through the air from one respiratory tract to another via large respiratory droplets or via fine respiratory aerosols. These four major modes of transmission (direct contact, indirect contact/fomite, droplet and aerosol) are often the foci of transmission control; for example, infection prevention and control measures in health-care settings are designed specifically for each mode. Some respiratory viruses, including influenza viruses, coronaviruses and rhinoviruses, can be recovered from faeces or infect cells in the gastrointestinal tract , suggesting infection may spread via faeces; for example, via aerosolization during toilet flushing. Studies have shown SARS-CoV-2 in ocular secretions and influenza virus infection by ocular exposure, suggesting respiratory viruses might also be transmitted via exposure to the eyes,(Leung,. (2021)).

SARS-CoV-2 RNA has also been detected in other biological samples, including the urine and feces of some patients.(Guan, *et al* .,2020)&(Zheng, *et al* .,2020). One study found viable SARS-CoV-2 in the urine of one patient.(Sun, *et al*

.,2020) Three studies have cultured SARS-CoV-2 from stool specimens. (Wang *et al* ., 2020),(Xiao, *et al* .,2020)&(Zhang *et al* ., 2020).

however, there have been no published reports of transmission of SARS-CoV-2 through feces or urine. Some studies have reported detection of SARS-CoV-2 RNA, in either plasma or serum, and the virus can replicate in blood cells. However, the role of bloodborne transmission remains uncertain; and low viral titers in plasma and serum suggest that the risk of transmission of SARS-CoV-2 – implications for infection prevention precautions: Scientific brief -3- transmission through this route may be low. (Wang *,et al* ., 2020)& (Le Chang, *et al.*,2020) .

Currently, there is no evidence for intrauterine transmission of SARS-CoV-2 from infected pregnant women to their fetuses, although data remain limited. WHO has recently published a scientific brief on breastfeeding and COVID-19.(Breastfeeding and COVID-19. Geneva: World Health Organization; 2020)This brief explains that viral RNA fragments have been found by RT-PCR testing in a few breast milk samples of mothers infected with SARS-CoV-2, but studies investigating whether the virus could be isolated, have found no viable virus. Transmission of SARS-CoV-2 from mother to child would necessitate replicative and infectious virus in breast milk being able to reach target sites in the infant and also to overcome infant defense systems. WHO recommends that mothers with suspected or confirmed COVID-19 should be encouraged to initiate or continue to breastfeed.(Breastfeeding and COVID-19. Geneva: World Health Organization; 2020) Evidence to date shows that SARS-CoV-2 is most closely related to known beta-coronaviruses in bats; the role of an intermediate host in facilitating transmission in the earliest known human cases remains unclear.(Andersen, *et al*.,2020)&(Zhou, *et al* .,2020) .

When do people infected with SARS-CoV-2 infect others? Knowing when an infected person can spread SARS-CoV-2 is just as important as how the virus spreads (described above). WHO has recently published a scientific brief outlining

what is known about when a person may be able to spread, based on the severity of their illness.(Criteria for releasing COVID-19 patients from isolation Geneva: World Health Organization; 2020) In brief, evidence suggests that SARS-CoV-2 RNA can be detected in people 1-3 days before their symptom onset, with the highest viral loads, as measured by RT-PCR, observed around the day of symptom onset, followed by a gradual decline over time.(Pan *et al* .,2020),(He, *et al* ,.2020)&(Wölfel. *et al* .,2020) The duration of RT-PCR positivity generally appears to be 1-2 weeks for asymptomatic persons, and up to 3 weeks or more for patients with mild to moderate disease,(He *et al* .,2020),(Wölfel, *et al* .,2020)&(Qi, *et al* ., 2020).

In patients with severe COVID-19 disease, it can be much longer.(Pan *et al* .,2020). Detection of viral RNA does not necessarily mean that a person is infectious and able to transmit the virus to another person. Studies using viral culture of patient samples to assess the presence of infectious SARS-CoV-2 are currently limited. (Criteria for releasing COVID-19 patients from isolation Geneva: World Health Organization; 2020) Briefly, viable virus has been isolated from an asymptomatic case,(Arons, *et al* .,2020) .

From patients with mild to moderate disease up to 8-9 days after symptom onset, and for longer from severely ill patients. (Criteria for releasing COVID-19 patients from isolation Geneva: World Health Organization; 2020) Full details about the duration of viral shedding can be found in the WHO guidance document on "Criteria for releasing COVID-19 patients from isolation". (Criteria for releasing COVID-19 patients from isolation Geneva: World Health Organization; 2020) Additional studies are needed to determine the duration of viable virus shedding among infected patients.

1.8. Sign and symptoms of Covid-19

Patients with SARS-CoV-2 infection can experience a range of clinical manifestations, from no symptoms to critical illness. In general, adults with SARS-CoV-2 infection can be grouped into the following severity of illness categories; however, the criteria for each category may overlap or vary across clinical guidelines and clinical trials, and a patient's clinical status may change over time.

Asymptomatic or Presymptomatic Infection*:* Individuals who test positive for SARS-CoV-2 using a virologic test (i.e., a nucleic acid amplification test [NAAT] or an antigen test) but who have no symptoms that are consistent with COVID-19.

Mild Illness: Individuals who have any of the various signs and symptoms of COVID-19 (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell) but who do not have shortness of breath, dyspnea, or abnormal chest imaging.

Moderate Illness: Individuals who show evidence of lower respiratory disease during clinical assessment or imaging and who have an oxygen saturation (SpO2) ≥94% on room air at sea level.

Severe Illness: Individuals who have SpO2 <94% on room air at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO2/FiO2) <300 mm Hg, a respiratory rate >30 breaths/min, or lung infiltrates >50%.

Critical Illness: Individuals who have respiratory failure, septic shock, and/or multiple organ dysfunction.

Patients with certain underlying comorbidities are at a higher risk of progressing to severe COVID-19. These comorbidities include being aged ≥65 years; having cardiovascular disease, chronic lung disease, sickle cell disease, diabetes, cancer, obesity, or chronic kidney disease; being pregnant; being a cigarette smoker; being a

transplant recipient; and receiving immunosuppressive therapy.(COVID, C. (19). and Your Health. Centers for Disease Control and Prevention.) Health care providers should monitor such patients closely until clinical recovery is achieved.

The optimal pulmonary imaging technique has not yet been defined for people with symptomatic SARS-CoV-2 infection. Initial evaluation for these patients may include a chest X-ray, ultrasound screening, or, if indicated, a computed tomography scan. An electrocardiogram should be performed if indicated. Laboratory testing includes a complete blood count with differential and a metabolic profile, including liver and renal function tests. Although inflammatory markers such as C-reactive protein (CRP), D-dimer, and ferritin are not routinely measured as part of standard care, results from such measurements may have prognostic value.(Tan ,et al .,2020)and (Casas-Rojo,et al.,2020)

Asymptomatic or Presymptomatic Infection

Asymptomatic SARS-CoV-2 infection can occur, although the percentage of patients who remain truly asymptomatic throughout the course of infection is variable and incompletely defined. It is unclear what percentage of individuals who present with asymptomatic infection progress to clinical disease. Some asymptomatic individuals have been reported to have objective radiographic findings that are consistent with COVID-19 pneumonia.(Verdoni,*et al .,2020)and(*Zhang *,et al .,20*20) Increasing the availability of virologic testing for SARS-CoV-2 and reliable serologic assays for SARS-CoV-2 antibodies will help determine the true prevalence of asymptomatic and presymptomatic.

Mild Illness

Patients with mild illness may exhibit a variety of signs and symptoms (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell). They do not have shortness of breath, dyspnea on exertion, or abnormal imaging.

Moderate Illness

Moderate illness is defined as evidence of lower respiratory disease during clinical assessment or imaging, with SpO2 ≥94% on room air at sea level. Given that pulmonary disease can progress rapidly in patients with COVID-19, patients with moderate disease should be closely monitored.

Severe Illness

Patients with COVID-19 are considered to have severe illness if they have SpO2 <94% on room air at sea level, PaO2/FiO2 <300 mm Hg, a respiratory rate >30 breaths/min, or lung infiltrates >50%. These patients may experience rapid clinical deterioration. Oxygen therapy should be administered immediately using a nasal cannula or a high-flow oxygen device.

Critical Illness

Critically ill patients may have acute respiratory distress syndrome, septic shock that may represent virus-induced distributive shock, cardiac dysfunction, an exaggerated inflammatory response, and/or exacerbation of underlying comorbidities. In addition to pulmonary disease, patients with critical illness may also experience cardiac, hepatic, renal, central nervous system, or thrombotic disease.

As with any patient in the intensive care unit (ICU), successful clinical management of a patient with COVID-19 includes treating both the medical condition that initially resulted in ICU admission and other comorbidities and nosocomial complications. For more information.

Infectious Complications in Patients With COVID-19

Some patients with COVID-19 may have additional infections that are noted when they present for care or that develop during the course of treatment. These coinfections may complicate treatment and recovery. Older patients or those with certain comorbidities or immunocompromising conditions may be at higher risk for these infections. The use of immunomodulators such as dexamethasone, interleukin-6

inhibitors (e.g., tocilizumab, sarilumab), or Janus kinase inhibitors (e.g., baricitinib, tofacitinib) to treat COVID-19 may also be a risk factor for infectious complications; however, when these therapies are used appropriately, the benefits outweigh the risks.

1.9 Pathogenesis of SARS-CoV-2

The virus mainly infects type 2 pneumocytes in the lung (Zhu *et al*., 2020).The pathogenic mechanism, that produces pneumonia seems to be particularly complex. The data to indicate that the viral infection is capable of producing an excessive immune reaction in the host. In some cases, a reaction takes place which as a whole is labeled a 'cytokine storm'. The effect is extensive tissue damage. The protagonist of this storm is interleukin 6 (IL-6). IL 6 is produced by activated leukocytes and acts on a large number of cells and tissues. It is able to promote the differentiation of B lymphocytes, promotes the growth of some categories of cells, and inhibits the growth of others. It also stimulates the production of acute phase proteins and plays an important role in thermoregulation, in bone maintenance and in the functionality of the central nervous system. Although the main role played by IL-6 is pro-inflammatory, it can also have anti-inflammatory effects. In turn, IL-6 increases during inflammatory diseases, infections, autoimmune disorders, cardiovascular diseases and some types of cancer. It is also implicated into the pathogenesis of the cytokine release syndrome (CRS) that is an acute systemic inflammatory syndrome characterized by fever and multiple organ dysfunction.(Cascella *et al.*,2020).

ACE2 levels in the nasal epithelium increase with age , which may contribute to the differential susceptibility of older individuals to COVID-19 (Bunyavanich *et al.*, JAMA 2020).

Following viral transmission, SARS-CoV-2 attaches to the surface of the epithelial membrane of the oral cavity, the mucosal membranes of the conjunctiva or the otic canal. ACE 2 protein, which is highly expressed on multiple human cells including type II alveolar cells (AT2), oral, esophageal, ileal epithelial cells, myocardial cells, proximal tubule cells of the kidneys as well as urothelial cells of the bladder (Zou, *et al* ,.2020) .

believed to mediate the internalization of SARS-CoV2. The spike (S) protein of SARS-CoV2 is cleaved by a cellular enzyme named furin at the S1/S2 site. This cleavage is essential for viral entry to the lung cells(Hoffmann, *et al* ,.2020). The activated S protein is primed by the TMPRSS2 and finally attaches ACE 2 receptors to enter the host cells. The genetic sequence of SARS-CoV-2 is homologous with the SARS-CoV, and the structure of (S) protein of these viruses is highly similar. They both use the same receptor to enter the host cell; however, SARS-CoV-2 binds ACE 2 receptors with tenfold higher affinity(Wrapp, *et al* ,2020).

Experimental studies suggest that the ACE 2/angiotensin (1–7) has a fundamental role in inflammation and signaling pathways contributing to tissue injury (Rodrigues Prestes, *et al* 2017). The physiological role of ACE 2 is the degradation of angiotensin II and the production of angiotensin (1–7), which counteracts ACE II (Tikelli., & Thomas,. (2012).

Following the viral replication in the host cell, downregulation of ACE 2 inhibits breakdown of angiotensin II into angiotensin (1–7). Disturbance in ACE 2/angiotensin (1–7) axis explains particular clinical features of COVID-19, such as hypokalemia, vasoconstriction (Zhao, *et al* ,. 2029) .

development of acute respiratory distress syndrome (ARDS) . Interestingly, the extent of ACE 2 expression in the gastrointestinal (GI), cardiovascular, genitourinary, endocrine (pancreas) and genitourinary (testis) systems is extremely higher than that in the predominant target of the virus, the respiratory system (Zou, *et al* ., 2020).

Evidence has not shown the presence of SARS-CoV-2 in some organs enriched with ACE 2 receptors, such as expressed prostatic secretion of COVID-19 patients (Zhang, *et al* ., 2020). Hence, there is no correlation between the virus infectivity and the level of ACE 2 expression. Limited evidence suggests that ACE 2 expression is attenuated in females compared with the males which could justify the higher number of COVID-19 cases in men (Zhao *et al* .,2020, Dashraath, *et al* .,2020).

Wang et al. identified CD 147 receptors as a novel route for the invasion of the virus. They showed that anti-CD147 humanized antibody inhibits the viral entry to host cells (Walls, *et al* ., 2020).

The severity of COVID-19 is positively correlated to the level of inflammatory cytokines such as interleukins (IL-2, IL-6, IL-7, IL-10), GCSF, IP-10, MCP-1, MIP-1A and TNF-α. In patients with severe disease, a significant reduction in lymphocyte count is observed (Zheng, *et al* .,2020),(Diao, *et al*., 2020), (Li, (2020). Flow cytometric analysis of severe COVID-19 patients demonstrates a remarkable reduction of lymphocytic T Cells (CD4+ and CD8+) and natural killer (NK) cells. Besides, an increase in the expression of natural killer group 2A (NKG2A), PD-1 and T-cell immunoglobulin mucin-3 (Tim-3) is associated with functional exhaustion of T lymphocytes in the early stage of the disease (Zheng, *et al* .,2020)&(Diao, *et al.*, 2020).

NKG2A is an inhibitory member of the NKG2 family and is expressed on NK cells, natural killer T (NKT) cells and a subset of CD8+ T cells. Interaction of NKG2A with Human Leukocyte Antigen- E (HLA-E) can inhibit the activation of NK cells, and T cells (Creelan,. & Antonia,. (2019)). PD-1 is expressed on both the T lymphocytes and NK cells. It is involved in subsiding immune responses and promoting self-tolerance through suppressing the activity of T cells and promoting differentiation of regulatory T cells (Salmaninejad, *et al* ., 2019).

T-cell immunoglobulin mucin-3 (Tim-3) is a co-inhibitory receptor that is expressed on IFN-γ producing T cells, and innate immune cells (e.g., macrophages and dendritic cells). It plays a key role in inhibiting T helper 1 cells (Th1) responses and the expression of cytokines such as TNF-α and IFN-γ (Das, & Kuchroo,. (2017)).

1.9.1 cytokine storm

In 2019–2020 a new coronavirus named SARS-CoV-2 was identified as the causative agent of a several acute respiratory infection named COVID-19, which is causing a worldwide pandemic. There are still many unresolved questions regarding the pathogenesis of this disease and especially the reasons underlying the extremely different clinical course, ranging from asymptomatic forms to severe manifestations, including the Acute Respiratory Distress Syndrome (ARDS). SARS-CoV-2 showed phylogenetic similarities to both SARS-CoV and MERS-CoV viruses, and some of the clinical features are shared between COVID-19 and previously identified beta-coronavirus infections. Available evidence indicate that the so called "cytokine storm" an uncontrolled over-production of soluble markers of inflammation which, in turn, sustain an aberrant systemic inflammatory response, is a major responsible for the occurrence of ARDS. Chemokines are low molecular weight proteins with powerful chemoattractant activity which play a role in the immune cell recruitment during inflammation.(Coperchini,. *et al* ,.2020).

Severe disease is associated with lymphopenia and an uncontrolled systemic inflammatory response called a cytokine storm, which ultimately leads to multiple organ failure and death,(Channappanavar., & Perlman,. ,2017).

COVID-19 infection is accompanied by an aggressive inflammatory response with the release of a large amount of pro-inflammatory cytokines in an event known as "cytokine storm." The host immune response to the SARS-CoV-2 virus is hyperactive resulting in an excessive inflammatory reaction. Several studies

analyzing cytokine profiles from COVID-19 patients suggested that the cytokine storm correlated directly with lung injury, multi-organ failure, and unfavorable prognosis of severe COVID-19 , The immune system has an exquisite mechanism capable of responding to various pathogens. Normal anti-viral immune response requires the activation of the inflammatory pathways of the immune system; however, aberrant or exaggerated response of the host's immune system can cause severe disease if remains uncontrolled ,(Ragab, *et al*,.2020).

Cytokines are an essential part of the inflammatory process. Cytokines are produced by several immune cells including the innate macrophages, dendritic cells, natural killer cells and the adaptive T and B lymphocytes , During an innate immune response to a viral infection, pattern recognition receptors (PRRs) recognize different molecular structures that are characteristic to the invading virus. These molecular structures are referred to as pathogen associated molecular patterns (PAMPs). Binding of PAMPs to PRRs triggers the start of the inflammatory response against the invading virus resulting in the activation of several signaling pathways and subsequently transcription factors which induce the expression of genes responsible for production of several products involved in the host's immune response to the virus, among which are the genes encoding several pro-inflammatory cytokines. The major transcription factors that are activated by PRRs are nuclear factor kB, activation protein 1, interferon response factors three and seven. These transcription factors induce the expression of genes encoding inflammatory cytokines, chemokines and adhesion molecules. This sequence of events results in recruitment of leukocytes and plasma proteins to site of infection where they perform various effector functions that serve to combat the triggering infection, (Thompson *et al* 2011).

Three of the most important pro-inflammatory cytokines of the innate immune response are IL-1, TNF- α, and IL-6. Tissue macrophages, mast cells, endothelial, and epithelial cells are the major source of these cytokines during innate

immune response. The “cytokine storm” results from a sudden acute increase in circulating levels of different pro-inflammatory cytokines including (IL-6, IL-1, TNF- α) and interferon. This increase in cytokines results in influx of various immune cells such as macrophages, neutrophils, and T cells from the circulation into the site of infection with destructive effects on human tissue resulting from destabilization of endothelial cell to cell interactions, damage of vascular barrier, capillary damage, diffuse alveolar damage, multiorgan failure, and ultimately death. Lung injury is one consequence of the cytokine storm that can progress into acute lung injury or its more severe form ARDS. (Shimizu M ,2019), The pathophysiology of unusually high pathogenicity for SARS-CoV or MERS-CoV has not been completely understood. Early studies have shown that increased amounts of proinflammatory cytokines in serum (eg, IL1B, IL6, IL12, IFNγ, IP10, and MCP1) were associated with pulmonary inflammation and extensive lung damage in SARS patients. MERS-CoV infection was also reported to induce increased concentrations of proinflammatory cytokines (IFNγ, TNFα, IL15, and IL17). the noted that patients infected with 2019-nCoV also had high amounts of IL1B, IFNγ, IP10, and MCP1, probably leading to activated T-helper-1 (Th1) cell responses. Moreover, patients requiring ICU admission had higher concentrations of GCSF, IP10, MCP1, MIP1A, and TNFα than those did not requiring ICU admission, suggesting that the cytokine storm was associated with disease severity. However, 2019-nCoV infection also initiated increased secretion of T-helper-2 (Th2) cytokines (eg, IL4 and IL10) that suppress inflammation, which differs from SARS-CoV infection. Further studies are necessary to characterise the Th1 and Th2 responses in 2019-nCoV infection and to elucidate the pathogenesis.(Huang, *et al* 2020).

1.9.2. chemokine storm

Chemokines are immunoregulatory mediators to overcome pathogens (Majumdar and Murphy, 2020; Zhu *et al*., 2020). Despite chemokines are important to attract immune cells and clear the viruses, over releasing of these chemokines lead to hyper inflammation and its adverse sequel where chemokines can directly been associated with the acute respiratory distress which is the main cause of death complicated almost 40% of critical and severe COVID-19 cases (Hue *et al*., 2020; Khalil, Elemam and Maghazachi, 2021). Recent studies and literatures have shown a direct correlation between chemokines and different stages of COVID-19(Chi *et al*., 2020; Hue *et al*., 2020; Ozsurekci *et al*., 2021). Interestingly, some viruses like large DNA ones, able to deceive the immune system and escape through the chemokines where these viruses produce molecules resembling the chemokines, that leads to impaired signaling regulatory process and immune system (Alcami, 2003; Canedo-Marroquín *et al*., 2020).

Important highlight the chemokine signature of SARS and MERS since they share structural features and clinical presentation with COVID-19. However, studies have shown notable differences among the three viruses such as the receptors used to infect host cells, susceptibility to type I IFN, and the cytokines and chemokines involved in the immunopathology of the lungs. Like other viral infections, the production of chemokines is an important anti-viral response responsible for infiltrating immune cells towards infected lungs as part of the immune response against corona viruses. Although chemokines are vital to attract immune cells to clear the virus, exacerbated expression leads to excessive inflammation and consequently ARDS, a common complication for SARS, MERS and COVID-19(Alosaimi, *et al* .,2021)-(Alosaimi, *et al* .,2020).

Regarding chemokine receptors, SARS-CoV-2 upregulated CCR1, CCR2 and CCR5 on the human thoracic dorsal root ganglion indicating the impact of inflammatory mediators on activating the sensory neurons of the lungs. This could possibly suggest that pharmacological inhibition of these receptors might suppress the hyperinflammation in critical COVID-19 patients (Mehlotra,. (2020). Moreover, host genomic factors are important elements that can impact the infection and mortality rate due to SARS-CoV-2. For instance, the frequency of CCR5 Δ32 showed significant positive correlation with COVID-19 infection and mortality rate/million especially in an African population, (Panda, *et al* .,2020)-(Solloch, *et al* .,2020)

1. 10. CC chemokine receptors -1 (CCR1)

Chemokines constitute a family of small heparin-binding proteins which orchestrate the infiltration of leukocytes during inflammation, but also directly influence other physiological and pathophysiological processes. In humans, more than 40 chemokines are known binding to around 18 G-protein-coupled receptors. A non-redundant role of certain chemokines and their receptors has been identified within the last years in inflammation and host defense. Among chemokine receptors, the CC chemokine receptors CCR1 and CCR2 have been shown to play a crucial role in these processes. Importantly, these receptors have already been targeted by specific antagonists in early human trials for autoimmune and infectious diseases. Although most of these antagonists failed to show any significant efficacy in the clinic, the knowledge of their biological effects could henceforth offer new avenues with optimal strategies for producing successful therapeutics.(Zimmermann *et al* .,2014).

Interactions between secreted immune proteins called chemokines and their cognate G protein–coupled receptors regulate the trafficking of leukocytes in inflammatory responses. The two-site, two-step model describes these interactions. It involves initial

binding of the chemokine N-loop/3 region to the receptor's N-terminal region and subsequent insertion of the chemokine N-terminal region into the transmembrane helical bundle of the receptor concurrent with receptor activation..(Sanchez et al .,2018).

The COVID-19 risk variant is associated with the expression of CCR1,CCR5 (Zhou, et al .,2020) . Although increased by several folds, CXCL1, CXCL12, CCL11 and CCL27 did not show significant differences among the three groups of patients and remained steady over the different tested time points. This suggests that these chemokines contribute to the common pulmonary inflammation and respiratory symptoms in all COVID-19 patients (Xu,. et al.,2020)

1.11 CC chemokine receptors -5 (CCR5)

C-C chemokine receptor type 5 ,also known as CCR5 or CD195, is a protein on the surface of white blood cells that is involved in the immune system as it acts as a receptor for chemokine .(Jiao, et al .,2019) .

CC chemokine receptor 5 (CCR5) is a seven-transmembrane, G protein-coupled receptor (GPCR) which regulates trafficking and effector functions of memory/effector T-lymphocytes, macrophages, and immature dendritic cells. It also serves as the main coreceptor for the entry of R5 strains of human immunodeficiency virus (HIV-1, HIV-2). Chemokine binding to CCR5 leads to cellular activation through pertussis toxin-sensitive heterotrimeric G proteins as well as G protein-independent signalling pathways. Like many other GPCR (G protein coupled receptor), CCR5 is regulated by agonist-dependent processes which involve G protein coupled receptor kinase (GRK)-dependent phosphorylation, β-arrestin-mediated desensitization and internalization. (Oppermann, M.2004) CCR5 is a receptor for a number of inflammatory CC-chemokine including CCL3/MIP-1-alpha, CCL4/MIP-1-beta and RANTES and subsequently transduces a signal

by increasing the intracellular calcium ion level & May play a role in the control of granulocytic lineage proliferation or differentiation. Participates in T-lymphocyte migration to the infection site by acting as a chemotactic receptor.(Sharapova et al .,2018).

Severe-to-critical COVID-19 is associated with adysregulated host immune response to the virus, which is thought to lead to pathogenic immune dysregulation and end-organ damage. Presently few effective treatment options are available to treat COVID-19. Leronlimab is a humanized IgG4, kappa monoclonal antibody that blocks C–C chemokine receptor type 5 (CCR5). It has been shown that in patients with severe COVID-19 treatment with leronlimab reduces elevated plasma IL-6 and chemokine ligand 5 (CCL5), and normalized CD4/CD8 ratios.(Agresti, et al .,2021).

Interestingly, SARS-COV-2 infected lungs express more CCL4, CCL8, and CCL11, the three ligands shared the same receptor, which is CCR5 , Top immune cells that express CCR5, Lung macrophages in severe COVID-19 infection orchestrate local inflammation by recruiting inflammatory monocytic cells and neutrophils, whereas, in moderate COVID-19 infection, macrophages produce more T cell attracting chemokine SARS-CoV-2 infection of alveolar macrophage can drive the “cytokine storm” that further damages multiple organs other than the lung, as in the case of heart and kidney, Interestingly, the CCR5 Δ32 allele was found to be an important genetic marker of SARS-CoV-2 related death .(M., *et al* .,2020).

Most studies investigating the role of cytokines and chemokines in the pathogenesis of COVID-19 revealed a broad array of elevated inflammatory mediators during the cytokine storm without specifying the exact time points of their increase during the infection. Therefore, it is crucial to analyze the temporal changes of chemokines over the course of the disease in order to catch the window of treatment when designing drugs that target critical immune molecules. Recently, simultaneous detection of 48 cytokines, chemokines and growth factors was performed using multiplex system on mild, severe and fatal COVID-19 group of patients in order to investigate the kinetic changes of chemokines.

The levels of CCL4 and CCL5 were shown to be upregulated in all three groups of patients, but negatively correlated with disease severity, as the expression of these chemokines was significantly higher in mild cases.

This suggests that CCL4 and CCL5 are likely to be associated with recovery and resolution of inflammation possibly through the activation of cytotoxic T cells and release of CCL5 upon antigen presentation (Xu, *et al* .,2020), (Zhao, *et al* .,2020).

Furthermore, elevated CCL5 levels remained consistently high during the 4-week follow up period (Zhao ., 2020). However, contradictory data exist regarding CCL5, as some studies showed the presence of this chemokine in severe patients as well as its close association with disease progression,(Li, *et al* .,2020).

1.12. Diagnosis And Imaging of COVID-19

1.12.1 Molecular tests (RT-PCR)

Samples are collected from the upper respiratory tract via nasopharyngeal and oropharyngeal swabs and from the lower respiratory tract via expectorated sputum and bronchoalveolar lavage (only for mechanically ventilated patients). After being stored at 4°C, the samples are sent to the laboratory where amplification of the viral genetic material is done through a reverse-transcription process(Cascella. *et al* .,2020). This involves the synthesis of a double-stranded DNA molecule from the existing viral RNA by either reverse-transcription PCR (RT-PCR) or a real-time-PCR(Bhadra, *et a*l .,2015)&(Chan, *et al* .,2015).

Finally, the conserved portions of the SARS-CoV-2 genetic code are identified on the amplified genetic material,(Cascella, *et al* .,2020). The test is recommended to be repeated for verification in cases of a positive test and again to confirm viral clearance in COVID-19 positive cases. The sensitivity of these tests is

not very high, that is, approximately 53.3% of COVID-19-confirmed patients had positive oropharyngeal swabs, and about 71% of patients came out to be RT-PCR positive with sputum samples,(Zhang, *et al* .,2020)&(Fang, *et al*.,2020) The RT-PCR results usually show positivity after 2–8 days, (Huang, *et al* .,2020).

1.12.2 Serology

Till date, no effective antibody test has been developed. A centers for disease control and prevention (CDC) research on a test developed by the US Vaccine Research Centre at the National Institutes of Health is ongoing, which seems to have a specificity higher than 99% with a sensitivity of 96%, (Cascella, et al .,2020).

1.12.3 Blood tests

A normal or decreased white blood cell count (and lymphopenia) can be observed in many cases, which is also considered to be indicative of a worse prognosis. Increased levels of lactate dehydrogenase, C reactive protein, creatine kinase (CK MB and CK MM), aspartate amino-transferase and alanine amino-transferase can be seen, (Cascella, et al .,2020). Increased D-dimer levels and an elevated neutrophil-to-lymphocyte ratio are seen in some patients.(Yang, et al ., 2020). Coagulation abnormalities can be observed in severe cases, as indicated by increasing in prothrombin time and international normalised ratio.

1.12.4 Chest X-ray

Chest X-ray is usually inconclusive in the early stages of the disease and might not show any significant changes. As the infection progresses, bilateral

multifocal alveolar opacities are observed, which may also be associated with pleural effusion, (Cascella, et al .,2020).

1.12.5 CT

High-resolution CT (HRCT) is extremely sensitive and the method of choice for diagnosing COVID-19 pneumonia, even in initial stages of the illness. The most commonly seen features are multifocal bilateral 'ground-glass' areas associated with consolidation and a patchy peripheral distribution, with greater involvement of the lower lobes. A 'reversed halo sign' is also seen in some patients, which is identified as a focal area of patchy opacities surrounded by a peripheral ring with consolidation. Other findings include pleural effusion, cavitation, calcification, and lymphadenopathy, (Cascella, et al .,2020).

1.13 Treatment of COVID-19

1. 13.1 Antibiotics

Although not always recommended in viral pneumonia, an optimum and effective antibiotic regimen helps prevent or manage secondary bacterial infections and sepsis. Macrolides such as azithromycin are quite effective in preventing pulmonary infections in patients with viral pneumonias, in addition to having a significant anti-inflammatory effect on the airways ,(Bacharier, et al .,2015)

1. 13.2 Corticosteroids

Steroids can be used for a short period of time, that is, 3–5 days in patients who show progressive deterioration of oxygen saturation, increased activation of the pro-inflammatory response and rapid worsening of features on chest imaging.

Methylprednisolone was the first and only steroid indicated initially, at a dose not exceeding 0.5–1 mg/kg/day for moderate cases and 1–2 mg/kg/day for severe cases. Higher doses were not recommended in view of the delay in viral clearance due to steroid mediated immunosuppression,(Huang, et al .,2020), (Parasher. (2021)), (Arabi, et al .,2018)&(Lee, et al., 2001).

Recently, dexamethasone has also been found to be effective for decreasing mortality in severe and critically ill cases,(World Health Organization. WHO welcomes preliminary results about dexamethasone use in treating critically ill COVID-19 patients. 2020).

1. 13.3 Antiviral drugs

The following antiviral drugs have been put to use for COVID-19 patients so far.

1. 13.3.1 Remdesivir (CIPREMI/COVIFOR)

It was initially suggested by some preclinical studies that remdesivir has in vitro activity against multiple RNA viruses (including Ebola) and could be beneficial for both prophylaxis and treatment of coronavirus infections, (Cascella, et al .,2020), (Parasher, (2021)),(Gordon, et al.,2020).

Remdesivir is a broad-spectrum antiviral agent, and acts by blocking the action of viral RNA-dependent RNA polymerase. This causes evasion of proofreading by viral exoribonuclease, causing a significantly decreased production of viral RNA, (Tchesnokov, et al .,2019). In a mouse model of SARS-CoV, remdesivir was observed to reduce the lung viral load and improve pulmonary function.(Sheahan, et al .,2017).

It was used to treat the first case of COVID-19 infection in the USA, who showed rapid improvement after 1 day of remdesivir treatment, (Holshue, et al .,2020). In two separate studies, although remdesivir was seen to be superior to placebo in decreasing rates of lower respiratory tract infections and shortening hospital stay, there was no significant difference seen between a 5-day course and a 10-day course of remdesivir,(Beigel,et al .,2020)&(Goldman, et al .,2020).

In comparison, therapeutic doses of lopinavir (LPV)/ritonavir (RTV) although did improve pulmonary function, but were not able to reduce virus replication or prevent severe lung damage. Thus, it is indicated that remdesivir has shown more potential than LPV/RTV in the treatment of COVID-19,(Sheahan. et al.,2020)&(Martinez, . (2020)).

It may be considered in patients with moderate disease at a loading dose of 200 mg intravenous over 1–2 hours on day 1, followed by 100 mg intravenous daily for 5–10 days. Contraindications to the use of remdesivir include use in children, pregnant or lactating females, and patients with severe hepatic or renal impairment, (Parasher. (2021)).

Thus, it is implied that remdesivir is best suited for hospitalised patients with COVID-19 having moderate-to-severe disease, and requiring supplemental oxygen therapy. On May 1, 2020, the US Food and Drug Administration (USFDA) gave emergency use approval for remdesivir in patients hospitalised with severe COVID-19; the final approval being given in light of tentative evidence of remdesivir efficacy in such patients. However, it is to be noted that treatment with remdesivir alone is not likely to be sufficient given the high mortality despite its use.

1. 13.4 Plasma exchange via convalescent plasma

It was observed that the COVID-19 virus isolated from the bronchoalveolar lavage fluid of a critically ill patient could be neutralised by plasma from several convalescent patients, (Zhou, et al ., 2020) This therapy may be considered in patients with severe disease who do not show improvement (oxygen requirement is progressively increasing) despite use of steroids. Some important requirements for this procedure include an adequate antibody titre in the convalescent plasma, ABO compatibility and cross-matching of the donor plasma. The recipient should be closely monitored for several hours post-transfusion for any transfusion-related adverse events and its use should be avoided in patients with IgA deficiency or Ig allergies. Dose ranges from 4 to 13 mL/kg, and usually, a single dose of 200 mL is given slowly over 2 hours,((Parasher. (2021)).

To ensure high efficacy via a high antibody titre, the convalescent plasma has to be collected within 2 weeks of patient recovery from COVID-19,(Zhai, et al .,2020).

Chapter Two

- Materials and Methods

Materials and methods

2.1. study design and setting

This is a cross-sectional study done at the Consultation Clinic at Al Zahra Hospital, the Internal Medicine Department of Imam Hussein Teaching City. During the period from the beginning of December 2020 – to the end of January 2021 .

2.2 Subjects:

Ninety Iraqi patients suffering from COVID-19 disease were included in this study. Diagnosis of patients based on clinical characteristics & PCR result & classified in to moderate and sever-critical according to WHO criteria (CT scans involvement : moderate < 50%, sever-critical >50% ,SPO2 saturation: moderate ≥90% , severe cases <90%)(WHO, Clinical Management of COVID-19,2020) .

The patients consist of 50 male and 40 female with age range from (20-85)year old as shown in figure 2.1.

2.2.1. inclusion and exclusion criteria:

Included criteria:

Patients with proved COVID-19 by PCR , laboratory results & CT involvement (moderate & sever-critical. All patients were selected according to convenient sampling.

Excluded criteria:

1- History of Cancer
2- History of Autoimmune disease

2.2.2 Ethical and Scientific Approval:

Ethical approval: The study protocol was be sent to the ethical committee of Karbala College of Medicine & the relevant ethical committee in Karbala health directorate. In addition, verbal and written approval was be taken from the participants before taking the sample. Health measures and safety was taken during sampling.

2.2.3. Data Collection: The demographic and clinical data were collected through an interview which was done for all patients through a detailed questionnaire. (Appendix 3 questionnaire).

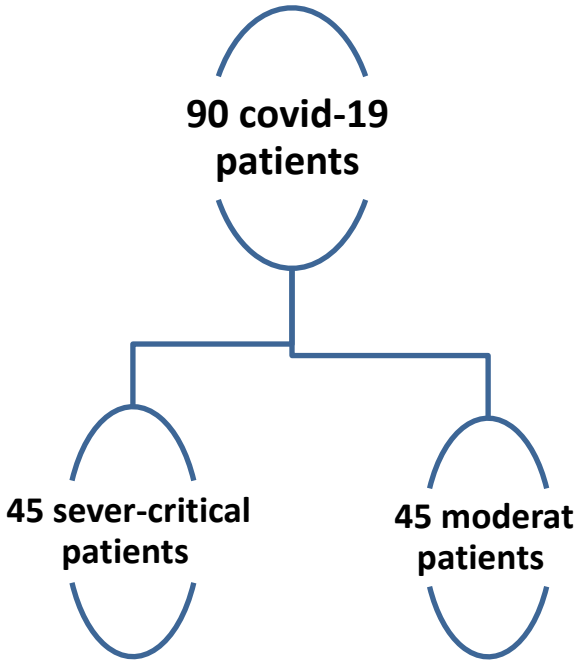


Figure 2.1 Study Subject distribution

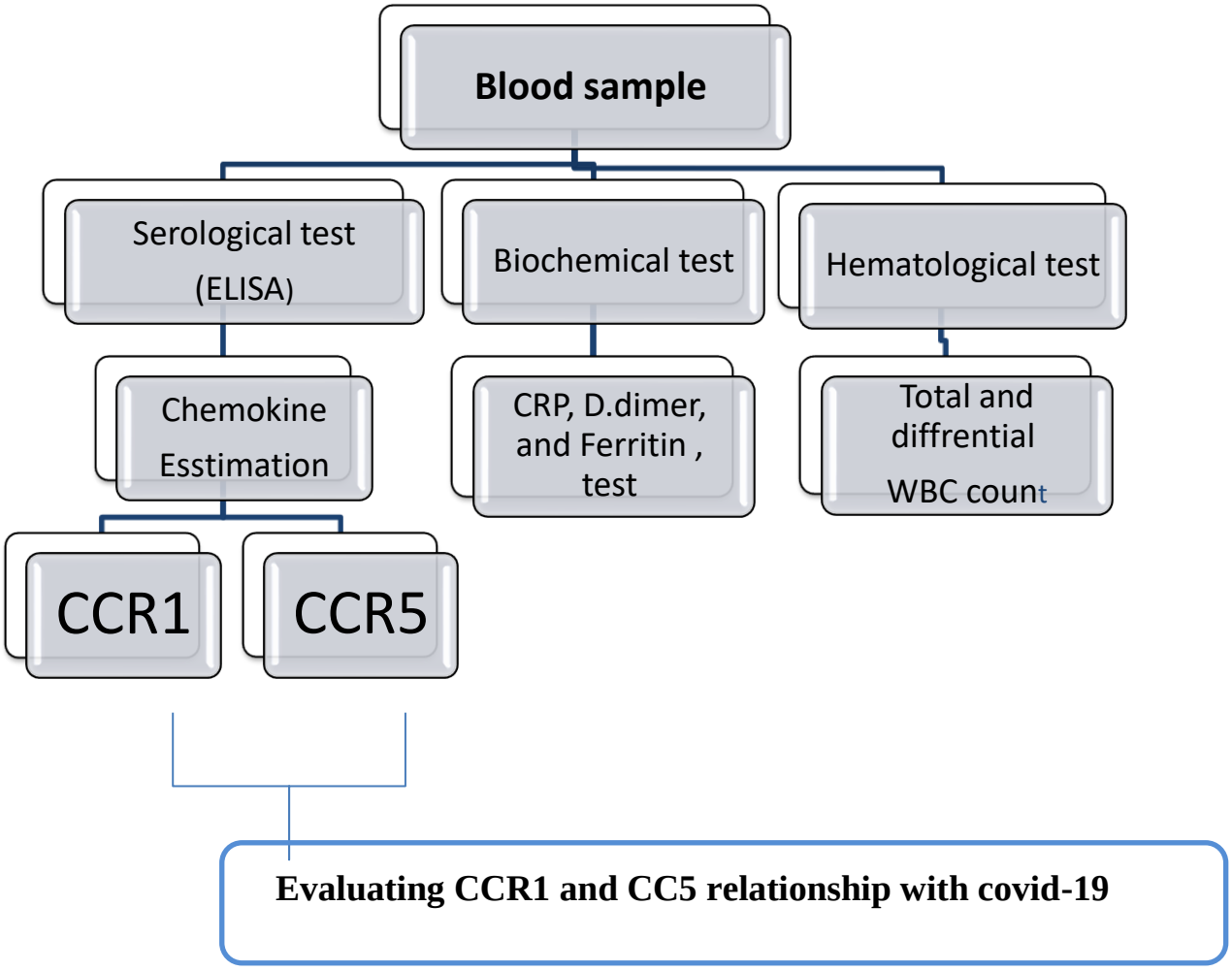


Figure 2. 2 The Study Plan

2.4 Materials

2.4.1 Equipment, and Instruments

The instruments and equipment used in this study with their remarks are list in table (2.1).

Table (2.1): Instruments and equipment.

Equipment &Instruments	Manufacturing Company	Origin
Automated blood analyzer	Sysmex	Germany
Centrifuge	Kokusan	Japan
Cool box	VB	China
Deep freezer	Teka	Spanish
EDTA Tubes 2.5 ml	ALS	
ELISA Incubator	BOEKEL	Germany
ELISA printer	Epson	
ELISA Reader	HumaReader HS	Germany
Eppendorf Tube 0.5 ml		
Fully-auto chemiluminescence immunoassay analyzer	Maglumi 800	Germany
Gel Tubes 6 ml	ALS	China
Micropipette set	SLAMED	Germany

Multichannel micropipette set	SLAMED	Germany
Pipette tip		China
Roller mixer	medispec	China
Sodium citrate 4.5ml	Golden vas	China
spectrophotometer	Mindray BA 88A	China
syringe 10 ml	Arrow	Egypt

2.5 Laboratory Methods

2.5.1.Sample Collection:

Ten milliliters of venous blood were collected using 10ml disposable syringe. The blood sample was immediately Distribute in sodium citrate tube 3ml for D. dimer test , EDTA tubes 2.5ml for complete blood picture and 5ml of blood was transformed into gel tube and left to clot for 15 minutes in room temperature (20-25)°C. Then gel tube centrifuged from 2500 to 3000 rpm for 10 min period to isolate , the isolated serum was distribute into aliquots (0.5ml) in tightly closed Eppendorf tubes, and then the tubes were stored at -20ºC until time of analysis.

2.5.2 Complete blood count

The blood was taken in EDTA tube and analyzed by automated blood analyzer devise .

2.5.3 CRP – Turbidimetric (Latex Turbidimetry)

Anti-CRP-coated latex particles accumulate when they interact with samples containing C-reactive protein (CRP). Latex particle agglutination is

proportional to the CRP concentration in the sample and can be measured by turbidity metering..

2.5.3.1 Reagents Composition of CRP

R1Diluent. Tris buffer, 20 mmol/L, pH 8.2.

R2 Latex. Latex particles coated with goat anti-human C RP, pH 7.3

CAL Calibrator. (Ref. 3931205). Human serum. CRP concentration is stated on the label vial and it is traceable to the Certified Reference material ERMDA470 (IRMM).

2.5.3.2 Material Required

-Thermostatic bath at 37ºC.

- Spectrophotometer or photometer thermostatable at 37ºC with a 540 ± 20 nm filter.

2.5.3.3. PROCEDURE

2.5.3.3.1 Preliminary Procedure

Prewarm the working reagent and the photometer (cuvette holder) to 37 ºC.

2.5.3.3.2 Analytic Procedure

1. Using distilled water zero the instrument at 540 nm.

2. Pipette into a cuvette: Sample / Calibrator 5 µL and Working Reagent 1.0 mL

3. Mix well and record the absorbance's in mediately (A1) and after 2 minutes (A2) after the sample addition.

2.5.3.4 Calculation

$$\frac{(A2\text{-}A1)\ \text{sample}}{(A2\text{-}A1)\ \text{calibrator}} \times \text{CAL conc.} = \text{mg/L CRP}$$

2.5.3 Human ferritin(FE)

2.5.3.1.Principle of the assay

Sandwich immunoassay: use of an anti-ferritin monoclonal antibody to label ABEI, and use another monoclonal antibody to label FITC. Low temperature average temperatures rise up, with low average temperatures at 37°C, then a one-time wash cycle. The ABEI is then numbered, incubated and sandwich shaped, and then washed a second time. Next, the starting reagents are added and a chemical flash reaction begins. The optical signal is then taken up by a photomultiplier such as RLU within 3 seconds and proportional to the ferritin concentration present in the sample.

2.5.3.2.Kit component

The kit components of ferritin are listed in table (2-2)

Table (2.2): Material Supplies

Reagent Integral for 100 determinations	
Nano magnetic microbeads: Tris buffer, 1.2% (W/V), 0.2%NaN3, coated with sheep anti- Fitc polyclonal antibody	2.5ml
Calibrator, low : bovine serum, 0.2%NaN3。	2.5ml
Calibrator, high: bovine serum, 0.2%NaN3。	2.5ml
Fitc Label: anti-Ferritin monoclonal antibody labeled FITC, containing BSA, 0.2%NaN3	12.5ml
Abei Label: anti-Ferritin monoclonal antibody labeled ABEI, containing BSA, 0.2%NaN3	22.5ml
Diluent ： 0.9% NaCl	25ml
All reagents are provided ready-to-use	

2.5.3.3 Preparation for Analysis

Patient sample with a cloudy or turbid appearance must be centrifuged prior to testing. after centrifugation, avoid the lipid layer (if present) when pipetting the specimen into a sample cup or secondary tube.

- Samples must be mixed thoroughly after thawing by low speed vortexing or by gently inverting, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results. Multiple freeze-thaw cycles of specimens should be avoided.
- All samples (patient specimens or controls) should be tested within 3 hours of being placed on board the MAGLUMI System.

2.5.3.4 TEST PROCEDURE

To ensure proper test performance, strictly adhere to the operating instructions of the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI. Each test parameter is identified via a RFID tag on the Reagent Integral.

40µl +100µl +20µ	Sample,calibrator or controls FITC Label Nano magnetic microbeads
10 min	Incubation
400µl each time	Cycle washing
+200µl	ABEI label
10 min	Incubation
400µl each time	Cycle washing
3 sec	Measurement

2.5,3.5 Calculation of Results

The analyzer automatically calculates the ferritin concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in ng/ml.

2.5.4 Human D-Dimer

2.5.4.1 PRINCIPLE OF THE TEST

Sandwich immunoluminometric assay; Use an anti-D-dimer monoclonal antibody to label ABEI, and use another monoclonal antibody to label FITC. Sample, Calibrator or Control, ABEI Label, FITC Label and magnetic microbeads coated with anti-FITC are mixed thoroughly and incubated at 37°C , forming a sandwich; after sediment in a magnetic field, decant the supernatant, then cycle washing for 1 time Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of D-DIMER present in controls or samples.

2.5.4.2 KIT COMPONENTS

The kit component of D. Dimer are found in table(2.3)

Table (2.3): Material supplies

Reagent Integral for 100 determinations	
Nano magnetic microbeads: Tris buffer, 1.2%(W/V), 0.2%NaN33, coated with sheep anti- Fitc polyclonal antibody	2.5ml
Calibrator, low	2.5ml
Calibrator, high	2.5ml
AbeiLabel: anti-D-dimer monoclonal antibody labeled ABEI, contains BSA, 0.2%NaN3	6.5m
Fitc Label: anti-D-dimer monoclonal antibody labeled FITC, contains BSA, 0.2%NaN3	6.5m
All reagents are provided ready-to-use	

2.5.4.3Test Procedure

To ensure proper test performance, strictly adhere to the operating instructions of the Maglumi Fully Auto Analyzer. Each test parameter is identified via a barcode on the Reagent Integral.

20µl +40µl +40µl +20µl	Sample,calibrator or controls ABEI Label FITC Label Nano magnetic microbeads
10 min	Incubation
400µl each time	Cycle washing
3 sec	Measurement

2.5.4.4 Calculation of Results

The analyzer automatically calculates the D-DIMER concentration in each sample by means of a calibration curve which is generated by a 2- point calibration master curve procedure. The results are expressed in IU/ml.

2.5.5 ELISA measurement of CCR1 & CCR5

Sera of covid-19 patients were assessed for level of 2 chemokine receptors (CCR1 and CCR5) The chemokine receptors levels obtained by means of ELISA method that based on similar principles.

2.5.5.1 Principles of Assay

Kit is a sandwich enzyme-linked immune sorbent assay designed for quantitative measurement of natural or recombinant antigens in human serum. In which the coating antibody

(Capture Antibody) adsorbed onto wells of 96-well plate. The plate has been pre-coated with human chemokine receptors(CCR1, CCR5) antibody. chemokine receptors present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human chemokine receptors Antibody is added and binds to chemokine receptors in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated chemokine receptors antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human chemokine receptors . The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

2.5.5.2 Kits

Table 2.4. shows kits.

Table (2.4): Kits and Solutions

Kit	Company	Country
Human CC- chemokine Receptor 1 ELISA kit	BT LAB	China
Human CC- chemokine Receptor 5 ELISA kit	BT LAB	China
Sterilizers		

2.5.5.3 Kit Contents

Components of kit as in table 2.6.

Table(2.5) :components of ELISA kits

Quantity	Components
Standard Solution (48ng/ml)	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Biotinylated human chemokine receptors Antibody	1ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pics

2.5.5.4 Assay Procedure

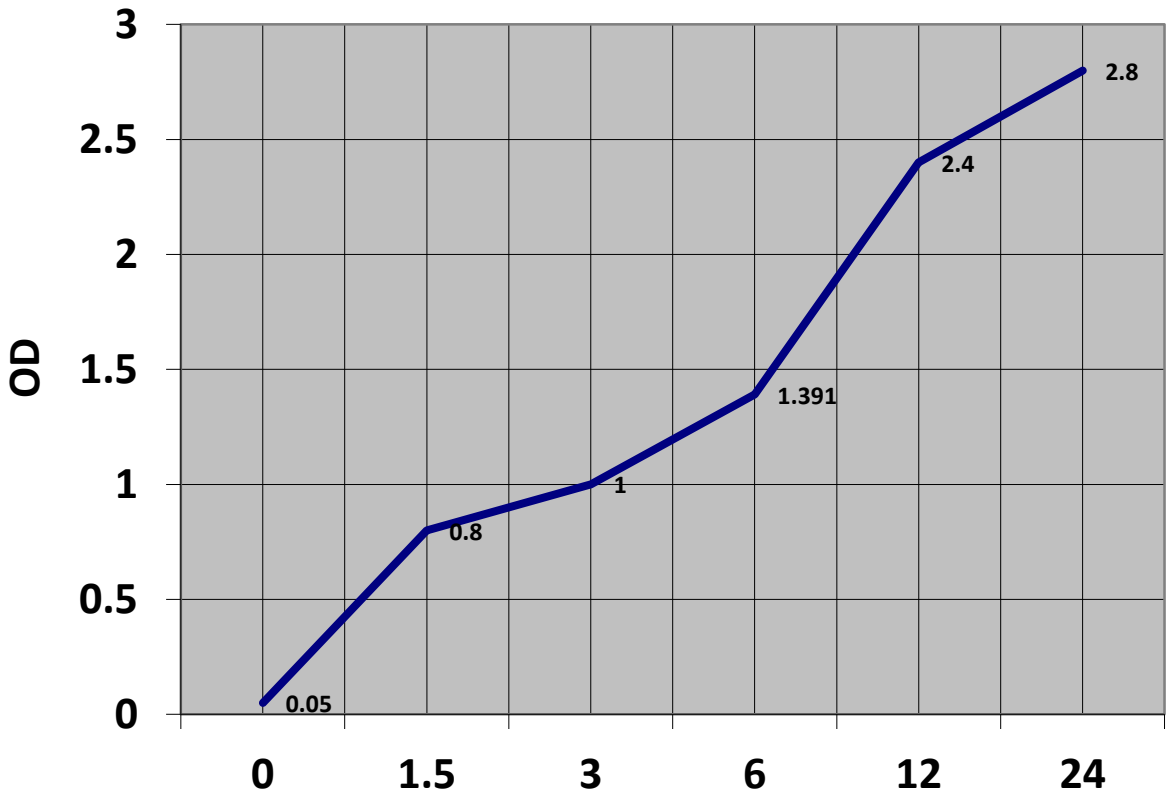
The preparation of all reagents, standard solutions and samples were in room temperature before use. The assay was performed at room temperature. It was required that in order to determine the number of strips, it to insert the strip in the frames that were being used. Otherwise, the unused strips were stored at 2-8°C. There was an addition of 50µl standard to standard well. Also, there was an addition of 40µl sample to sample wells and then an addition of 10µl anti-CCR1 antibody to sample wells, then addition of 50µl streptavidin-HRP to sample wells and standard wells. The Mixing well was designed as follows; the plate was covered with a sealer and an incubation of 60 minutes at 37°C.

The next step of the process was to remove the sealer and wash the plate 5 times with a wash buffer, then soaking the wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. Though for automotive washing the aspiration of all wells and washing5 times with wash buffer, overfilling wells with wash buffer. Then, Blotting the plate onto paper towels or other absorbent material. Furthermore, there was an addition of 50µl substrate solution A to each well and then addition of 50µl substrate solution B to each well. Afterwards, there was an incubation of plates covered with a new sealer for 10 minutes at 37°C in the dark. In order to stop the process, there was an addition of 50µl Stop Solution to each well, the blue color changed into yellow immediately. Finally, the determination of the optical density (OD value) of each well was immediately detected using a micro plate reader set to 450 nm within 10 minutes after adding the stop solution.

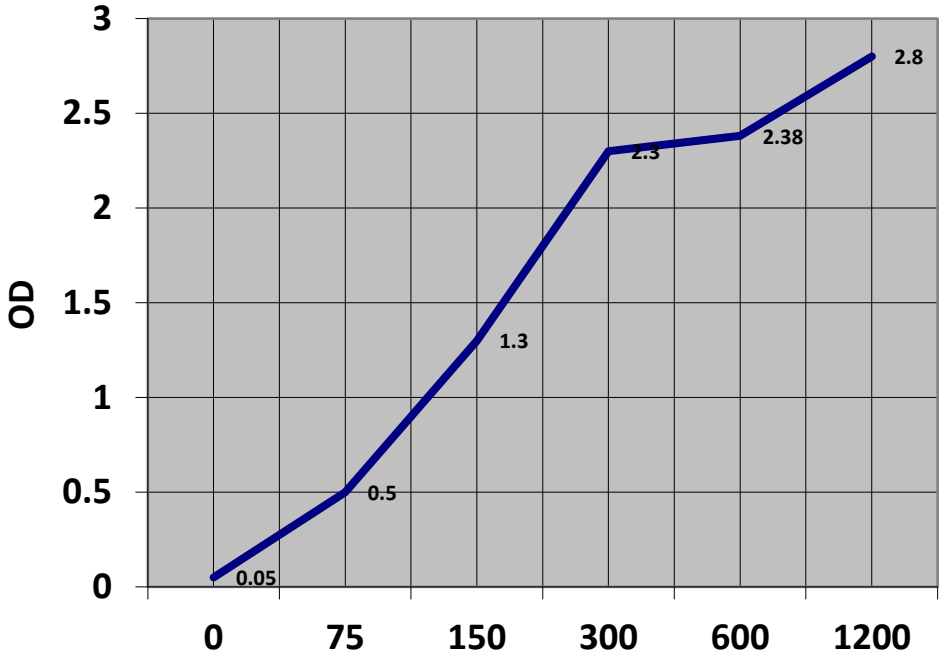
The second marker (CCR5) was subjected to the same approach and completeness same as the first marker(CCR1)

2.5.5.5 Results Calculation

The samples concentrations calculated by a standard curve fitting equation that performed in the same procedure for each chemokine receptors , (Figures 2.3. & 2.4.).



Figures2-3 standardCurve of CCR1



Figures2-4 standardCurve of CCR5

2.6. Statistical Analysis:

Data of the COVID-19 patients were transferred into computerized database with statistical package; the statistical package for social sciences (SPSS) version 26 used in all statistical analysis and testing. Chi-square test used to assess the differences between both groups in categorical variables such as gender, age groups, residence, …., etc. Student's t test used to compare two means of any parameter across the studied groups. Analysis of variances (ANOVA) test was applied to compare mean CCR1 and CCR2 across the CT severity score of lung involvement. Bivariate correlation tests Pearson's (for two scale variables) and Spearman's (for one scale and other categorical

variables) tests were applied. Correlation coefficient (R) was calculated in both tests . Statistically, R value ranged between zero (complete no correlation) and One (perfect correlation) and the higher R value close to one indicates the stronger correlation. Sign of R reflects the direction of correlation; negative signed R reflects an inverse (negative correlation) while R value with no sign (i.e. positive) reflects a direct correlation. Receiver operating characteristic curve analysis used to assess the validity of CCR1 and CCR2 as predictor of severity of COVID 19 in two ways according to clinical grouping (severe and Moderate) and according to CT severity score for lung involvement. ROC curve is a plot for the sensitivity (True positive rate) against 1-specificity (False positive rate) so it is directly calculate the sensitivity but indirectly found specificity as a result of (1-False positive rate). Area under the ROC curve is an indicator of validity of a test to predict an outcome; AUC of less than 0.600 means that a test failed to predict an outcome, 0.600 – 0.699 indicates fair prediction, 0.700 to 0.799 good prediction, 0.800 to 0.899 very good and 0.900 or more indicates excellent prediction. Level of significance set at 0.05 as a cutoff point for significant difference or correlation. Finally, results expressed in tables and figures with an explanatory paragraph for each using MS Word and Excel software version 2013. (Al-Mosawe, mohammed. and Fayadh ,2021)

Chapter Three

Results

3. Results :

3.1. Demographic characteristics of the studied groups

A total of 45 patients with severe & critical and 45 patients with moderate COVID-19 disease were enrolled in this study. Patients with severe form of disease were significantly older than those with moderate disease. The mean age was 67.1 ± 11.2 years and 53.1 ± 10.6 years, for sever –critical and moderate patients respectively, on the other hand, age distribution in decade interval showed that severity of disease increased with advancing age, in both comparisons, *P. value* was significant < 0.05.

No statistically significant differences were found between both groups in gender, residency, smoking status or history of CVD, *(P. value > 0.05*), while hypertension was significantly more frequent in patients with severe COVID-19, (*P. value < 0.05*, significant), all these findings are displayed in (Table 3.1)

Table 3.1. Demographic characteristics of the studied groups

Variable		Severe & critical COVID-19 (n = 45)		Moderate COVID-19 (n = 45)		P. value
		No.	%	No.	%	
Age (year)	≤ 40	1	2.2	5	11.1	<0.001 sig *
	41 - 50	3	6.7	14	31.1	
	51 - 60	6	13.3	16	35.6	
	61 - 70	19	42.2	10	22.2	
	> 70	16	35.6	0	0.0	
	Mean (SD)	67.1 (11.2)		53.1 (10.6)		<0.001 sig**
Gender	Male	27	60.0	21	46.7	0.205 ns
	Female	18	40.0	24	53.3	
Residency	Urban	37	82.2	38	84.4	0.780 ns
	Rural	8	17.8	7	15.6	
Smoker		11	24.4	15	33.3	ns 0.352
History of Hypertension		38	84.4	30	66.7	**0.030 sig**
History of CVD		21	46.7	16	35.6	0.248 ns

CVD: Cardiovascular disease, SD: standard deviation of mean
*Chi-square test used in comparison** Student's t test used in comparison.

Laboratory investigations revealed that patients with severe CVID-19 had significantly higher white blood cells (WBC) count , neutrophil cells count and lower lymphocyte cells count , (*P. value < 0.05*). Platelets count was not significantly different between both groups, (*P>0.05*). Hemoglobin level was significantly higher in severe than moderate COVID-19 patients, (*P<0.05*).

Serum ferritin was significantly much higher in severe than moderate patients; 1423.3 ng/ml vs. 793.5 ng/ml, respectively, (*P<0.05*). So as for D-dimer and LDH levels , where they were significantly higher in severe COVID-19 group,

(P<0.05). No significant difference in CRP levels between both groups, (P>0.05), (Table 3.2).

Table 3.2. Laboratory parameters of the studied groups

	Severe & critical COVID-19 (n = 45)		Moderate COVID-19 (n = 45)		P. value*
	Mean	SD	Mean	SD	
WBC x 10^3 cell/ml	16.1	8.1	11.9	4.1	**0.002 sig**
Neutrophil x 10^3 cell/ml	87.0	4.7	81.4	5.7	**< 0.001 sig**
Lymphocyte x 103 cell/ml	7.6	3.6	12.6	6.5	**< 0.001 sig**
Platelets x 10^3 cell/ml	266.3	141.4	257.9	91.0	0.740 ns
Hemoglobin (g/dL)	13.4	2.4	12.2	2.3	**0.016 sig**
S. ferritin (ng/ml)	1423.3	973.6	793.5	534.9	**0.001 sig**
CRP (mg/L)	78.7	62.4	69.7	50.4	0.452 ns
D-dimer (µg/ml)	1377.3	973.6	1031.8	805.8	**0.013 sig**
LDH (U/L)	598.8	194.7	444.6	467.4	**<0.001 sig**
*Student's t test used in all comparisons					

3.2. CCR1 & CCR5 serum levels among studied groups

As shown in (Table 3.3), the mean CCR1 was significantly lower in patients with severe than those with moderate COVID-19, the mean CCR1 was 1.92 ± 0.66 and 4.29 ± 1.817, respectively, (P<0.001). So as for CCR5, it was significantly lower in severe COVID-19 patients than moderate disease patients, the mean CCR 5 was 66.3 ± 35.7 vs. 113.1 ± 47.8, respectively, (P<0.001), (Table 3.4). These findings indicated that lower CCR1 and CCR5 associated with more severe disease status.

Table 3.3. Comparison of CCR1 between severe & critical COVID-19 and Moderate COVID-19 groups

CCR1	Severe& critical COVID-19 (n = 45)	Moderate COVID-19 (n = 45)	P. value
Mean	1.92	4.29	<0.001 sig
SD	0.66	1.817	
Minimum	0.784	2.000	
Maximum	3.171	15.488	

Table 3.4. Comparison of CCR5 between the studied groups

CCR5	Severe & critical COVID-19 (n = 45)	Moderate COVID-19 (n = 45)	P. value
Mean	66.3	113.1	<0.001
SD	35.7	47.8	
Minimum	23.0	74.3	
Maximum	254.0	882.000	

3.3. Correlation between CCR1, CCR5 & other study variables

Bivariate correlation analysis using Pearson's and Spearman's correlation tests revealed that in severe-critical COVID-19 group, an inverse (negative) significant correlation between CCR1 level and WBC count (correlation coefficient (R) = -0.281, *P. value* = 0.031) and a direct (positive) significant correlation between CCR1 and LDH(R=0.44, p.value=0.002) levels. No other significant correlations were found between CCR1 and other parameters, *(P>0.05*), (Table 3.5).

Regarding the correlation between CCR5 and other parameters in severe-critical COVID-19 group, a significant direct (positive) correlation was found between CCR5 and WBC count (R = 0.651, P<0.001), Neutrophil count (R = 0.335, *P = 0.024*), an inverse (negative) correlation with lymphocyte count (R = -0.331, *P = 0.026).* A direct (positive) correlation with CRP (R = 0.410, P = 0.005), and a direct correlation with LDH (R = 0.320, P = 0.032). No significant correlations with other parameters and variables, (P>0.05), (Table 3.6).

Table 3.5. Matrix of Correlation between CCR1 and other parameters of patients with severe-critical COVID-19 (n = 45)

Parameter	Correlation with CCR1	
	R	P. value
Age	-0.024	0.875
Gender	0.101	0.509
WBC x 10^3 cell/ml	-0.281	**0.031 sig**
Neutrophil x 10^3 cell/ml	0.091	0.551
Lymphocyte x 103 cell/ml	-0.098	0.522
Platelets x 10^3 cell/ml	-0.154	0.311
Hemoglobin (g/dL)	0.082	0.594
S. ferritin (ng/ml)	0.009	0.955
CRP (mg/L)	-0.223	0.140
D-dimer (µg/ml)	0.244	0.106
LDH (U/L)	0.44	**0.002 sig**
CCR5	-0.022	0.888
R: Correlation coefficient		

Table 3. 6. Matrix of Correlation between CCR5 and other parameters of patients with severe & critical COVID-19 (n = 45)

Parameter	Correlation with CCR5	
	R	P. value
Age	0.051	0.740
Gender	-0.269	0.074
WBC x 10^3 cell/ml	0.651	**< 0.001 sig**
Neutrophil x 10^3 cell/ml	0.335	**0.024 sig**
Lymphocyte x 10^3 cell/ml	-0.331	**0.026 sig**
Platelets x 10^3 cell/ml	0.163	0.285
Hemoglobin (g/dL)	-0.071	0.643
S. ferritin (ng/ml)	0.284	0.059
CRP (mg/L)	0.410	**0.005 sig**
D-dimer (µg/ml)	0.268	0.075
LDH (U/L)	0.320	**0.032 sig**
CCR1		
R: Correlation coefficient		

In moderate COVID-19 group, only one significant correlation was found by bivariate correlation test, this was between CCR1 and WBC count . It was significant direct correlation , (R = 0.302, P = 0.044), (Table 3.7).

In the same group, CCR5 showed no significant correlations with all variables and parameters, in all correlations , P. value > 0.05, not significant, (Table 3.8)

Table 3.7. Matrix of Correlation between CCR1 and other parameters of patients with Moderate COVID-19 (n = 45)

Parameter	Correlation with CCR1	
	R	P. value
Age	0.074	0.630
Gender	0.031	0.841
WBC x 10^3 cell/ml	0.302	0.044
Neutrophil x 10^3 cell/ml	0.100	0.512
Lymphocyte x 103 cell/ml	-0.059	0.701
Platelets x 10^3 cell/ml	0.084	0.581
Hemoglobin (g/dL)	-0.232	0.125
S. ferritin (ng/ml)	0.145	0.343
CRP (mg/L)	-0.086	0.572
D-dimer (µg/ml)	0.228	0.132
LDH (U/L)	-0.059	0.701
Lung involvement (%)	0.141	0.356
CCR5	0.178	0.242

Table 3.8. Matrix of Correlation between CCR5 and other parameters of patients with Moderate COVID-19 (n = 45)

Parameter	Correlation with CCR5	
	R	P. value
Age	0.094	0.538
Gender	-0.196	0.197
WBC x 10^3 cell/ml	-0.002	0.991
Neutrophil x 10^3 cell/ml	-0.119	0.435
Lymphocyte x 103 cell/ml	0.037	0.811
Platelets x 10^3 cell/ml	0.068	0.658
Hemoglobin (g/dL)	-0.111	0.468
S. ferritin (ng/ml)	0.021	0.890
CRP (mg/L)	0.193	0.205
D-dimer (µg/ml)	-0.059	0.700
LDH (U/L)	0.029	0.852
Lung involvement (%)	0.112	0.466
CCR1	0.178	0.242

3.4Diagnostic utility of CCR1 & CCR5

To assess the validity of CCR1 and CCR 5 in predicting severity of COVID-19, Receiver Operating Characteristics (ROC) curve analysis was performed and revealed that CCR1 was good predictor of severe-critical form of disease, at an optimal cutoff point of 2.32, the area under the ROC curve (AUC) was 0.937 that produced a sensitivity, specificity and accuracy rates of 82.2%, 100% and 91.1%, respectively. With 100% positive predictive value (PPV) and 84.9% negative predictive value (NPV), (Figure 3.1 and Table 3.9).

For CCR5, it was also good predictor of severe-critical COVID-19 disease form with an AUC of 0.852 and at an optimal cutoff point of 83.5 it was 71.1% sensitive, 91.1% specific and 81.1% accurate, with 88.9% PPV and 75.9% NPV, (Figure 3.1and Table 3.10), however, CCR5 was lower than CCR1 in predicting severe disease.

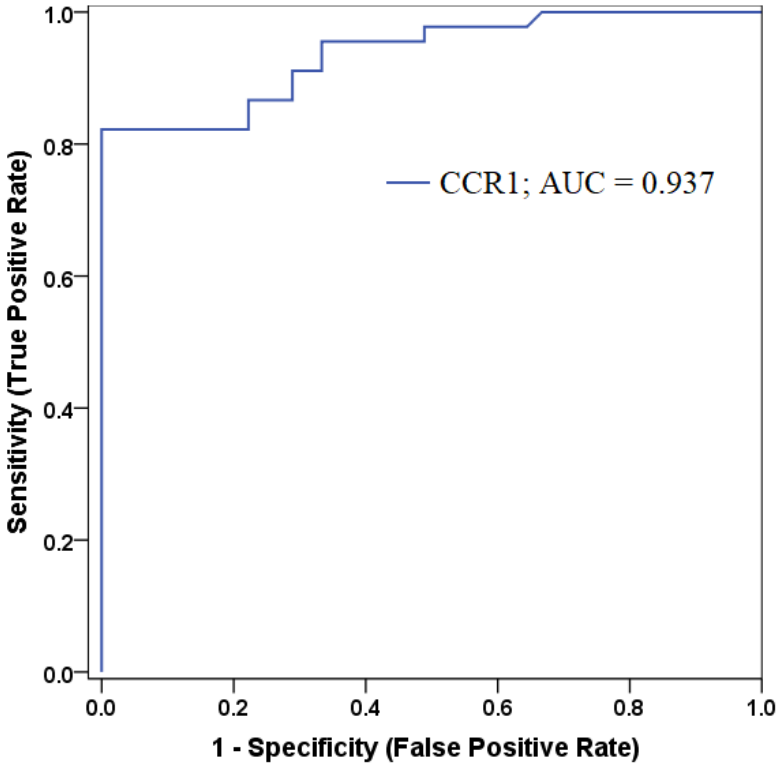


Figure 3.1. Receiver Operating Characteristics (ROC) curve analysis for the validity of CCR1 in prediction of Severe COVID-19

Table 3.9. Validity parameters and cutoff point of CCR1 in prediction of severe & critical COVID-19

Cutoff point	2.32
Area under ROC curve (AUC)	0.937
Sensitivity	82.2%
Specificity	100.0%
Accuracy	91.1%
Positive predictive value	100.0%
Negative predictive value	84.9%

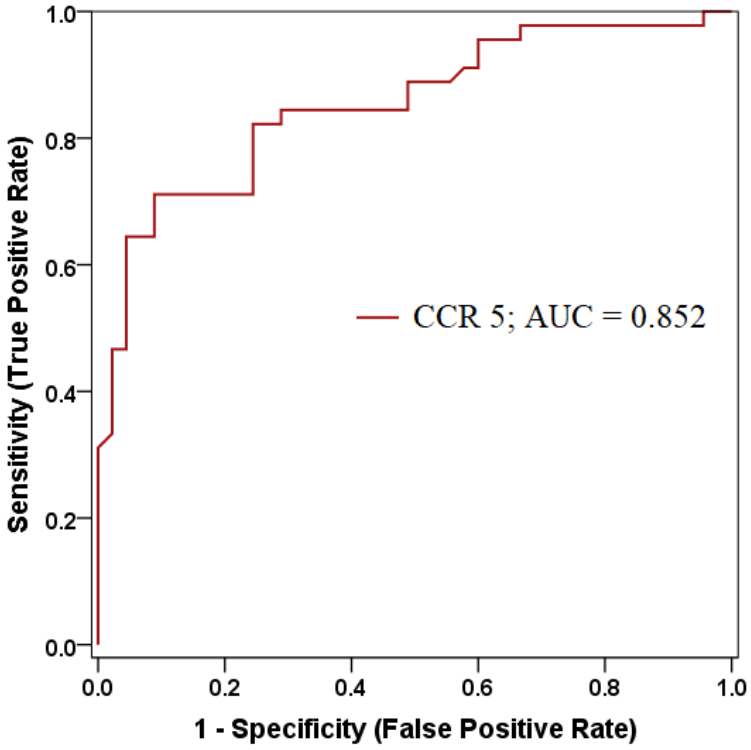


Figure 3.2. Receiver Operating Characteristics (ROC) curve analysis for the validity of CCR5 in prediction of Severe COVID-19

Table 3.10. Validity parameters and cutoff point of CCR5 in prediction of severe & critical COVID-19

Cutoff point	83.5
Area under ROC curve (AUC)	0.852
Sensitivity	71.1%
Specificity	91.1%
Accuracy	81.1%
Positive predictive value	88.9%
Negative predictive value	75.9%

3.5. Comparison of CCR1 & CCR5 levels according to CT scan severity score

Further analysis is performed to assess the validity of CCR1 and CCR5 in predicting the advanced lung involvement which assessed by CT scan severity scores. The CT severity score ranged as score 1 (where lung involvement < 5%), score 2 (5 – 25%), score 3(26 – 50%), score 4 (51-75%) and score 5 (lung involvement > 75%), the higher score indicates the more severe disease Scores of lung involvement, according to CT severity score (Al-Mosawe, *et al*., 2021).

Distribution of all 90 patients according to these severity scores revealed that none of the patients had score 1, 5 patients (5.6%) had score 2, 34 (37.8%) at score 3, 29 (32.2%) at score 4 and 22 (24.4%) at score 5 of CT severity score (Table 3.11).

Comparison of mean CCR1 level across the CT severity scores, showed that CCR1 level reduced with advancing severity score; in patients with score 2, mean CCR1 was 4.1 ± 2.7, in patients with score 3 , mean CCR1 level was 3.8 ± 2.2 , in

those with score 4 the mean CCR1 was 2.8 ± 1.3 and the least CCR1 level of 2.2 ± 0.7, in those with CT severity score 5, (*P. value < 0.001*),(Table 3.12 and Figure 3.3).

Almost similar trend was found with CCR5 across the CT severity score, where the mean CCR 5 decreased significantly with advancing CT severity score, it was 125.1 ± 74.3 in patients with score 2, 103.7 ± 30.8 in patients with score 3, 86.3 ± 36.6 in those with score 4 and the lower CCR 5 level in patients with CT severity score 5,6407±23.9 (*P. value <0.001*), (Table 3.13 and Figure 3.4).

Table 3.11. Computed tomography (CT) scan severity score according to lung involvement of COVID-19 patients (N=90)

CT Severity score*	No. of patients	%
Score 1	0	0.0
Score 2	5	5.6
Score 3	34	37.8
Score 4	29	32.2
Score 5	22	24.4
Total	90	100.0

Table 3.12. Comparison of CCR1 level according to CT scan severity score of lung involvement of COVID-19 patients (N=90)

CT Severity score	No. of patients	CCR1	
		Mean	SD
Score 2	5	4.1	2.7
Score 3	34	3.8	2.2
Score 4	29	2.8	1.3
Score 5	22	2.2	0.7
P. value	90	< 0.001 sig	

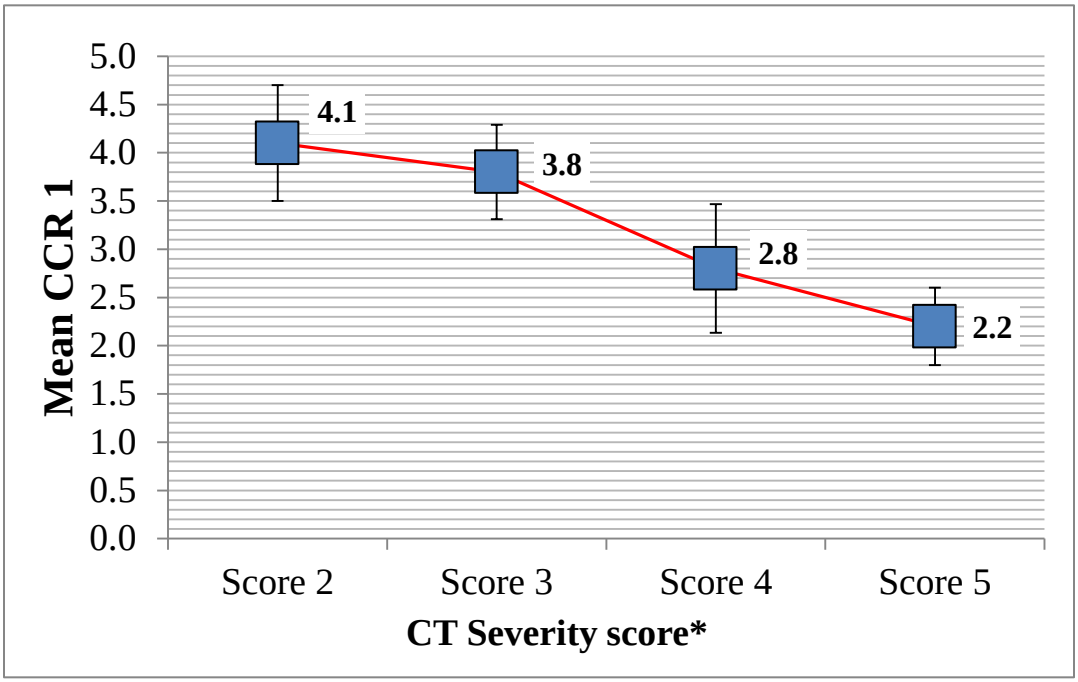


Figure 3.3. Mean values and 95% confidence intervals of CCR1 according to CT severity score of lung involvement of COVID-19 patients (N=90)

Table 3.13. Comparison of CCR5 level according to CT scan severity score of lung involvement of COVID-19 patients (N=90)

CT Severity score	No. of patients	CCR5	
		Mean	SD
Score 2	5	125.1	74.3
Score 3	34	103.7	30.8
Score 4	29	86.3	36.6
Score 5	22	64.7	23.9
P. value	90	< 0.001 sig	

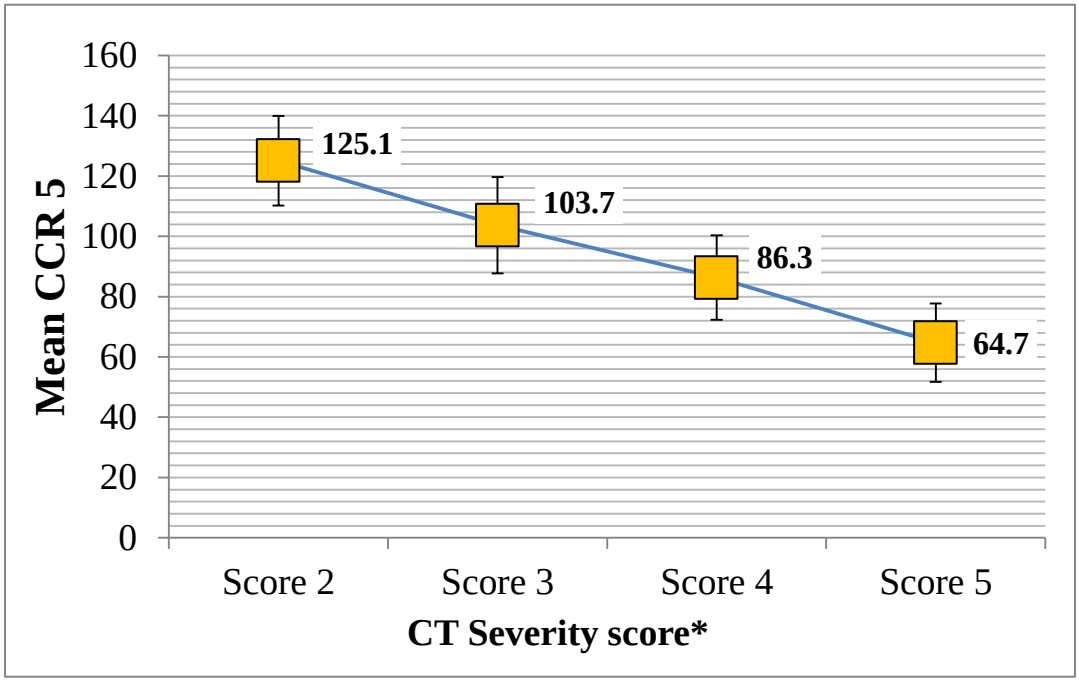


Figure 3.4. Mean values and 95% confidence intervals of CCR5 according to CT severity score of lung involvement of COVID-19 patients (N=90)

Furthermore, ROC analysis revealed that both CCR1 and CCR5 were good predictors of severe lung involvement assessed by CT severity scores. At an optimal cutoff point of 2.32 CCR1 produce an AUC of 0.895, sensitivity of 72.5%, specificity of 100%, accuracy of 86.3%, PPV of 100% and NPV of 78.4%, (Figure 3.5 and Table 3.14).

Regarding CCR5, the AUC was 0.843 at an optimal cutoff point of 83.5, giving a sensitivity of 71%, specificity of 100%, accuracy of 85.5%, PPV of 100% and NPV of 77.5%, (Figure 3.6 and Table 3.15).

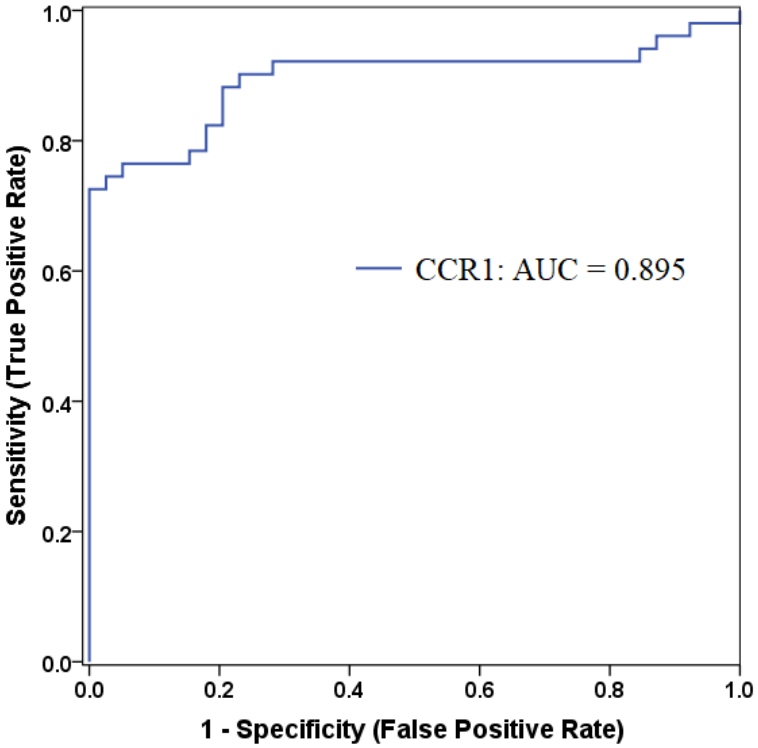


Figure 3.5. Receiver Operating Characteristics (ROC) curve analysis for the validity of CCR1 in prediction of advanced lung involvement CT severity score (score 4 and 5)

Table 3.14. Validity parameters and cutoff point of CCR1 in prediction of advanced lung involvement CT severity score (score 4 and 5)

Cutoff point	2.32
Area under ROC curve (AUC)	0.895
Sensitivity	72.5%
Specificity	100.0%
Accuracy	86.3%
Positive predictive value	100.0%
Negative predictive value	78.4%

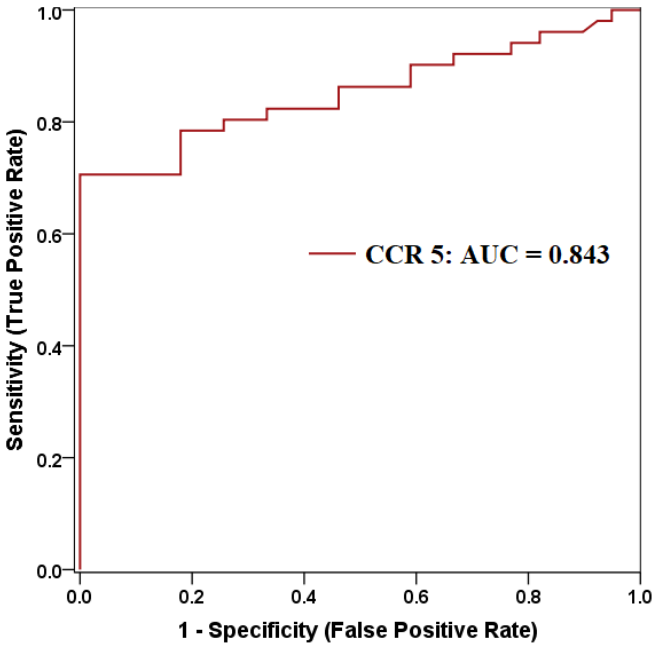


Figure 3.6. Receiver Operating Characteristics (ROC) curve analysis for the validity of CCR 5 in prediction of advanced lung involvement CT severity score (score 4 and 5)

Table 3.15. Validity parameters and cutoff point of CCR5 in prediction of advanced lung involvement CT severity score (score 4 and 5)

Cutoff point	83.5
Area under ROC curve (AUC)	0.843
Sensitivity	71.0%
Specificity	100.0%
Accuracy	85.5%
Positive predictive value	100.0%
Negative predictive value	77.5%

3.6. CCR1, CCR5 serum levels & outcome of studied groups

Regarding the outcome of the patients, unfortunately all patients with severe and one patient with moderate COVID 19 were died. From other point of view, 10 patients with moderate COVID 19 needed admission to ICU and 34 patients were discharged for home, (Table 3.16).

Moreover, comparison of CCR1 and CCR5 between died and survived patients revealed that CCR 1 and CCR5 were significantly higher in survived than died patients, in both comparisons, *P<0.001*, (Table 3.17)

Table 3.16 . Outcomes of patients with severe and moderate COVID-19

Outcome	Severe& critical (n = 45)		Moderate (n = 45)		Total
	No.	%	No.	%	
Home discharge	0	0.0	34	75.6	34
Admitted to ICU	45	0.0	10	22.2	10
Died	45	100.0	1	2.2	46

Table 3.17. Comparison of CCR1 and CCR5 between survived and died patients

Parameter	Outcome				P. value
	Survived (N = 44)		Died (N = 46)		
	Mean	SD	Mean	SD	
CCR1	4.17	2.73	2.09	1.30	< 0.001
CCR5	113.68	123.19	66.85	35.46	< 0.001

Chapter Four

Discussion

4.1. Demographic characteristics of the studied groups

Coronavirus disease appeared in 2020 as a cause of frequent and severe lethal pneumonia. Like many other infectious diseases, COVID-19 activate the immune response and host antiviral defense, if this process not well regulated it provokes the immunopathology which is life threatening. (Bartleson,*et al*. (2021)

The demographic and clinical characteristics of these patients revealed that patients with severe and critically ill cases were significantly older than those with moderate disease . These findings agreed that reported in previous studies from Iraq and other countries, (Al-Malkey and Al-Sammak, 2020; Poletti *et al*., 2021).

The current study found that males were more likely to have more severe/critical disease than females. However, the difference was statistically insignificant and this could be due to small sample size, previous studies documented significant higher incidence and more severe diseases among males, Jin et al.(2020) documented that males were about 2.4 folds more likely to have COVID-19 than females and more severe disease in males with higher mortalities

In the current study , no significant differences were found in residency and smoking. This could be attributed to the homogenous studied population and living environment. However, other studies documented conflicting results regarding smoking and residency (Lippi and Henry, 2020; Patanavanich and Glantz, 2020). In present study, higher proportion of severe/critical COVID-19 patients had history of hypertension, which agreed that reported by Mubarik et al(2021). In China. The association between hypertension and severity and mortality of COVID-19 was also documented in other studies and the prevalence of

hypertension among COVID-19 patients varied according to age of patients with higher rate in elderly (Leiva Sisnieguez, Espeche and Salazar, 2020; Wang ., 2020).

In present study , higher proportion of severe cases had history of CVD, nonetheless. The difference did not reach the statistical significance while other studies reported an association between severity of COVID-19 and CVD (Mai, Del Pinto and Ferri, 2020; Hessami *et al*., 2021).It could be due to small sample size in our study or variation between included populations.

4.2. Laboratory investigations of the studied groups

In the present study , laboratory investigations revealed that patients with severe-critical CVID-19 had significantly higher WBC count , neutrophil cells count , lower lymphocyte cells count. Platelets count was not significantly different between both groups while hemoglobin level was significantly higher in severe than moderate COVID-19 patients. This was not unexpected due to nature of this disease, however, in COVID-19 patients , levels of these parameters change in all forms of the disease, and their levels increase with the severity, therefore some studies tried to considered these parameters as markers for disease severity , but conflicting results were registered, (Elshazli *et al.*, 2020; Velavan and Meyer, 2020; Imran *et al.*, 2021; López-Escobar *et al*., 2021).

During the study , serum ferritin was significantly much higher in severe-critical than moderate patients, so as for D-dimer and LDH levels , where they were significantly higher in severe COVID-19 group. These parameters, have is shown to be crucial biomarkers for disease severity in many studies .Currently, clinicians classify severity of disease according to the values of S. ferritin and D-dimer, and also the management plan

based on their levels (Gómez-Pastora *et al.*, 2020; Yao *et al*., 2020; Ye *et al*., 2020; Yu *et al.*, 2020).

No significant difference in CRP levels between both groups, this may be because its positivity almost equal in moderate and severe cases. However in previous studies when the CRP compared across all severity forms (mild, moderate, severe and critical) cases, CRP appeared to be significant and promising marker; Pepys et al.(2021) Introduced that CRP could predict the outcome of COVID-19 but is it or not therapeutic target still need further evaluation. Chen et al(2020). documented that CRP was positively associated with severity of COVID-19 .

4.3. CCR1 & CCR5 serum levels among studied groups

It is documented that higher levels of CCR1 or CCR5, present in mild and severe cases of COVID-19. From other point of view, it has been reported that CCR1and CCR5, appeared to have protective effect in mouse infected with MA15-SARS-CoV and in human dendritic cells infected with SARS-CoV. On the other hand, and inhibition of these receptors improves the outcome of patients (Li et al., 2021; Patterson *et al*., 2021). Additionally, deficiency of these two receptors associated with decreased immune cells recruitment into the lung leading to more severe disease and higher mortality (Khalil, Elemam and Maghazachi, 2021).

In SARS-CoV-2, recent clinical trials documented that inhibition of CCR1 and CCR5 have a significant effect in reducing the inflammatory cytokines in severe and critical cases of COVID-19 and also reduce the viremia of patients . The blockade of these receptors could lead to preventing pulmonary transmission of proinflammatory leukocytes and diminish activation of immune pathogenicity in human (Bonville *et al*., 2004; Seethamraju *et al.*, 2020; Yang *et al*., 2020;

Agresti *et al.*, 2021; Patterson *et al.*, 2021). However, the role of chemokines in general and CCR1 and CCR5 in special, in COVID-19 still under debate among scientific community.

The current study tried to assess the association of chemokine receptor 1 and chemokine receptor 5 with COVID-19 severity among Iraqi patients. Hence, a total of 90 patients were enrolled in this study ; 45 cases with moderate and 45 cases with severe and critical stages of disease.

The current study discovered that the mean levels of CCR1 and CCR5 were significantly lower in Severe-critical COVID-19 cases than those with moderate disease, with a highly significant difference. Which agreed that reported (Bini, Kirke and Schramm, 2021). and Fernand et al. from Austria and Australia, respectively. On the other hand, clinical trials showed that lower levels of these receptors associated with more severe disease. (Patterson *et al.*, 2021) concluded that inhibition of CCR5 in patients with severe or critical COVID-19, lead to significant decrease in cytokines, and significant increase in CD8 T-cells and further reduction in viral load after 14 days. In earlier study conducted by (Sheahan *et al.*, 2008). In 2008 on SARS-CoV infection revealed that in genetically deficient mice for CCR1, CCR2 and CCR5 infected with SARS-CoV, had higher weight loss and more damage in their lungs which support the fact that more severe COVID-19 associated with lower levels of CCR1 and CCR5. Also these findings indicated the importance of chemokines in immunopathogenesis of SARS-CoV. However, clinical trials enrolling , mild, moderate and severe cases of COVID-19 still ongoing to assess the ameliorating efficacy of CCR5, CCR1 antagonist in However, different chemokines inhibitors have been tested to assess the exacerbated proinflammatory response in COVID-19 patients. Treatment target severe

and poor prognosis via inhibition of chemokines signaling. CCR5/CCR2b antagonist have shown to reduce virus replication (Okamoto, Toyama and Baba, 2020). Moreover, CCR5 blocking antibody has shown to reduce severity and viremia in advanced COVID-19 cases (Patterson *et al*., 2020). In fact, CCR5 blocking antibody has been developed to for inhibition of HIV entry by blocking the binding of CCR5 to HIV gp120, without blockade to CCR5-lignad binding, but in COVID-19 the virus do not use CCR5 for its entry , and may act in alternative mechanism (Bonville *et al*., 2004; Seethamraju *et al*., 2020; Yang *et al*., 2020; Agresti *et al*., 2021; Patterson *et al*., 2021).

4.4. Correlation between CCR1, CCR5 & other study variables

Significant correlation was appeared between WBC count and CCR1 & LDH level with CCR1 in sever-critical cases. Also, a significant association between WBC count and CCR1 in moderate cases. The association between WBC count and CCR1 could be to the association between chemokines receptors and leukocytes trafficking or other unexplained factor, because the immunopathogenesis of COVID-19 is still not well clarified (Olson and Ley, 2002; Jacques and Apedaile, 2020).

For CCR5 correlation with other parameters in sever-critical cases, a positive correlation was found for WBC & neutrophil count while negative correlation for lymphocyte count was obtained. A formerstudies showed that sever COVID-19 cases have low lymphocyte and high neutrophil count compared to other groups (Ding *et al*.,2020; Guan *et al*., 2020; Yang *et al*., 2021). Also, positive correlation with CRP & LDH

levels with CCR-5 in sever-critical cases was present and these findings are similar to previous data reported by meta-analysis done by Hariyanto. *et al*., 2021.

4.5. Diagnostic utility of CCR1 & CCR5

From other point of view, both CCR1 and CCR5 appeared to be good predictor of severe COVID-19 , at a cutoff point of 2.32 , CCR1 and AUC of 0.937 which a large area under the ROC curve indicating higher validity and prediction ability that produce higher sensitivity. Specificity and accuracy. Similarly, CCR5 was good predictor with an AUC of 0.852 and at a cutoff point of 83.5, CCR5 was good sensitive, specific and accurate in a rate of 71.1%, 91.1% and 81.1%, respectively.

Studies concerned with the validity of CCR1 and CCR5 as predictor of COVID-19 severity are almost unavailable, only two studies concerned with this subject, (Fernand *et al.,* 2020). from Austria, documented that lower levels of CCR1 and CCR5 associated with more severe disease and that both parameters were good predictor and can be used as a marker to predict the outcome and disease severity , anyway, they preferred CCR5 than CCR1 because it is more popular. In other study from Australia, Bini et al(2021). reported that both CCR1 and CCR5 were significantly higher in severe and critical patients with COVID-19 than those with mild or moderate disease. Furthermore, role of chemokines and its association with COVID-19 are still unclear; but their role in other infectious disease are well studied such as with HIV infection (Barmania and Pepper, 2013).

4.6. Comparison of CCR1 & CCR5 levels according to CT scan severity score

Both CCR1 and CCR5 were good predictors of severity according CT scan severity scoring. Further analysis of severity of disease was

performed according to chest CT scan severity score (Wasilewski *et al*., 2020; Al-Mosawe, mohammed and Fayadh, 2021), observed, that both CCR1 and CCR5 were good predictor for severity of disease when assessed according to the CT scan. And there was an inverse correlation between levels of CCR1 and CCR5 from one side and Chest CT scan severity score. No studies available regarding this correlation, but in current study, this significant correlation and may need further assessment by future studies.

4.7. CCR1, CCR5 serum levels & outcome of studied groups

The current study documented a highly significant lower levels of CCR1 and CCR5 in died cases compared to survived ones. The majority of severe and critical cases died, which is supported the previously mentioned that critically ill COVID-19 cases were more likely to have lower levels of these receptors. However, higher mortality rates in severe and critically ill patients were reported globally where majority of cases admitted to Intensive Care units had poor prognosis, outcomes and high mortality rates. The higher mortality rates mainly due to more advanced disease, cytokine storm and more advanced pulmonary damage in addition to advancing age and comorbidities in such patients (Grasselli *et al.*, 2020; Moreira, 2020; Flythe *et al*., 2021).

From strength points of this study is the first study conducted in Iraq, and one of the little studies concerned with this issue, so it will be added to the growing scientifically sound database in the scientific community. However, the study is not free of limitations, among these is small sample size and short duration of the study in addition to restriction in obtaining data from authorized agencies, however, further studies can be conducted in better conditions and overcome these limitation.

Conclusion and Recommendation

Conclusions:

1. The demographic characteristics of severe and moderate COVID-19 patients in this study did not much different than epidemiological picture of Iraqi patients according to previous literatures.
2. Severity of disease was significantly associated with advancing age of patient.
3. Severity of disease was not associated with gender, residency or smoking status.
4. Hypertension and cardiovascular diseases were more frequent in severe and critically ill cases than those with moderate disease.
5. Almost all laboratory , hematological and inflammatory markers levels were significantly different between severe and moderate cases.
6. Chemokine receptor -1 and Chemokine receptor -5 were significantly lower in severe- critical than moderate cases.
7. Both receptors were significantly associated with more severe disease according to clinical and chest CT scan severity score.
8. Chemokine receptor -1 and Chemokine receptor -5 were good predictors of severity of COVID-19 with good sensitivity, specificity and accuracy.

Recommendations:

In light of the study findings and conclusions, it is recommended that:

1. Chemokine receptors in general should be taken into account in assessment of severity of COVID-19, in particular CCR1 and CCR5.

2. CCR -1 and CCR-5 could be a promising marker for early prediction for the outcome of COVID-19 cases before they reach the critical status and therefore their outcome can be improved when early measures taken to prevent deterioration.

3. Further studies with larger sample size are highly suggested for further assessment and evaluation of validity of these receptors including other variables and also mild cases.

4- chemokine gene polymorphisms studies are highly suggested for assessment of covid-19 severity .

Chapter Five
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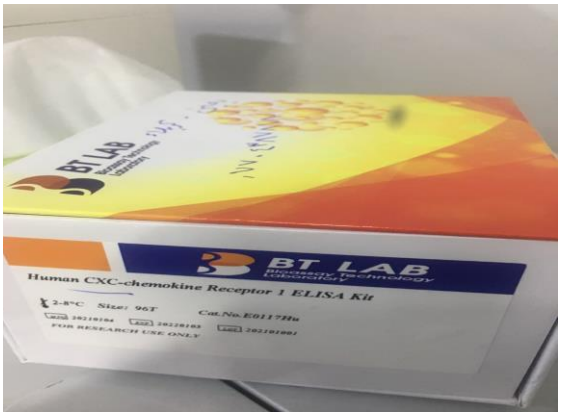
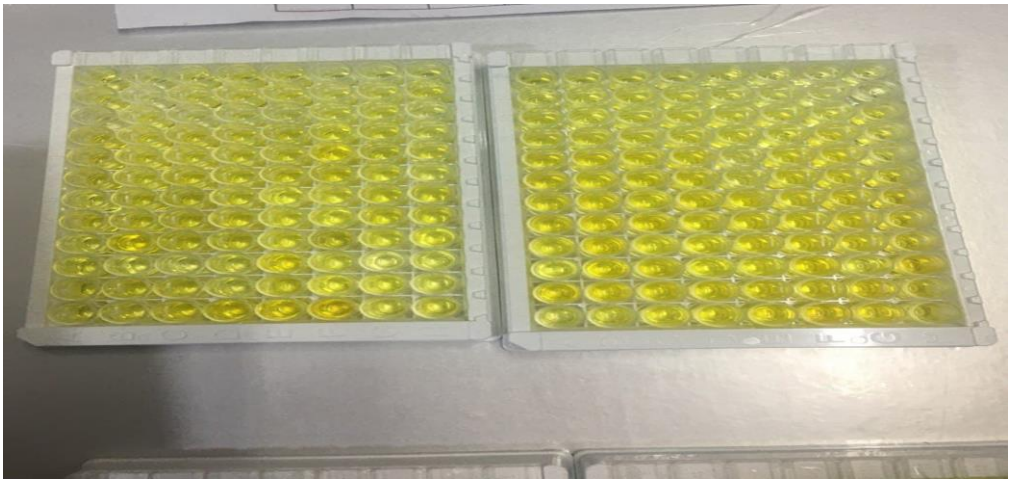
Appendix

Appendices1:

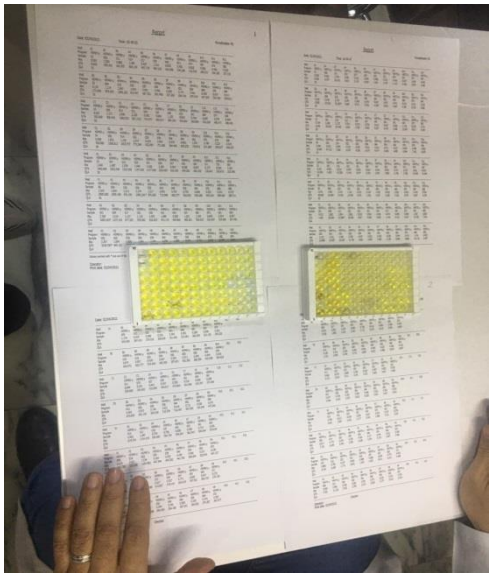
A. Patient sample



B. Kit of markers



c-micro plate of immunological markers



E-Result OF CCR1&CCR5

D-ELISA System

Appendices 2:

Snibe maglumi 800

ppendix3: Questionnaire Data

Case No.:

Name: Age: gender

Residency: Rural: Urban:

- Disease duration

- Smoker non smoker

- Hypertension

- Cardiovascular disease

- Fever - Cough

- Expectoration - Rhinorrhoea

- Myalgia or fatigue - Nausea and vomiting

- Sore throat - Shortness of breath

- Chest pain - Diarrhea

Lab. Data:

- CBC Leukocytes count

Neutrophils count Lymphocytes count

Platelets count - Hemoglobin

- Serum ferritin - C-reactive protein

- D-dimer - LDH

- C T scan

الملخص

فيروس كورونا المتلازمة التنفسية الحادة الوخيمة 2 (SARS-CoV-2) ، العامل المسبب لمرض فيروس كورونا 2019 (COVID-19) ، أصبح الآن وباءً حيث تم الإبلاغ عن ما يقرب من 219 مليون حالة حتى الآن. على الرغم من أن غالبية مرضى COVID-19 يعانون من أعراض خفيفة أو معتدلة فقط ، فإن مجموعة فرعية ستتطور إلى مرض شديد مصحوبًا بالتهاب رئوي ومتلازمة الضائقة التنفسية الحادة (ARDS) التي تتطلب تهوية ميكانيكية. تشير النتائج المستجدة إلى استجابة مناعية غير منتظمة تتميز بالالتهاب الجامح ، بما في ذلك متلازمة إطلاق السيتوكين (CRS). يُعتقد أن مرضى COVID-19 المصابين بأمراض خطيرة والذين يعانون من العاصفة الكيماوية لديهم تشخيص أسوأ وزيادة معدل الوفيات. في المرضى المصابين بفيروس SARS-CoV-2 ، تبدو العاصفة الكيميائية مهمة لإحداث العديد من المظاهر الحادة لـ COVID-19 مثل متلازمة الضائقة التنفسية الحادة. يبدو أن مستقبلات الكيموكين تلعب دورًا رئيسيًا لأنها تشارك في تطوير وتطور COVID-19 الأكثر خطورة والتي قد تهدد الحياة. إن فهم التسبب في العاصفة الكيماوية سيساعد ليس فقط في الكشف عن عوامل الخطر للحالة ولكن أيضًا الاستراتيجيات العلاجية لتعديل الاستجابة المناعية وتقديم نتائج محسنة لدى مرضى COVID-19 المعرضين لخطر كبير للإصابة بأمراض خطيرة. لذا ، فإن الهدف من الدراسة الحالية هو الكشف عن العلاقة بين مستقبلات الكيموكين (CCR1، CCR5) وشدة مرض COVID-19 مع دوره في التسبب في المرض والعلاج المحتمل في المستقبل. تم أخذ تسعين عينة دم من مرضى تم تشخيصهم إكلينيكيًا بفيروس كورونا. وأخذت العينات من كلا الجنسين (50) ذكر و (40) انثى تراوحت اعمارهم بين (20-85) وقسمت الى حالات متوسطة وحرجة شديدة. كان جميع المرضى المشمولين من قسم الطب الباطني ووحدة العناية المركزة في مستشفى الحسيني التعليمي في كربلاء / العراق خلال الفترة من ديسمبر 2020 حتى فبراير 2021. تم تحليل عينات الدم باستخدام مجموعات ELISA للمستقبلات الكيميائية CCR1 و CCR5. كان متوسط مستويات CCR1 و CCR-5 أقل بشكل ملحوظ في المرضى الذين يعانون من الحالات الحرجة الشديدة من أولئك الذين يعانون من COVID-19 المعتدل مع P <0.001. تم إجراء تحليل منحنى خصائص تشغيل جهاز الاستقبال (ROC) وكشف أن CCR1 و CCR-5 كانا مؤشرًا جيدًا على شكل حاد من المرض مع المنطقة الواقعة تحت منحنى = ROC (AUC) 0.937، 0.852 على التوالي مع حساسية وخصوصية عالية. للاستنتاج ، أشارت هذه النتائج إلى أن انخفاض CCR1 و CCR5 المرتبطين بحالة مرضية أكثر شدة وكلا المستقبلين يمكن اعتبارهما مؤشرات بيولوجية جيدة لشدة الإصابة بعدوى COVID-19.

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علاقة مستقبلات كيموكين المصلية 1 ومستقبلات كيموكين 5 مع شدة مرض كورونا(مرض كوفيد -19)

رسالة

مقدمة الى مجلس كلية الطب / جامعة كربلاء لنيل درجة الماجستير في علم الاحياء المجهرية الطبية بواسطة الباحثة

سجى ابراهيم جاسم

بكالوريوس علوم الحياة / جامعة كربلاء 2011

بإشراف

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2021م **1443هـ**