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Correlation of serum Bradykinin and Membrane Attack Complex with severity of covid- 19 regarding Computed Tomography finding

A THESS

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By

Aqeel Salman Abd Al-salam AL-Khazraji

B.Sc. Clinical Analysis, College of Medical and Health Technologies.

Supervised by

Prof. Dr.
Alaa Saad AL-Attabi

College of Medicine

University of Karbala

2021 A.D

Prof. Dr.
Dhamiaa Maki Hamza

College of Medicine

University of Karbala

1443A.H

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

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صدق الله العلي العظيم

سورة المجادلة - الآية ١١

Certification

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

Professor

Dr. Alaa S. Alattabi

M.B.Ch.B F.I.C.M.S path/immunity & microbiology

College of Medicine

University of Karbala

Professor

Dr. Dhamiaa maki Hamza

PhD of microbiology

College of Medicine

University of Karbala

Chair of microbiology department

In view of the available recommendation, I forward this thesis for debate by the examination committee

Head of department

Signature:

Assist. Prof.

Dr. Sawsan Mohammed Jabbar AL-Hasnawi

College of medicine

University of Karbala

Dedication

To ... The one who taught me how to stand firmly above the ground.....

My esteemed father.

To... The source of love, altruism and generosity.

My esteemed mother

To... Those who I faced the difficulties by them my brothers and sisters

To ... My Wonderful Wife

To... My soul and the pulse of my heart

My sons (Aeham & Dhagum)

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Summary

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It causes serious respiratory illness such as pneumonia and lung failure. The study aimed to evaluate correlation of Bradykinin, membrane attack complex serum levels, to pulmonary computed tomography scoring in patients with coronaviruses (covid-19).

The study is a cross – sectional that included 90 patients. The participants comprising of 44% severe, 29% moderate and 17% mild patients with covid–19 (56 males and 34 females) who were attending in Al-Hussein medical city, Al-Hayat units, in Karbala, Iraq, were carried out for the period from October 2020 to January 2021. Subjects enrolled in the study were categorized into three group's patient severe, moderate and mild according to lung involvement diagnosed by pulmonary computed tomography scores under the supervision radiologist. Laboratory tests for serum ferritin, D. dimer, C - reactive protein and hematological parameter were. Sandwich ELISA for patients, were tested for specific serum human Bradykinin and membrane attack complex.

The result of the study revealed that the general mean age in studied groups (48.86±15.73) range (19 – 88) years, a total of 90 patients included 56 males at percentage of 62.2 % and 34 females at percentage of 37.8 %. (40 male, 5 females) are smoker and (16 males, 29 females) are non- smokers. According to the mean serum levels of bradykinin among patient groups were (3.58±1.5 ng/ml) in mild, moderate (8.77 ± 1.74 ng /ml) and severe (20.01±5.72ng /ml), with highly significant (P=0.0005) correlation with covid – 19. Serum level of membrane attack complex showed highly significant (P=0.0001) correlation. The mean serum levels of MAC were (218.75±130.37 ng /ml) in mild, moderate

(734.45±466.07 ng /ml), and severe (1130.14±648.78 ng/ml) in covid – 19 patient, the highly concentration of serum levels of bradykinin and membrane attack complex was noticed in severe group when compared with moderate and mild groups according to total lung involvement.

Moreover the mean of total lung involvement in mild (4.94 ± 2.436), moderate (12.59 ± 1.680) and severe was (19.98 ± 2.063), and that showed a highly significant correlation (P=0.0005) with covid – 19 patient groups; a highly total lung involvement in severe more than moderate and mild groups. Total lung involvement and serum levels of BK was significantly different between patient groups (P=0.002, P=0.0005, P=0.0005 respectively), there was a significant positive correlation of MAC with total lung involvement in mild group (P<0.05). On the other hand there was non-significant correlation of total lung involvement with MAC in moderate and severe groups (P>0.05).

Also there was non-significant correlation among total lung involvement, bradykinin and membrane attack complex with hematological parameters (P>0.05). On the other hand a positive significant correlation of total lung involvement with D. dimer and S. ferritin in the mild group (P<0.05) and positive significant correlation of MAC with D. dimer in mild group (P<0.05).There was positive significant correlation of MAC with S. ferritin in severe and mild groups (P<0.05). Significant positive correlation was observed between BK with D. dimer in severe group (P<0.05).

In addition, the levels of C. reactive protein (CRP), D. dimer and S. ferritin were increased in moderate and severe groups in comparison to mild group and given significant correlation (P=0.0005, P=.009, P=0.0005). Therefore C. reactive protein (CRP), D. dimer and S. ferritin could serve as markers for disease severity.

The results of this study have shown elevated serum levels of BK and MAC in severe group compared with moderate and mild groups of covid 19 patients; therefore, these markers may have an important role in diagnosis of severe covid- 19 patients. In conclusion this study revealed significant correlation of serum BK levels with severity of covid -19. The serum levels of membrane attack complex positive correlation with mild lung involvement while non-significant correlated with moderate and severe lung involvement.

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List of Abbreviations

Abbreviation	Meaning
3CLpro	3 – chymotrypsin like protease
AA	Acquired angioedema
ACE	Angiotensin- converting enzyme
ARDS	Acute respiratory distress syndrome
Arg	Arginine

B 1	Bradykinin receptor one
B 2	Bradykinin receptor two
BK	Bradykinin
C-C motif	Monocyte chemoattractant protein
C-CL3	Macrophage inflammatory protein
COPD	Chronic obstructive pulmonary disease
CoVs	Coronaviruses
CT	Computed tomography
DTH	Delayed hypersensitivity
FbDP	Fibrin degradation products
FER	Ferritin
FGF2	Fibroblast growth factor2
GCSF	Granulocyte colony-stimulating factor
Gly	Glycine
GMCSF	Granulocyte- macrophage colony stimulating factor
HA	Hereditary angioedema
IBV	Infectious bronchitis virus
IL-10	Interleukin ten

IL-13	Interleukin thirteen
IL-1B	Interleukin one beta
IL-2	Interleukin two
IL-4	Interleukin four
IL-6	Interleukin six
IL-7	Interleukin seven
IL-8	Interleukin eight
IL-9	Interleukin nine
INFy	Interferon gamma
IP10	Interferon gamma induced protein ten
KDa	Kilo Dalton
KKS	Kallikrein-Kinin system
m RNA	Messenger ribonucleic acid
MAC	Membrane attack complex
MBL	Mannan-binding lectin
MCPI	Monocyte chemoattractant protein one
MERS-CoV	Middle east respiratory syndrome coronaviruses
MIP1a	Macrophage inflammatory protein one alpha

MIP1b	Macrophage inflammatory protein one beta
MOF	Multiple organ failure
NOD-like receptor	Nucleotide – binding leucine- rich repeat receptor
ORF	Open reading frame
PDGFB	Platelet-derived growth factor subunit B
RIG-1 like receptor	Retinoic acid-inducible rich repeat receptor
RNA	Ribonucleic acid
SARS	Severe acute respiratory syndrome
Ser	Serine
SPR	Solid phase receptacle
STR	Strip
TMPSS2	Transmembrane protease serinr2
TNFa	Tumor necrosis factor alpha
VEGFA	Vascular endothelial growth factor A

Chapter One

Introduction And Literature Review

1.1. Introduction:

Coronavirus is one of the main pathogens that primarily attack the human respiratory system. Previous outbreaks of coronaviruses include the severe acute respiratory syndrome and the middle east respiratory syndrome which have been previously characterize as agents that are a great public health threat. In late December 2019, a group of patients was admitted to hospitals with an initial diagnosis of pneumonia of an unknown etiology. These patients were epidemiological linked to a seafood and wet animal wholesale market in Wuhan, Hubei Province, China (Rothan & Byrareddy 2020).

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. On electron microscopy, these viruses show a characteristic appearance that resembles a crown (corona in Latin means crown) due to the presence of club-shaped surface protein projections (Pal *et al.*, 2020). The CoVs are pleomorphic, measure between 80 and 160 nm in length, and have a small genome measuring 27-32 Kilobase with a unique replication strategy (Sahin *et al.*, 2020).

Transmission is generally transmitted through direct or indirect contact of mucous membranes (eyes, nose, or mouth) with infectious respiratory droplets or fomites. Transmission risks increase with period and proximity with the contacts infected persons (Pal *et al.*, 2020). Patients with COVID-19 present primarily with increased body temperature, myalgia or fatigue, and dry cough. Although most patients are thought to have appropriate prognosis, older people and those with chronic underlying conditions may have worse outcomes (Chen *et al.*, 2020).

Patients with severe disease may evolve dyspnea and hypoxemia within one week after onset of the illness, which may rapidly progress to acute respiratory distress syndrome (ARDS) or end-organ failure (Chen *et al.*, 2020).

The main pathogenesis of COVID-19 infection as a respiratory system targeting virus was severe pneumonia, combine with the incidence of ground-glass opacities, and acute cardiac injury. Significant high blood level of cytokines and chemokine's were noted in patients with COVID-19 infection that included interleukin1-β , interleukin 7, interleukin 8, interleukin 9, interleukin 10, basic fibroblast growth factor 2, granulocyte colony stimulating factor, granulocyte macrophage colony stimulating factor, interferon gamma γ, interferon inducible protein 10, monocyte chemoattractant protein 1, microphage inflammatory protein 1α, microphage inflammatory protein 1β, platelets derived growth factor B, tumor necrosis factor α, and vascular endothelial growth factor A. Some of the severe cases that were admitted to the ICU show high level of pro-inflammatory cytokines including interleukin 2, IL7, IL10, GCSF, IP10, MCP1, MIP1α, and TNFα that are cause to develop disease severity (Huang *et al.*, 2020) .

Bradykinin [BK-(1-9)] is a peptide-hormone of the kallikrein-kinin system (KKS) which actions were first characterized by an elegant study by Rocha e Silva and coworkers in 1949. Bradykinin (BK), a non-peptide of sequence Arg1 -Pro2 -Pro3 -Gly4 -Phe5 -Ser6 -Pro7 -Phe8 -Arg9 is a member of the kinins, a group of peptides ubiquitously produced by the action of kallikreins on circulating kininogens. Other members of the kinin family include kallidin (KD) (Lys0 -BK) and the metabolites of KD and BK: desArg9 - BK and desArg9 –KD (Souza-Silva *et al.*, 2020, Rasaeifar *et al.*, 2019). Bradykinin is stimulate by different inflammatory mediator such as interleukins (IL) (IL-4, IL-6, IL-8, and IL-13) and tumor necrosis factor-alpha (TNF-α). Angiotensin-converting enzyme metabolizes BK and blocks its effect on B2 receptors, whereas ACE2 metabolizes

des-Arg9-BK and Lys-des-Arg9-BK and blocks their effect on B1 receptors (Sodhi *et al.*, 2018).

In SARS-CoV-2 infection, BK and its metabolites are augmented due to down-regulation of ACE2 by SARS-CoV-2; therefore dysregulation of this pathway may lead to acute pulmonary damage, fluid extravasation, leukocyte recruitment, and the development of ARDS via activation of B1 receptors, which are overexpressed by the pro-inflammatory conditions. Activation of BK system in the acute viral respiratory infection increases the risk of capillary permeability and development of multiple organ failure (MOF), exposure to SARS-CoV-2 infection reduces the expression of ACE2 and increases the activity and level of Des-Arg973-BK (DABK).Elevate signaling through DABK/BKB1R system leads to vascular–alveolar fluid extravasation, leukocyte extravasation, and ARDS development (Tolouian *et al.*, 2020).

Membrane attack complex is a multi-meric assembly of proteins consisting of C5b, C6, C7, C8 and multiple copies of C9, which forms the membrane spanning pore (Morgan 2016). The membrane attack complex is the end product of a complex series of biochemical interactions in which initially soluble complement proteins bind and undergo dramatic structural rearrangements to form a transmembrane pore. The resulting membrane attack complex pores are a hetero-oligomer formed from the irreversible, stepwise assembly of 7 different polypeptide chains: C5b, C6, C7, C8 (a hetero-trimer comprised of C8α, C8β and C8γ) and C9, where 18 copies of C9 are required to complete the pore .activation of complement leads to the generation of C5b via the cleavage of C5 by membrane-bound C5-convertase enzymes (Heesterbeek *et al.*, 2019). C5b is a metastable intermediate that rapidly sequesters C6. Recruit of C7 unfurls a lipophilic domain upon binding, while integration of C8 into the assembly is accompanies by an initial insertion into the membrane. The C5b-8 initiator complex then binds C9 and undergoes unidirectional, clockwise oligomerization

(with 18 copies of C9) to complete an 11 nm wide transmembrane pore (Parsons *et al.*, 2019, Serna, M. *et al.*, 2016).

Human C-reactive protein (CRP) was identified as a plasma protein which, in the presence of Ca^{2+}, precipitated C-polysaccharide (PnC) isolated from the cell wall of *Streptococcus pneumoniae*. The precipitation was due to the binding of CRP to phosphocholine (PCh) moiety present in PnC. In animals, we define a protein as CRP if it has at least two of the following three characteristics: First, it is a cyclic oligomer of almost identical subunits of molecular weight 20–30 kDa. Second, it binds to PCh in a Ca^{2+}-dependent manner. Third, it exhibits immunological cross-reactivity with human CRP (Pathak, A., & Agrawal, A. 2019).

D-dimer molecules are generated through the degradation of cross-linked fibrin during fibrinolysis. D-dimer generation requires the activity of three enzymes: thrombin, activated factor XIII (factor XIIIa), and plasmin. The process starts when thrombin generated by the coagulation system converts soluble fibrinogen to fibrin monomers. These monomers then form fibrin polymers through noncovalent interactions based on allosteric changes within the protein as a result of thrombin cleavage of fibrinopeptides from the N-terminal domain. Fibrin is strengthened through interactions with factor XIII, which, after activation by thrombin, cross-links the D domains of adjacent fibrin monomers. Plasmin digestion of the fibrin clot results in the D-dimer molecule (Johnson *et al*., 2019)

Ferritin is a spherical protein made of 24 subunits. These subunits are composed of heavy (FtH) and light (FtL) chains and their proportional contribution to the hollow spherical shell varies among tissues. Ferritin was traditionally considered to be a cytosolic protein with the mere function of iron storage (Zarjou *et al*., 2019**)**.

1.2. Aim of the study:

This study aimed to evaluate correlation of bradykinin, membrane attack complex serum levels, to severity of pulmonary computed tomography scoring in patients with coronaviruses (covid-19). Those were achieved by the following objectives:

1- Determination the concentration of BK and MAC in serum of patients with covid-19 by ELISA kit.
2- Determination the pulmonary CT. scoring in patients with coronaviruses (covid-19).
3- Determination the concentration of S. ferritin, D. dimer and CRP in serum of patients with covid – 19.
4- Determination correlation of BK, MAC with S. ferritin, D. dimer and CRP in serum of patients with covid – 19.
5- Determination correlation of BK, MAC and CT scoring in disease by statistical package for Social sciences (SPSS) using.

1.3. Coronaviruses:

Coronavirus disease (COVID-19) is an infectious disease cause by a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that initially started in Wuhan province in China and has now affected > 200 countries worldwide and declared a Pandemic (Huang *et al.*, 2020). The virus mainly affected the respiratory system causes flu-like diseases with symptoms such as a cough, fever, and more severe cases (Zou *et al.*, 2020).

Coronavirus are a group of envelope viruses with non-segmented, single-strand, positive sense RNA genomes. A part from infecting a variety of economically important vertebrates (such as pigs and chickens), six coronaviruses have been known to infect human host and cause respiratory disease. Among them, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle

East respiratory syndrome coronavirus (MERS-CoV) are zoonotic disease and highly pathogenic coronaviruses that have resulted in regional and global outbreaks (of the International 2020).

1.4. Etiology:

Coronaviruses (CoVs) are a large family of RNA viruses that are found diversely in animal species. They are known to cause diseases of the respiratory, hepatic, nervous system, and gastrointestinal systems in humans. Under the electron microscope, they impart a crown-like appearance due to the presence of envelope spike glycoproteins .CoVs belong to the Roniviridae, Arteriviridae, and Coronaviridae families (Cascella *et al.*, 2021 , Chen *et al.*, 2020).

The Coronaviridae family can be classified into four genera of alpha-COV, beta-COV, delta-COV, and gamma-COV .Gene characterization has helped identify that bats and rodents are the gene source of alpha-COV and beta-COV, On the other hand, avian species are deemed as genetic sources of delta-COV and gamma-COV (Cascella *et al.*, 2021).

CoVs are responsible for 5-10% of acute respiratory infections, it has been estimated that 2% of the population are deemed healthy carriers of these viruses. Some common human CoVs include HCoV-OC43, HCoV-HKU1, HCoV-229E, and HCoV-NL63 , In the immune-competent, these CoVs clinically present with self-limiting respiratory infections and common colds , In the elderly and immune-compromised, they can involve the lower respiratory tracts , Other human CoVs such as MERS-CoV, SARS-CoV, and SARS-CoV-2 present with pulmonary and extra-pulmonary features (Cascella *et al.*, 2021, Chen *et al.*, 2020). SARS-CoV-2, which is responsible for the COVID-19 pandemic, is a type of beta-COV. Genomic characterization studies of the new strain have

indicated an 89% nucleotide match with bat SARS-like CoVZXC21, There is also an 82% nucleotide match with the human SARS virus (Chan *et al.*, 2020).

1.5. Epidemiology:

Coronaviruses (CoVs) have been related to significant disease outbreaks in the East Asia and the Middle East. The severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) began to appear in 2002 and 2012, respectively. Recently, a new coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causes coronavirus disease 2019 (COVID-19), appears in late 2019, and it has posed a global health threat, causing an ongoing pandemic in many countries and territories (Rodriguez-Morales *et al.*, 2020).

Coronavirus belongs to the family Coronaviridae (subfamily Coronavirinae), the member of which infect a wide range of hosts, produce symptoms and illnesses range from the common cold to the severe and eventually fatal diseases , such as SARS, MERS, and, presently, COVID-19. SARS-CoV-2 is considered one of the seven members of the CoV family that infected humans (Zhu *et al.*, 2020). It belongs to the same lineage of Coronaviruses that cause SARS; however, this novel virus is genetically distinct. Until 2020, six CoVs were known to infect humans, includes human CoV 229E (HCoV-229E), HCoV-NL63, HCoV-OC43, HCoV-HKU1, SARS-CoV, and MERS-CoV. Although SARS-CoV and MERS CoV have resulted in outbreaks with high mortality, others remain associated with mild upper-respiratory-tract illnesses (Wei *et al.*, 2020).

Newly progress Coronaviruses pose a high threat to global public health. The current emergence of COVID-19 is the third Coronavirus outbreak in humans over the past 2 decades. It is no coincidence that predicted potential SARS- or MERS-like CoV outbreaks in China following pathogen transmission

from bats (Munster *et al.*, 2020, Fan *et al.*, 2019). Coronaviruses are appear in China and spread rapidly everywhere of the country and, later, to other countries. Due to the severity of this outbreak and the prospect of spreading on an international scale, the WHO declared a global health emergency on 31 January 2020; subsequently, on 11 March 2020, they announced it a pandemic situation. At present, we are not in a position to effectively treat COVID-19, since neither confirmed vaccines nor specific antiviral drugs for treating human CoV infections are available (Lu 2020).

1.6. Classification of coronaviruses:

Orthocoronavirinae is a subfamily of the virus. superkingdom; Riboviria (clade); Orthornavirae (kingdom); Pisuviricota (phylum); Pisoniviricetes (class); Nidovirales (order); Cornidovirineae (suborder), and Coronaviridae (family) according to the Coronaviridae Study Group (CSG) of the International Committee on Taxonomy of Viruses (ICTV) (Guruprasad 2020).

According to the International Committee on Taxonomy of Viruses, coronavirus classify under the order Nidovirales, family Coronaviridae, and subfamily Coronavirinae. Depend on early serological and later genomic evidence; Coronavirinae is divided into four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus. Four discrete pedigrees (A, B, C, and D) have been assigned within the genus Betacoronavirus. Among the six known human coronaviruses, HCoV-229E and HCoV-NL63 belong to Alphacoronavirus, while HCoV-OC43 and HCoV-HKU1 belong to lineage A, SARS-CoV to lineage B, and MERS-CoV to lineage C Betacoronavirus: (Woo *et al.*, 2012) as seen in figure (1.1).

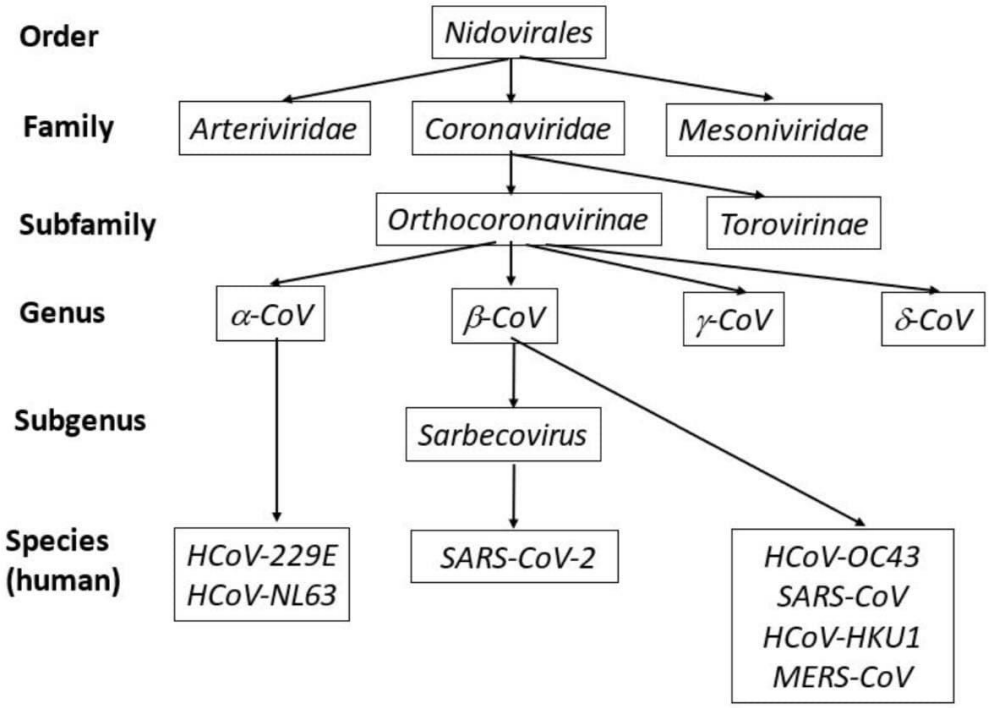

Figure (1.1): Coronaviruses classification. (Malik 2020).

1.7. Structure of coronaviruses:

The genome of coronaviruses is a non-segmented, single-stranded RNA molecule with positive sense (mRNA), which is the same sense as the mRNA. Structurally it's similar to most eukaryotic mRNAs, in having 5'caps and 3′ poly-adenine tails. One of the distinctive features of the coronavirus genome its remarkably large size range from 26 to 32 kb. For comparison, this is approximate three times the size of alphavirus or flavivirus genomes and four times the size of picornavirus genomes. Indeed, the size of the coronavirus genome is among the largest known viral genomic RNAs. The genome contain multiples ORFs, encoding a fixed array of structural and the non-structural proteins, as well as a variety of accessory proteins which differs in number and sequence among the coronavirus (Chen *et al.*, 2020).

Coronavirus particles consisting of four or five structural proteins along with various minor components including non-structural proteins (nsp). Human coronaviruses have a same to genome structure and protein organization, including an open reading frame ORF1a/b to encode 16 nonstructural proteins (nsp1 through nsp16) (Chen *et al.*, 2020).

The large overlapping poly-proteins, ORF1a and ORF1b, commonly refer to pp1a and pp1b, include approximately 2/3 of the genome and encode the replicase poly-protein. These poly-proteins are cleaves by papain-like cysteine protease (PLpro, resides within nsp3) and 3C-like serine protease (3CLpro, also known as main protease Mpro, resides within nsp5) to produce non-structural proteins, including RNA-dependent RNA polymerases (RdRp, resides within nsp12) and helicase (Hel, resides within nsp13). The remaining 1/3 of the genome principally encodes four structural proteins that are : spike (S), envelope (E), membrane (M), and nucleocapsid (N), and some also encode a hemagglutinin esterase (HE) protein which are encoded by other ORFs at the 3′ polyadenylated (Chen *et al.*, 2020) as in figure (1.2).

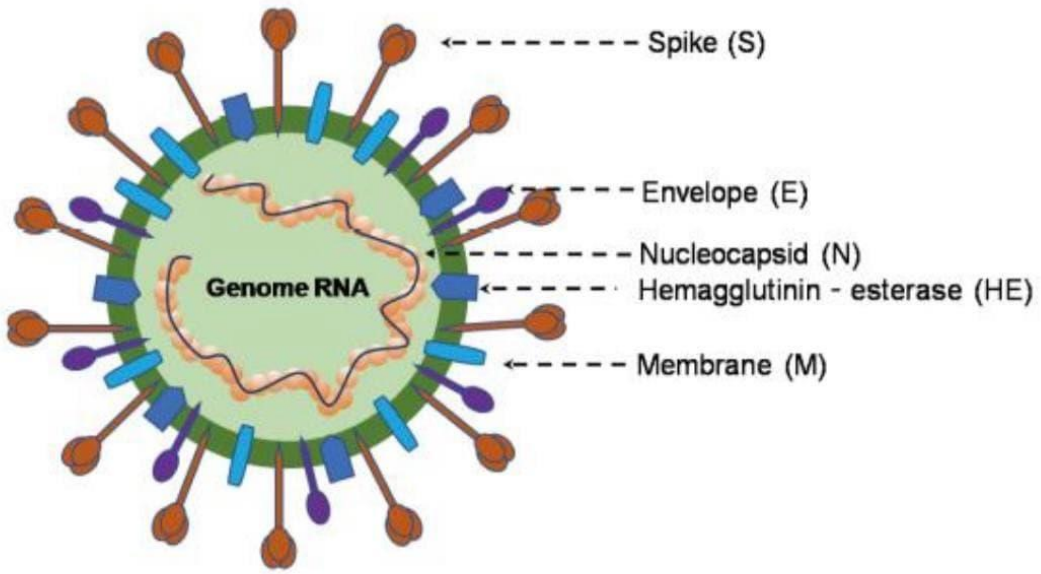

Figure 1.2: Coronavirus structure (Abdullahi, A. M. 2020).

1.71. Nucleocapsid (N) protein

The coronavirus nucleocapsid (N) proteins are the structural phosphoprotein of 43–46 KDa, a component of the helical nucleocapsid. The main functions of the N protein is to package the viral genome into a ribonucleoprotein (RNP) particle in order to protected the genomic RNA and for its integration into a viable virion. The N protein is thought to bind to the genomic RNA in a beads-on-a-string fashion. In addition, it also reaction with the viral membrane protein during virion assembly and plays a critical role in improve the efficiency of virus transcription and assembly. The N protein undergoes rapid phosphorylation following its synthesis. N protein is involved in the host's immune response and inhibits the production of interferon (Artika *et al.*, 2020).

1.7.2. The haemagglutinin esterase (HE) protein:

Haemagglutinin esterase is forms a disulfide-linked homodimer and forms the short spicules on the virion surface. HE allows the initial adsorption of the virus to the cell membrane, but requires the subsequent interaction of the S protein. Also has esterase activity allows viral particles to be released from the infected cell by removing acetyl groups of membrane (Ashour *et al.*, 2020).

1.7.3. Accessory proteins:

The eight SARS-CoV ORFs encoding for the accessory proteins are 3a, 3b, 6, 7a, 7b, 8a and 9b. Interestingly, these accessory proteins are found to be specific for SARS-CoV and have no important homology to accessory proteins from other coronaviruses. The protein 3a is the largest accessory protein and is thought to play a role as a structural ingredient of the SARS-CoV. It has been demonstrated to be integrated into the virus-like particles (VLPs) although it is not necessary for the VLP formation. In addition, the 3a protein has been shown

to interrelate with the SARS-CoV structural proteins (M, S, E,) and the accessory protein 7a and may facilitate the SARS-CoV assembly. The 3a protein may also play roles in elude the host immune system. Moreover, it has been proposed that it function as an ion channel through the use of its transmembrane domains (Artika *et al.*, 2020).

The protein 3b has been indicated to have the ability to influence necrosis and apoptosis and is also able to inhibit the host antiviral response by repressing type-I interferon production. The 3b protein is considered as an interferon antagonist. The protein 6 is integrated in VLPs when co-expressed with the SARS-CoV structural proteins (S, M, E). The physical interacted of protein 6 with these structural proteins is hypothesized to be critical for its assembly into the VLP. The p6 protein has been identifying as a β-interferon antagonist. The protein 7a is a minor structural protein which may facilitate viral assembly. It has been suggested that the 7a protein also important in SARS-CoV pathogenesis by inducing inflammatory responses (Artika *et al.*, 2020, McBride *et al.*, 2014).

1.7.4. Envelope (E) protein:

The envelope (E) protein is a small integral membrane polypeptide, range from 76 to 109 amino acid remnants with molecular weight of 8.4–12 KDa. The E protein plays essential roles in a number of aspects of the coronavirus replication cycle, such as assembly, budding, envelope formation, and pathogenesis. Interestingly, although the protein is highly expressed inside the infected cells, only a small fraction of the protein is integrated into the viral envelope (Schoeman & Fielding 2019).

Consequently, the protein is only a small constitutive of the virus particle. Due to its small size and limited quantity, the E protein was identifying much later compared to the other coronaviruses structural proteins. Its primary and secondary structure indicates that the E protein has a short hydrophobic N

terminus of 7–12 amino acid remnant, followed by a transmembrane domain (TMD) of 25 amino acids, and ends with a long hydrophilic carboxy terminus (Schoeman & Fielding 2019).

1.7.5. Membrane (M) protein:

The membrane (M) glycoprotein is the most abundant envelope protein of coronaviruses play a critical role in the virion assembles through M-M, M-spike (S), and M-nucleocapsid (N) protein interactions. Generally, its length is 217–230 amino acids. It's a triple-spanning membrane protein with a short amino-terminal domain existing on the exodomain of the virus (in the virion exterior, equivalent to the lumen of intracellular organelles) and a long carboxy-terminal domain in the endodomain of the virion (in the virion interior, equal to the cytoplasmic extent of intracellular membranes) (Perrier *et al.*, 2019).

The resultant polypeptides, in the preglycosylated forms, are of 25–30 KDa (221–262 amino acids) and the detect glycosylated forms are of higher molecular weights. The C-terminal domains of the MERS-CoV and IBV M proteins have been shown to containing signals for the trans-Golgi network and the endoplasmic reticulum-Golgi intermediate compartment (ERGIC)/cis-Golgi localization, of host cells respectively (Artika *et al.*, 2020 , Perrier *et al.*, 2019).

1.7.6. Spike (S) protein:

The coronavirus spike (S) protein is the large glycosylated transmembrane protein range from about 1162 to 1452 amino acid remnant. Monomers of the S protein, before to glycosylation, are 128–160 KDa (Hulswit *et al.,* 2016).but molecular masses of the glycosylated forms of the full-length monomer are 150–200 KDa. Following translation, the proteins fold into a metastable perfusion form and convene into a homotrimer forming the coronavirus special surface spike of crown-like appearance.

The S protein is the most external envelope protein of the coronavirus. The S glycoprotein plays critical roles in mediated virus attachment to the host cell receptors and facilitates merging between viral and host cell membranes (Hulswit *et al.*, 2016).

The S protein is a protein forms trimeric spicules on the surface of the virion, it is cut by a cellular protease and results in separation into two S1 and S2 subunits. The S1 subunit is responsible for binding to the cell receptor and forms the globular head of the structure, the S2 subunit is involved in fusion with the cell membrane and forms the stem. S1 has cell receptor binding domains (RBDs); these sites are highly conserved and are related to interspecific transmission and viral pathogenesis (Chan *et al.*, 2020, Fehr & Perlman 2015). Protein S mediate cell-cell fusion between infected and adjacent, uninfected cells resulting in formation of syncytia or multinucleated giant cells, a strategy that viruses use to spread (Malik 2020).

1.8. Replication life of coronaviruses:

1.8.1. Viral entry and membrane fusion:

The infection of coronaviruses is beginning by the binding of the virus particles to the cellular receptors which leads to viral entry followed by merging of the viral and host cellular membranes, The membrane fusion event allows the release of the viral genome into the host cells cytoplasm, a process known as uncoating, which makes the viral genome available for translation. Coronavirus entry is facilitating by the trimeric transmembrane spike (S) glycoprotein, which mediates receptor binding and merging of the viral and host membranes. The interaction between the S protein and the cellular receptor is a main determinant of host species range and tissue tropism (Artika *et al.*, 2020).

The S1 subunit (domain) of the coronavirus S proteins plays important roles in mediating the S protein binding to the host receptor. This S1 subunit

shows the most diversity among coronaviruses and partly accounts for the wide host range of this virus family. Coronaviruses show complex patterns regarding receptor recognition and the diversity of receptor usage is one of the most profound features of coronaviruses (Walls *et al.*, 2017, Li 2016) as in figure (1.3).

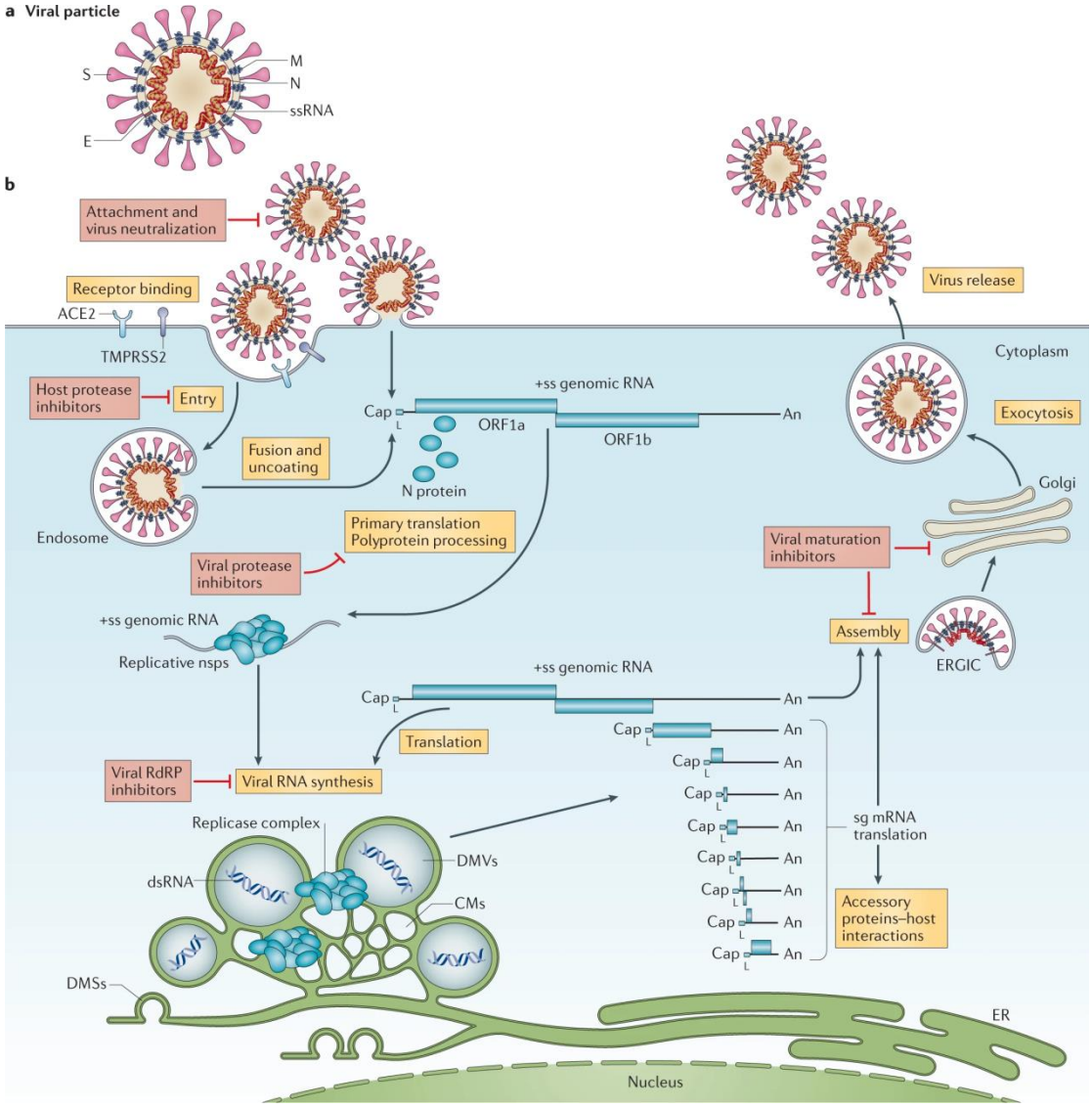

Figure 1.3: Replication life of coronaviruses (V'kovski *et al*., 2020).

1.8.2. Coronavirus genome expression:

Entry of coronaviruses into host target cells depends on the binding of spike glycoprotein to the cellular receptor and priming of S protein by host cell proteases. Like SARS-CoV, SARS-CoV-2 uses the ACE2 receptor for

internalization and TMPRSS2 serine proteases for S protein priming (Hoffmann *et al.*, 2020).

Similar to SARS-CoV, the extra-pulmonary spread of SARS-CoV-2 may be seen due to the widespread tissue expression of the ACE2 receptor. In addition, studies revealed that the spike protein of SARS-CoV-2 exhibits 10–20 times higher affinity as compared to that of SARS-CoV. Binding of spike protein to the ACE2 receptor results in conformational changes in spike protein that leads to the fusion of viral envelop protein with host cell membrane following entry via endosomal pathway (Coutard *et al.,* 2020 , Wrapp *et al.,* 2020).

This event is followed by the release of viral RNA into the host cytoplasm that undergoes translation and generates replicase polyproteins pp1a and pp1b that further cleaved by virus encoded proteinases into small proteins. The replication of coronavirus involves ribosomal frame shifting during the translation process and generates both genomic and multiple copies of subgenomic RNA species by discontinuous transcription that encodes for relevant viral proteins. Assembly of virion takes place via interaction of viral RNA and protein at endoplasmic reticulum (ER) and Golgi complex. These virions are subsequently released out of the cells via vesicles (Hoffmann *et al.*, 2020).

The replication of the coronavirus genome is viewed as the most essential aspect of the coronavirus biology. As the largest group of RNA virus, coronaviruses require RNA synthesis machinery with the sincerity to faithfully replicate their RNA. Coronavirus replication is achieved by employed complex mechanisms including various proteins encoded by both viral and host cell genomes. Evolutionary, the virus genome contains comparatively constant replicative genes which are indispensable for viral replication (Artika *et al.*, 2020) as in figure (1.4).

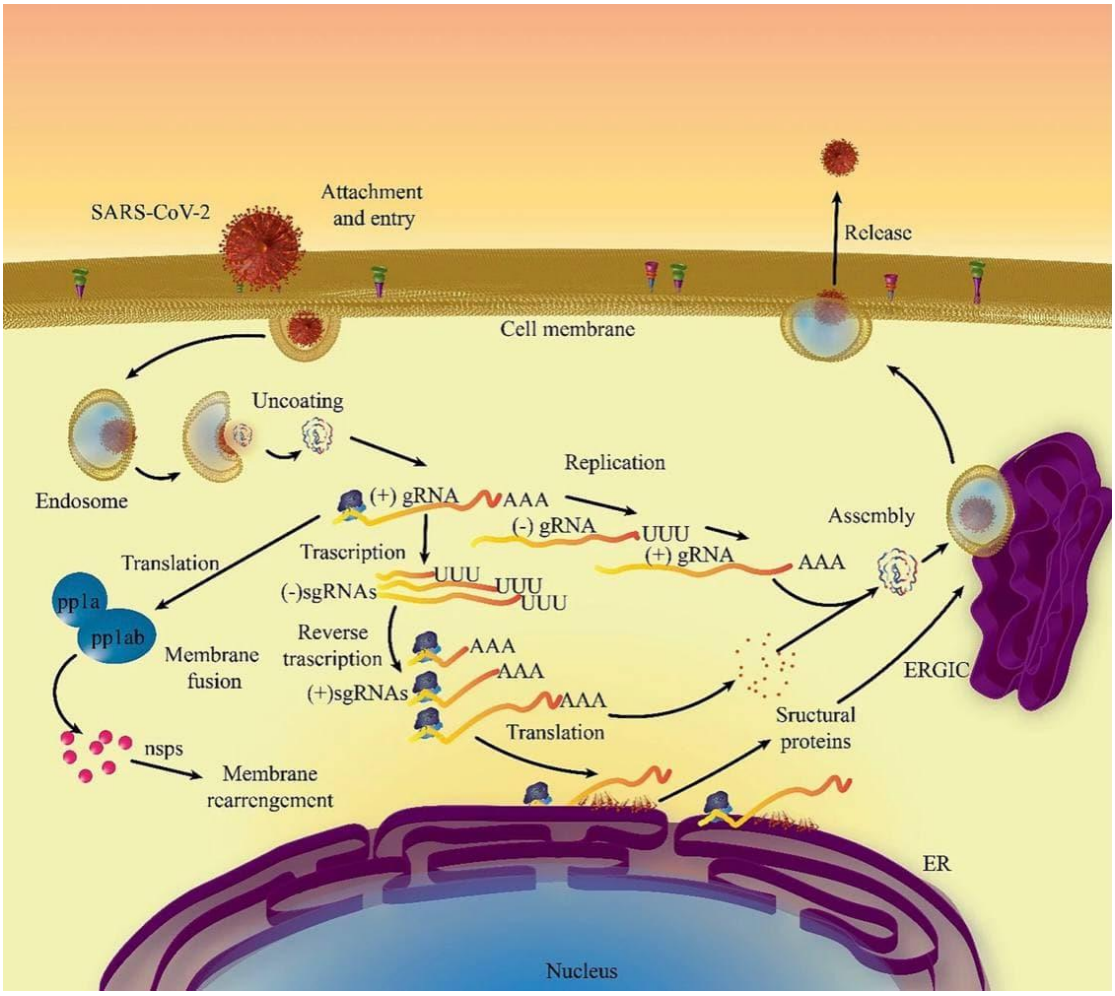

Figure 1.4: Entry and replication of SARS – CoV2 (Mirzaei *et al.*, 2020).

1.8.3. Virion Assembly and Budding:

One of the distinctive features of coronaviruses is the location of their virion assembly. For most enveloped viruses, virion assembly takes place at the host cells plasma membrane. For coronaviruses, however, virion budding and assembly occurs at the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). Coronaviruses, therefore, obtain their membrane envelope from ERGIC (Schoeman & Fielding 2019). For efficient coronavirus virion assembly, the three membrane (enveloped) proteins must be retained near the ERGIC. In fact, the M, E and some S proteins have intracellular trafficking signals which target these structural proteins to the budding site where they accumulate. Therefore, the efficiency of viral proteins incorporation into coronavirus virions is determined

by protein trafficking to the ERGIC and protein–protein interactions at the ERGIC (Ujike & Taguchi 2015).

Most of the protein-protein interactions required for coronavirus assembly are mediated by the M proteins. The coronavirus packaging signal (PS), a *cis*-regulatory element encoded within the viral RNA, functions in packaging the viral genome into the ribonucleocapsid (Woo *et al.*, 2019). The nucleocapsid (N) phosphoprotein plays a fundamental role during viral self-assembly and one of its critical functions is to form the viral genome into a helical ribonucleocapsid (ribonucleoprotein, RNP). Viral N–N self-interactions are thought to be necessary for formation of the ribonucleocapsid and subsequent assembly of the viral particles (Chang *et al.*, 2014).

The generation of mature virions involves insertion into the endoplasmic reticulum (ER) of the coronavirus structural proteins, S, E, and M. These proteins travel along the secretory pathway into the ERGIC and are inserted into the membrane of the ERGIC. The ERGIC is also a location where the viral genomes are encapsidated by the N protein. The structural proteins then interact with the encapsidated viral genomes and assemble into mature coronavirus particles by budding (Fehr & Perlman, 2015).

1.9. Transmission:

Modes of transmission traced in an imported case are through droplet transmission, fecal-oral route, conjunctiva and fomites. Additionally, local transmission can be traced back to the patient's bodily fluids such as respiratory droplets, saliva, feces, and urine. The virion is stabilized at lower temperatures, i.e., 4°C has higher survival than 22°C (Xu *et al.*, 2020 , Ong, *et al.*, 2020 , Kampf *et al.*, 2020) . As SARS-CoV-2 virions are shed throughout the clinical course, patients with COVID-19 can spread the infection prior to symptom presentation, during the symptomatic course and during the clinical recovery period. Addition considerations that must be made regarding the residence time of

the SARS-CoV-2 virion on surface. The half-life of SARS-CoV-2 in aerosols, copper, cardboard, stainless steel, and plastic are 1.5 h, 1 h, 3.4 h, 5.6 h, and 6.8 h, respectively(Van Doremalen *et al.,* 2020). The viable residence time of SARS-CoV-1 in aerosols, copper, cardboard, stainless steel, and plastic are 3 h, 4 h, 24 h, 48 h, and 72 h, respectively (Van Doremalen *et al.,* 2020).

1.10. Pathogenesis of COVID-19:

1.10.1. Innate immunity responses to coronavirus:

The innate immunity is a well-maintained defense mechanism for accelerated recognition and control of pathogens and subsequent promotion of the adaptive immune response. Sufficient trigger of the innate immunity depends on detecting the Pathogen-associated molecular patterns (PAMPs) via pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptor (NLR), C-type lectin-like receptors, and free-molecule receptors in the host cytoplasm following the promotion via PAMPs, PRRs absorb adaptor proteins that comprise complex signaling pathways involving several kinases. This signaling pathway eventually results in the promotion of the essential transcription factors, such as the nuclear factor interferon regulatory factor 3 (IRF3), Nuclear factor-kappa B (NF-κB), and Activator Protein-1(AP-1) (Mirzaei *et al.,* 2020).

Pathogen-associated molecular patterns (PAMPs) recognition via TLRs can happen in cell membranes, lysosomes, endosomes, as well as endocytolysosomes. Overall, various TLRs could trigger different biological reactions via subsequent stimulation of several adapter proteins, like myeloid differentiation factor 88 (MyD88), TIR-domain-containing adapter-inducing interferon-β (TRIP), Toll/interleukin-1 receptor (TIR) domain-containing adapter protein (TIRAP) as well as TRIF-related-adaptor-molecule (TRAM). Increased neutrophils and

diminished lymphocytes also associate with the disease severity and mortality in COVID-19 patients (Wu *et al.*, 2020, Yamamoto *et al.*, 2004).

These clinical characteristics intimated the likelihood of involvement of highly pro-inflammatory statuses in the disease progression and severity. This early sharp rise in the serum levels of pro-inflammatory cytokines has also been reported in SARS-CoV and MERS-CoV infection, implying a potential similar cytokine storm-mediated disease severity. The robust innate immune response toward viral infection relies profoundly on the interferon (IFN) type I responses and its downstream cascade that completes in managing viral replication and initiation of the effective adaptive immune response (Prompetchara *et al.*, 2020) as seen in figure (1.5).

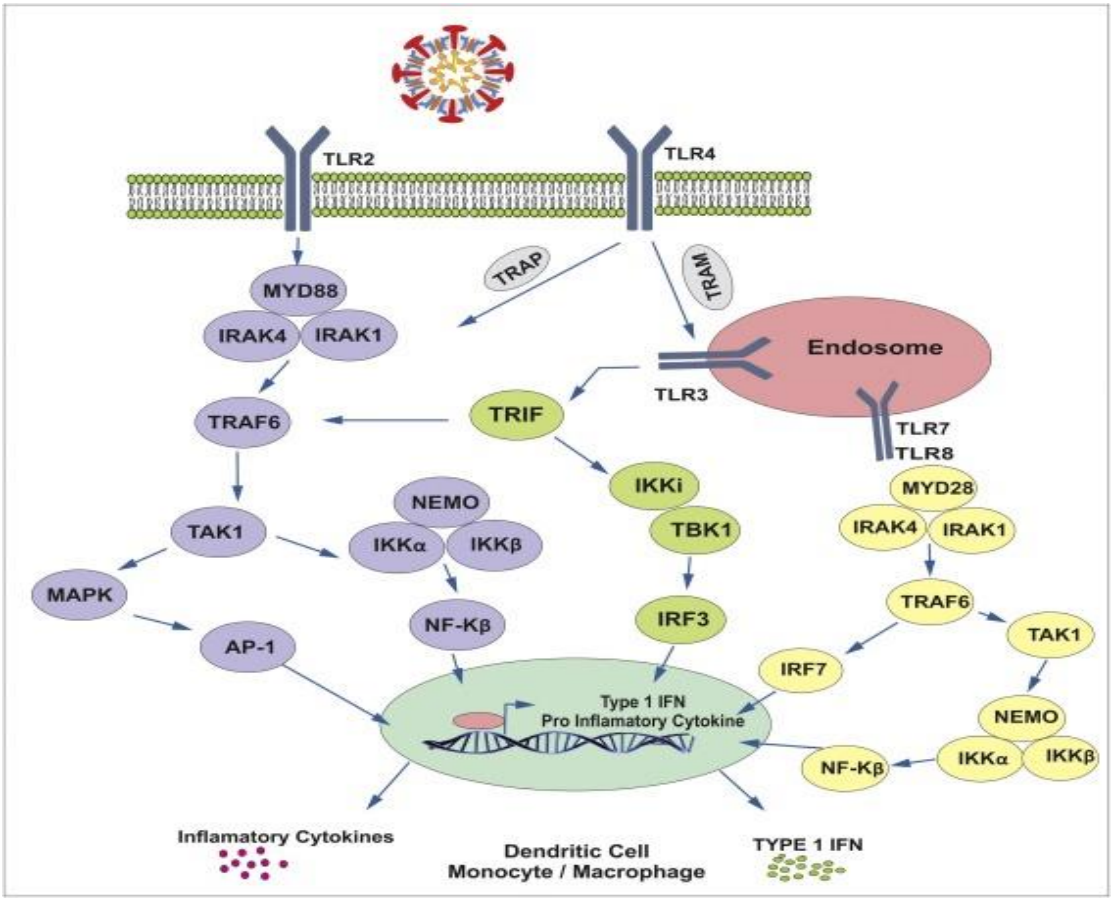

Figure 1.5: Innate immunity responses to coronavirus (Hosseini *et al.*, 2020).

1.10.2. Antigen presentation in coronavirus infection:

The virus enters the cells; its antigen will be presented to the antigen presentation cells (APC), which is a central part of the body's anti-viral immunity. Antigenic peptides are presented by major histocompatibility complex (MHC; or human leukocyte antigen (HLA) in humans) and then recognized by virus-specific cytotoxic T lymphocytes (CTLs). Hence, the understanding of antigen presentation of SARS-CoV-2 will help our comprehension of COVID-19 pathogenesis (Li *et al.,* 2020).

Unfortunately, there is still lack of any report about it, and we can only get some information from previous researches on SARS-CoV and MERS-CoV. The antigen presentation of SARS-CoV mainly depends on MHC I molecules, but MHC II also contributes to its presentation. Previous research shows numerous HLA polymorphisms correlate to the susceptibility of SARS-CoV, such as HLA-B∗4601, HLA-B∗0703, HLA-DR B1∗1202, and HLA-Cw∗0801 ,whereas the HLA-DR0301, HLA-Cw1502 and HLA-A∗0201 alleles are related to the protection from SARS infection In MERS-CoV infection, MHC II molecules, such as HLA-DRB1∗11:01 and HLA-DQB1∗02:0, are associated with the susceptibility to MERS-CoV infection (Hajeer *et al.,* 2016).

Macrophages and dendritic cells play a crucial role in the innate immune and adaptive immune responses. These cells can stimulate T lymphocytes and B lymphocytes; therefore induce coupling promoting innate and adaptive immunity (Mirzaei *et al.,* 2020). The immature dendritic cell has a high migration capacity, and mature dendritic cells could considerably trigger T cells in the central link of regulation and maintaining the immune responses. Dendritic precursor cells are differentiated into dendritic cells via adjusting inducers; such as granulocyte–macrophage colony-stimulating factor, interleukin-4 (IL-4), and tumor necrosis factor-alpha (TNF-α). CD4 positive T cells and CD8 positive T cells have an essential function by adjusting immune reactions toward viruses and the risk of autoimmunity and inflammation. CD4 positive T cells elevate the production of

virus-specific antibodies via stimulating T-dependent B cell activation and, CD8 positive T cells are cytotoxic and can kill the cells infected with the virus (Mirzaei *et al.*, 2020).

1.10.3. Adaptive immune responses to coronavirus:

The adaptive immune system consists of two branches: the humoral immune response arm (production of antibodies by B cells) and the cellular immune response arm (activities carried out by cytotoxic $CD4^+$ and $CD8^+$ T cells). Both typically require antigen presentation in conjunction with major histocompatibility complex (MHC) and a costimulatory signal for full activation .There are three possible outcomes of viral infection: Early clearance of the pathogen either directly or by phagocytosis, overwhelming infection with failure to control, persistent infection where a balance between the pathogen and the host is achieved (Shokri *et al.*, 2019).

T cells, CD4+ and CD8 + T cells play a critical antiviral role through promoting the secretion of pathogen-specific antibodies by inducing T-dependent B cells and killing the virus infected cells, respectively (Maloir *et al.*, 2018).

In infection of MERS-CoV, T cells play critical roles in controlling the pathogenesis. Furthermore; T cells from human peripheral blood mononuclear cells, human lymphoid tissues, and the spleen of common marmosets were highly susceptible to MERS-CoV. MERS-CoV induces substantial apoptosis in the infected T cells that involve the activation of the intrinsic and extrinsic caspase-dependent apoptosis pathways, resulting in high pathogenicity of the virus. MERS-CoV can directly infect and replicate productively in macrophages and dendritic cells, which results in their malfunction and failure to present virus antigen to T. downregulation of antigen presentation pathways (decreased MHC I and II, costimulatory molecules) in macrophages and dendritic cells during MERS-CoV infection would strongly inhibit the activation of T cells (Shokri *et al.*, 2019).

1.10.4. Humoral and cellular immunity:

Antigen presentation subsequently stimulates the body's humoral and cellular immunity, which are mediated by virus-specific B and T cells. Similar to common acute viral infections, the antibody profile against SARS-CoV virus has a typical pattern of IgM and IgG production. The SARS-specific IgM antibodies disappear at the end of 3months , while the IgG antibody can last for a long time, which indicates IgG antibody may mainly play a protective role and the SARS-specific IgG antibodies primarily are S-specific and N-specific antibodies (Li *et al.*, 2020 , De Wit *et al.*, 2016).

Comparing to humoral responses, there are more researches on the cellular immunity of coronavirus. The latest report shows the number of $CD4^+$ and $CD8^+$ T cells in the peripheral blood of SARS-CoV-2-infected patients significantly is reduced, whereas its status is excessive activation, as evidenced by high proportions of HLA-DR (CD4 3.47%) and CD38 (CD8 39.4%) double-positive fractions (Xu *et al.*, 2020).

Similarly, the acute phase response in patients with SARS-CoV is associated with severe decrease of $CD4^+$ T and $CD8^+$ T cells. Even if there is no antigen, $CD4^+$ and $CD8^+$ memory T cells can persist for four years in a part of SARS-CoV recovered individuals and can perform T cell proliferation, DTH response and production of IFN-γ .Six years after SARS-CoV infection, specific T-cell memory responses to the SARS-CoV S peptide library could still be identified in 14 of 23 recovered SARS patients (Li *et al.*, 2020).

1.11. Cytokine Storm in COVID-19:

Cytokine storm (CS) is a critical life-threating condition requiring intensive care admission and having a quite high mortality. CS is characterized by a clinical presentation of overwhelming systemic inflammation, hyperferritinemia, hemodynamic instability, and multi-organ failure, and if left untreated, it leads to death. The trigger for CS is an uncontrolled immune response resulting in

continuous activation and expansion of immune cells, lymphocytes, and macrophages, which produce immense amounts of cytokines, resulting in a cytokine storm. The CS clinical findings are attributed to the action of the pro-inflammatory cytokines like IL-1, IL-6, IL-18, IFN-γ, and TNF-α (Shimizu 2019).

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 (Thompson *et al.*, 2011).

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, multi-organ 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 2019).

1.11.1. Mechanisms of cytokines storm by pathogenic human Coronaviruses infection:

Cytokines play an important role in immunopathology during viral infection. A rapid and well-coordinated innate immune response is the first line of defense against viral infection. However, dysregulated and excessive immune responses may cause immune damage to the human body (Channappanavar *et al.*, 2016).

The relevant evidences from severely ill patients with HCoVs suggest that pro-inflammatory responses play a role in the pathogenesis of HCoVs. In vitro cell experiments show that delayed release of cytokines and chemokine's occurs in respiratory epithelial cells, dendritic cells (DCs), and macrophages at the early stage of SARS-CoV infection. Later, the cells secrete low levels of the antiviral factors interferon's (IFNs) and high levels of pro-inflammatory cytokines (interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF) and chemokine's (C-C motif chemokine ligand (CCL)-2, CCL-3, and CCL-5) (Lau *et al.*, 2013).Like SARS, MERS-CoV infects human airway epithelial cells, THP-1 cells (a monocyte cell line), human peripheral blood monocyte-derived macrophages and DCs, and induces delayed but elevated levels of pro-inflammatory cytokines and chemokine's. After MERS-CoV infection, plasmacytoid dendritic cells, but not mononuclear macrophages and DCs (Tynell *et al.*, 2016, Scheuplein *et al.*, 2015).

Serum cytokine and chemokine levels are significantly higher in patients with severe MERS than patients with mild to moderate MERS. The elevated serum cytokine and chemokine levels in MERS patients are related to the high number of neutrophils and monocytes in the patients' lung tissues and peripheral blood, suggesting that these cells may play a role in lung pathology (Ng *et al.*, 2016 , Kim *et al.*, 2011). The production of IFN-I or IFN-α/β is the key natural immune defense response against viral infections, and IFN-I is the key molecule that plays an antiviral role in the early stages of viral infection. Delayed release of IFNs in the early stages of SARS-CoV and MERS-CoV infection hinders the body's antiviral response (Channappanavar *et al.*, 2019).

Afterward, the rapidly increased cytokines and chemokine's attract many inflammatory cells, such as neutrophils and monocytes, resulting in excessive infiltration of the inflammatory cells into lung tissue and thus lung injury. It appears from these studies that dysregulated and/or exaggerated cytokine and chemokine responses by SARS-CoV-infected or MERS-CoV infected cells could play an important role in pathogenesis of SARS or MERS (Smits *et al.*, 2010).

1.12. Coronavirus immune evasion:

To better survive in host cells, SARS-CoV and MERS-CoV use multiple strategies to avoid immune responses. The evolutionarily conserved microbial structures called pathogen-associated molecular patterns (PAMPs) can be recognized by pattern recognition receptors (PRRs). However, SARS-CoV and MERS-CoV can induce the production of double-membrane vesicles that lack PRRs and then replicate in these vesicles, thereby avoiding the host detection of their dsRNA (Li *et al.*, 2020).

Interferon-I (IFN-α and IFN-β) has a protective effect on SARS-CoV and MERS-CoV infection, but the IFN-I pathway is inhibited in infected. Accessory protein 4a of MERS-CoV may block the induction of IFN at the level of MDA5 activation through direct interaction with double-stranded RNA. Besides, ORF4a,

ORF4b, ORF5, and membrane proteins of MERS-CoV inhibit nuclear transport of IFN regulatory factor 3 (IRF3) and activation of IFN β promoter. The antigen presentation can also be affected by the coronavirus. For example, gene expression related to antigen presentation is down-regulated after MERS-CoV infection (Li *et al.*, 2020, Channappanavar *et al.*, 2019).

1.13. Clinical manifestation:

A wide range of clinical manifestations is seen in patients with SARS-CoV-2 from asymptomatic to symptomatic: mild, moderate, severe and critical cases. The asymptomatic patients have no typical clinical symptoms, but virus is detected. Other patients have mild flu-like symptoms and patients with moderate symptoms the disease begins in the upper respiratory tract and subsequently infects the lower respiratory tract causing pneumonia. Patients with severe disease after one week present with dyspnea, hypoxemia, shortness of breath and significant lung injuries. Later the disease progresses rapidly caused respiratory failure, and need mechanical ventilation, septic shock, difficult to correct metabolic acidosis, coagulation dysfunction, acute respiratory distress syndrome and the patient dead for multi-organ failure (Gao *et al.*, .2020).

The symptoms of COVID-19 infection appear after an incubation period of approximately 5.2 days .The period from the onset of COVID-19 symptoms to death ranged from 6 to 41 days with a median of 14 days .This period is dependent on the age of the patient and status of the patient's immune system. It was shorter among patients > 70-years old compared with those under the age of 70 (Li *et al.*, 2020, Wang *et al.*, 2020).

1.14. Complication of covid-19 infection:

The age and sex have been shown to affect the severity of complications of COVID-19. The rates of hospitalization and death are less than 0.1% in children but increase to 10% or more in older patients. Men are more likely to develop severe complications compared to women as a consequence of SARS-CoV-2 infection (Promislow 2020).

Patients with cancer and solid organ transplant recipients are at increased risk of severe COVID-19 complications because of their immunosuppressed status .The main complications reported in patients with SARS-CoV-2 may include, Coagulopathy, mainly disseminated intravascular coagulation, venous thromboembolism, elevated D-dimer and prolonged prothrombin time ,Laryngeal edema and laryngitis in critically ill patients with COVID-19, Necrotizing pneumonia due to superinfection caused by Panton-Valentine leucocidin secreting *Staphylococcus aureus* infection. This superinfection is usually fatal (Duployes *et al*., 2020).

Cardiovascular complications, including acute pericarditis, left ventricular dysfunction, acute myocardial injury (associated with increased serum troponin), new or worsening arrhythmias and new or worsening heart failure .Acute respiratory failure. Approximately 5% of COVID-19 patients require admittance to an intensive care unit because they develop severe disease complicated by acute respiratory distress syndrome (Kluge *et al.,* 2020)

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1.15. Prevention and Treatment:

The best means of protection is to stay away from the virus exposure. The preventive measure are: washing hands with water and soap for more than 2s or sanitizer with 70% alcohol, ventilate work or home spaces, disinfecting common spaces, avoid public places, avoiding travel, maintain a social distance of approximately 1 m, suspected or confirmed cases must remain at home in

quarantine. People who are in contact with infected patients/individuals wear face masks and tissue while sneezing/coughing and avoid touching, face, nose, and eyes or mouth (Islam *et al.*, 2020).

Supportive care is: hydration with serum, cough and fever management. Patients with shortness of breath should be hospitalized for oxygen supply or mechanical ventilation. Patients with diabetes mellitus, hypertension, chronic diseases or kidney problems should be carefully monitored for any organic deterioration and have care that patients do not acquire opportunistic infections (McArthur *et al.*, 2020). There is not specific antiviral drugs against COVID-19, however some treatment with antivirals and adjunctive therapies as convalescent plasma, tocilizumab, azithromycin and corticosteroids are used as supportive care for COVID-19 patients with promising results as described Remdesivir (R) ,Is an adenosine analogue that incorporates into nascent viral RNA chains resulting in pre-mature termination. In vitro studies have shown that R inhibits the replication of SARS-CoV-2 and in the non-human primates R penetrated efficiently in different organs of body (Jan *et al.*, 2020).

Favipiravir (Avigan) Is a guanine analogue that inhibits the viral RNA polymerase, first enters the infected cells through endocytosis and is then transformed into in an active phosphoribosylated form and its interrupts the nucleotide incorporation process during viral replication (Jean *et al.*, 2020).

1.16. Diagnosis:

1.16.1. Polymerase Chain Reaction:

Reverse transcription polymerase chain reaction–based SARS-CoV-2 RNA detection from respiratory samples (eg, nasopharynx) is the standard for diagnosis. However, the sensitivity of testing varies with timing of testing relative to exposure (Kucirka *et al.*, 2020). Factors contributing to false-negative test results include the adequacy of the specimen collection technique, time from

exposure, and specimen source. Lower respiratory samples, such as bronchoalveolar lavage fluid, are more sensitive than upper respiratory samples (Wang *et al.*, 2020).

1.16.2. Laboratory Findings:

Several serological tests can also aid in the diagnosis, including elevated serum C-reactive protein, lactate dehydrogenase, alanine aminotransferase, and aspartate aminotransferase. The most common hematological abnormality is lymphopenia (Rodriguez-Morales *et al.,* 2020, Guan *et al.,* 2020). In conjunction with coagulopathy, modest prolongation of prothrombin times, mild thrombocytopenia and elevated D-dimer values are common (Chen *et al.,* 2020).

1.16.3. Imaging:

Chest computed tomographic imaging findings are nonspecific and overlap with other infections, so the diagnostic value of chest computed tomographic imaging for COVID-19 is limited (Bernheim *et al.,* 2020). The characteristic chest computed tomographic imaging abnormalities for COVID-19 are diffuse, peripheral ground-glass opacities. Ground-glass opacities have ill-defined margins, air bronchograms, smooth or irregular interlobular or septal thickening, and thickening of the adjacent pleura (Shi *et al.*, 2020).

1.17. Immunological markers:

1.17.1. Bradykinin:

Bradykinin (BK) and related kinins are a family of small active peptides implicated in a series of biological effects. The non a peptide BK is cleaved from the high molecular weight kininogen (HMWK), via activation of plasma kallikrein. BK generation in plasma takes part in the intrinsic coagulation pathway activation, involving the interaction of Factor XII (FXII), prekallikrein (PPK) and Factor XI (FXI) with HMWK, leading to prothrombotic and inflammatory effects (Gao *et al.*, 2020).

Alternatively, kallidin (Lys-BK) is a decapeptide formed by the action of tissue kallikrein on low molecular weight kininogen (LMWK) precursor. In some circumstances, Lys-BK can be converted into BK following the cleavage of the amino-terminal lysine, by the action of plasmatic aminopeptidases. Both Lys-BK and BK are short-acting mediators that are rapidly degraded into inactive fragments by kininase 2, also called angiotensin-converting enzyme (ACE). Under certain circumstances, such as in an inflammatory milieu, there is an increased affinity of kininase 1 for kinins. In this context, this enzyme converts Lys-BK and BK into the active kinins, namely Lys-des-Arg^{9}-BK and des-Arg^{9}-BK, respectively (Gao *et al.*, 2020).

Bradykinin [BK-(1-9)] is a peptide-hormone of the kallikrein-kinin system (KKS) which actions were first characterized by an elegant study by Rocha e Silva and coworkers in 1949 (Souza-Silva *et al.*, ., 2020). Bradykinin (BK), a non-peptide of sequence Arg1 -Pro2 -Pro3 -Gly4 -Phe5 -Ser6 -Pro7 -Phe8 -Arg9 is a member of the kinins, a group of peptides ubiquitously produced by the action of kallikreins on circulating kininogens. Other members of the kinins family include kallidin (KD) (Lys0 -BK) and the metabolites of KD and BK: desArg9 - BK and desArg9 -KD. Kinins are produced in response to inflammation, trauma, burns, shock, allergy and some cardiovascular diseases, provoking changes in blood pressure and vasodilation, increased vascular permeability, stimulation of sensory neurons, vascular and bronchial smooth muscle contraction, intestinal ion secretion, release of prostaglandins and cytokines, and the production of nitric oxide1,2. Pharmacological actions of this family of compounds are mediated by at least two G-protein coupled receptors, named B1 and B2. The former is up-regulated during inflammation episodes or tissue trauma whereas, the latter is constitutively expressed in a variety of cell types. Members of the kinins family show a diverse pharmacological profile. Thus, whereas BK and KD exhibit higher affinity for the B2 receptor, the

desArg9 metabolites bind only to the B1 receptor, with desArg9 -KD being a potent B1 receptor agonist2 (Rasaeifar *et al.*, 2019).

The kinin-kallikrein system is a zymogen system that after activation leads to the release of the non-peptide bradykinin that after binding to the B2R on endothelial cells can lead to capillary leakage (Jurado-Palomo & Caballero 2017). BK is a peptide generated from endogenous kininogen (kallikrein) by specific proteolytic process. It is an important member of the vasodilator system that controls local blood flow. Two types of kallikrein, tissue kallikrein (TK) and plasma kallikrein (PK), release kinins. PK converts hepatic high-molecular-weight kininogen to BK, whereas TK converts hepatic low-molecular-weight kininogen to Lys-BK. Both BK and Lys-BK activate BK receptor type 2 (B2). ACE metabolizes BK to des-Arg9-BK and Lys-BK to Lys-des-Arg9-BK. Both of these metabolites activate BK receptor type 1 (B1). B2 receptors are normally found and responsible for vasodilatation, whereas B1 receptors are induced under pro-inflammatory conditions (Gralinski *et al.*, 2018).

BK is stimulated by different inflammatory mediators such as interleukins (IL) (IL-4, IL-6, IL-8, and IL-13) and tumor necrosis factor-alpha (TNF-α). ACE metabolizes BK and blocks its effect on B2 receptors, whereas ACE2 metabolizes des-Arg9-BK and Lys-des-Arg9-BK and blocks their effect on B1 receptors (Sodhi *et al.*, 2018). In SARS-CoV-2 infection, BK and its metabolites are augmented due to down-regulation of ACE2 by SARS-CoV-2; therefore dysregulation of this pathway may lead to acute pulmonary damage, fluid extravasation, leukocyte recruitment, and the development of ARDS via activation of B1 receptors, which are overexpressed by the pro-inflammatory conditions. Activation of BK system in the acute viral respiratory infection increases the risk of capillary permeability and development of multiple organ failure (MOF); exposure to SARS-CoV-2 infection reduces the expression of ACE2 and increases the activity and level of Des-Arg973-BK (DABK). Enhanced signaling through DABK/BKB1R system leads to vascular–alveolar fluid

extravasation, leukocyte extravasation, and ARDS development (Tolouian *et al.*, 2020).

Severe acute respiratory syndrome coronavirus 2 (SARSCoV-2) binds to target cells through the angiotensin-converting enzyme-2 (ACE-2) receptor (Zwaveling *et al.*, 2020). These receptors are expressed on epithelial cells of the lung, kidneys, intestine, and blood vessels (Wan *et al.*, 2020). ACE converts angiotensin I into angiotensin II by the removal of 2 peptides, which induces vasoconstriction and inactivates bradykinin, a known vasodilator. ACE-2 is suggested to counteract ACE in the Renin-angiotensin system (RAS) by converting angiotensin II into a metabolite, angiotensin 1-7, that leads to vasodilatation by stimulation of nitric oxide synthase (Tolouian *et al.*, 2020).

Interestingly, ACE-2 also hydrolyzes the active metabolite of bradykinin: des-Arg9 - bradykinin, which binds to bradykinin receptors type 1 (BKB1) that are expressed on endothelial cells in the lungs on bronchiolar exocrine cells and pneumocytes type II. Signaling through the BKB1 receptor can induce fluid extravasation and recruitment of leucocytes to the lungs (Sodhi *et al.*, 2018). The SARS-CoV-2 spike antigen binds to ACE2, which in turn internalizes, and thereby down-regulates the expression and function of ACE2. Subsequently, there is an increase of blood pressure and pulmonary edema that might evolve to angioedema, likely through generation of BK active metabolites, such as des-Arg^9-BK. In addition to the modulation of renin-angiotensin system (RAS), des-Arg^9-BK binds to B_1 receptors and enhances inflammation and vascular permeability, which is associated with acute lung injury (Sriram & Insel 2020).

The inflammatory setting evoked by the des–Arg^9–BK–B_1 receptor axis is related to a cytokine storm, with a marked increase in the production of several pro-inflammatory cytokines and chemokines (e.g., TNF, IL-1β, IL-6, CXCL5, CCL2 and CXCL1), finally mediating organ dysfunction (Sodhi *et al.*, 2018).

1.17.2. Membrane Attack Complex:

The complement system (CS) is an integral component of the innate immune response and comprises over 30 different proteins. This system can be activated by three different pathways: the classical, the lectin and the alternative pathway (Noris *et al.,* 2020, Tolouian *et al.,* 2020). All of these pathways converge on the formation of C3 convertases that cleave C3 to generate the pro-inflammatory peptide C3a and a large amount of C3b, which opsonizes pathogens proteins. C3b also forms C5 convertase, which induces release of the potent anaphylatoxin C5a, as well as the fragment C5b that is responsible for the formation of the membrane attack complex C5b–9 on target cells — the terminal event of complement activation (Noris *et al.*, 2020 , Ortiz 2020).

The membrane attack complex of complement (MAC) is a multi-meric assembly of proteins consisting of C5b, C6, C7, C8 and multiple copies of C9, which forms the membrane spanning pore (Morgan 2016). The MAC is the end product of a complex series of biochemical interactions in which initially soluble complement proteins bind and undergo dramatic structural rearrangements to form a transmembrane pore. The resulting MAC pore is a hetero-oligomer formed from the irreversible, stepwise assembly of 7 different polypeptide chains: C5b, C6, C7, C8 (a hetero-trimer comprised of C8α, C8β and C8γ) and C9, where 18 copies of C9 are required to complete the pore .activation of complement leads to the generation of C5b via the cleavage of C5 by membrane-bound C5-convertase enzymes. C5b is a metastable intermediate that rapidly sequesters C6 (Heesterbeek *et al.,* 2019, Parsons *et al.,* 2019). Recruitment of C7 unfurls a lipophilic domain upon binding, while integration of C8 into the assembly is accompanied by an initial insertion into the membrane. The C5b-8 initiator complex then binds C9 and undergoes unidirectional, clockwise oligomerization (with 18 copies of C9) to complete an 11 nm wide transmembrane pore (Serna *et al.,* 2016).

Key role of complement system (CS) activation in COVID-19. CS is one of the main actors of the innate immune system response and the activation of complement system (CS) protects the host against pathogens. However, uncontrolled CS activation can lead to tissue damage and persistent inflammation (Conigliaro *et al.*, 2019). The mechanisms of C3 regulation and the role of complement activation in SARS-CoV-2 pathogenesis can be explained by the close relationship with inflammatory cytokines including IL1β, IL-6 and tumor necrosis factor (TNF-α) , C3 and C5 synthesis can result up-regulated by exuberant pro-inflammatory cytokine production induced by SARS-CoV-2 (Conti *et al.*, 2020). The release of anaphylatoxins C3a and C5a promotes a chemotactic and proinflammatory milieu in the lungs as the main target tissue. Accordingly, the viral infection may turn on the complement with the formation of convertase enzymes that cleave the key proteins C3 and C5 with the formation of the MAC leading to microorganism lysis in a vicious circle (Conigliaro *et al.*, 2019).

In addition, monnon-binding lectin (MBL), which can be modulated by SARS-CoV S glycoprotein, takes part in the battle by activating the CS. Therefore, CS may have a role in both the early and late phase of the disease: the first immune defence-based phase, critical for pathogen clearance, and the second inflammation-driven damaging phase. Dysregulated CS activation is likely to play a crucial role in the pathogenesis of acute lung injury (Rivellese & Prediletto 2020, Guzik *et al.*, 2020). In COVID-19 associated tissue inflammatory injury may be the terminal pathway, which represents the common end-point of the three complement activation cascades (Wang *et al.*, 2015).

The binding of C3b to the classical/lectin or the alternative C3 convertases forms the C5 convertases that cleave C5, generating C5a and C5b. C5a is a potent anaphylatoxin that is involved in exacerbating inflammatory reactions. While C5b participates in the formation of C5b-9, which inserts itself into cell membranes, causing cell injury and dysfunction. C5a promotes monocyte and neutrophil attraction, aggregation and activation to generate an oxidative burst with the

release of reactive oxygen species (ROS) that exert a critical role in virus induced lung damage and mortality (Noris *et al.*, 2020). The interaction between C5a and C5aR on endothelial cells induces the upregulation of adhesion molecules that favour the adhesion of leukocytes and promote their transmigration into lung parenchyma. C5a has also been shown to initiate mast cell degranulation, initiate cytokine storms and increase vascular permeability (Wang *et al.*, 2015, Noris et *al.*, 2020).

1.18. Biochemical markers in COVID-19:

1.18.1. D. dimer:

D-dimer originates from the lysis of cross-linked fibrin with rising levels indicating the activation of coagulation and fibrinolysis **(**Zhang *et al.*, 2020**).**

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

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

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

1.18.2 S. ferritin:

Ferritin is a useful marker to predict the outcomes in COVID-19. Hyperferritinemia can activate macrophages, which increases the secretion of pro-inflammatory cytokines, and the subsequent inflammation is mainly

responsible for organ damage. Although ferritin is known as a positive acute phase reactant and serum level of ferritin intracellular protein increases during inflammation, dying cells may also release the ferritin (Gandini *et al*., 2020).

1.18.3. C. reactive protein (CRP):

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

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

Chapter Two Materials and Methods

2.1. Materials and Methods

2.1.1. Study design

The type of the study is cross – sectional study with a total of 90 patients diagnosed according total lung involvement by pulmonary computed tomography scoring into (44 severe, 29 moderate and 17 mild) who were attending in Al – Hussein – medical city and Al – Hayat unit in Karbala, Iraq. In period from October 2020 to January 2021. Under the supervision of radiologist specialists were included in this study Figure (2.1).

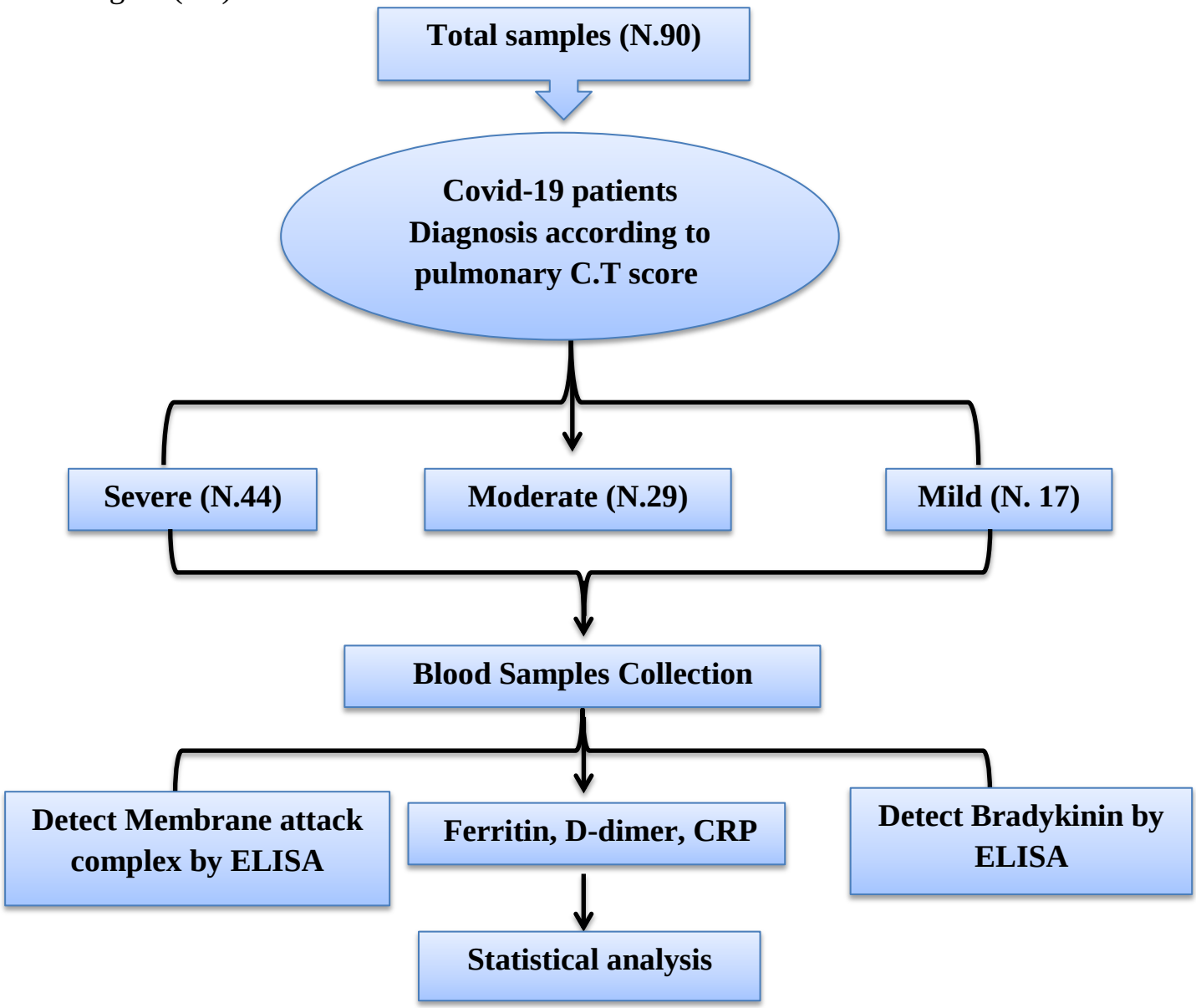

Figure (2.1) Flowchart of work the study design.

2.1.2. Patients

The study included ninety patients range from (19 – 88) years with coronaviruses (Covid–19) divided into three groups (severe, moderate and mild) according to lung involvement diagnosed by pulmonary computed tomography scores. Patients with covid – 19 were diagnosed by the pulmonary computed tomography scores by radiologist into 44% severe, 29% moderate and 17% mild. The total lung involvement was the sum of the individual lobar scores; each of the five lung lobes was visually scored on a scale of 1 to 5. score 1, less than 5% involvement ; score2, 5%–25% involvement; score 3, 25%–50% involvement; score4, 50%– 75% involvement; and score5, more than 75% involvement (Pan *et al*., 2020). The overall lung score out of 25 was classified as mild, moderate, and severe, depending on the score range from < 8 mild, 9 – 15 moderate and >15 severe based on the study model by Chung (Chung *et al*., 2020).

Mild patients with symptoms of acute upper respiratory tract infection, including fever, fatigue, myalgia, cough, sore throat, runny nose, and sneezing. Some cases may have no fever or have only digestive symptoms such as nausea, vomiting, abdominal pain, and diarrhea (de Souza *et al*., 2020).

Moderate patients presented as pneumonia. Frequent fever and cough, mostly dry cough, followed by productive cough, some may have wheezing, but no obvious hypoxemia or shortness of breath, and lung auscultation may have rhonchi (de Souza *et al*., 2020).

Severe patient's early respiratory symptoms such as fever and cough may be accompanied by gastrointestinal symptoms such as diarrhea. The disease usually progresses around 1 week, and dyspnea occurs, with central cyanosis. Oxygen saturation is less than 92%, with other hypoxia manifestations. Progress to acute respiratory distress syndrome or respiratory failure, and may also have shock,

encephalopathy, myocardial injury or heart failure, coagulation dysfunction, and acute kidney injury, including multiple organ dysfunction (de Souza *et al*., 2020).

The most common laboratory manifestation, normal or lower white blood cell counts, or thrombocytopenia, with elevation above threefold the upper normal level of at least two of the following markers: C reactive protein (CRP), ferritin, D-dimer, lactate dehydrogenase (LDH) and cardiac troponin (Caricchio *et al*., 2021).

Table (2.1): CT. Score

C.T severity	Lung involvement%	Score
< 8 mild	< 5 %	1
	5 – 25 %	2
8 – 15 moderate	25 – 50 %	3
	50 – 75 %	4
> 15 severe	> 75%	5
Total lung involvement = The sum of scores		

2.1.3. Inclusion Criteria and Exclusion criteria

Inclusion criteria:

1- All positive patients with covid-19 according to the real-time reverse transcriptase-polymerase chain reaction (RT-PCR) of nasal and pharyngeal swabs.
2- All positive patients with covid-19 according to lung involvement diagnosed by the pulmonary C.T scores.

Exclusion criteria:

1- Coronaviruses Patient on Steroid Treatment, others immunomodulator drugs.
2- Patient with autoimmune disease
3- Diabetic patients
4- Patients with inflammatory bowel disease
5- Asthmatic patients
6- Patients with chronic obstructive pulmonary diseases
7- Hypertensive patients on ACE inhibitors drugs.

2.1.4. Ethical Issue

The consents for the study had been taken from the ethical committee of Karbala College of Medicine and the relevant ethical committee in Karbala health directorate. In addition, verbal approval will be taken from the patients before take the sample. The information about each case collected from patients was taken with ethical considerations ,So to get blood sample, we already informed patients that we would use their blood for research purposes and most of them were cooperative and helpful . The permission had been taken from Al-Hussein medical city, Al-Hayat units, in holly karbala.

2.1.5. Blood Sample Processing

Blood sample were collected by venipuncture from 90 patients, five millimeters of venous were drawing by disposable syringe under sterilization technique and putting in gel tube then, allowed to clot; after that serum allow serum to clot for 10 – 20 minutes in gel tube at room temperature. Centrifuge at 2000 – 3000 RPM for 20 minutes. The serum has been collected in plain tube then stored at -20c to be used for ELISA test to determine concentration of BK and MAC according to the leaflet manufacture. Health measures and safety were taken when sampling (wearing a mask, gloves, goggles and face shield).

2.1.6. Laboratory Instruments in the Study

Instruments were used to detect levels of different kinds of parameters in serum of patients and control group. Different type of tool was mentioned in table (2.2)

Table (2.2): Laboratory instruments and apparatus used during this study.

Different had been used in this Laboratory instruments and apparatus used during this study as in table (2.2)

Instruments	Country
Automatic Washer	Germany
Centrifuge	Hitachi /Germany
Disposable syringes 5ml	China
Elisa system	Human / Germany
Gel tube	Lebanon
Gloves	T G S / Malaysia
Incubator	Germany
Micropipette(100-1000)	
Micropipette(10-100micron)	
Multichannel Pipette (250-50 microns)	
Plane tube	China
Spectrophotometer	Germany

Tip(micropipette yellow ,blue)	Lebanon
VIDAS	Germany
Vortex	Japan
Water bath	Chania

2.2. Materials (kits) used in the study: diagnostic kits had been used in this study table (2.3)

Table (2.3) List of Kits used

Kits	**Source**
C-Reactive Protein	Spain
D- dimer	France
Human bradykinin (BK) Kit	Bioassay (china)
Human membrane attack complex (MAC) Kit	
S. ferritin	France

2.2.1. Bradykinin (BK) Kit

Kit component

Table (2.4) Kit component of brdykinin

Components	Quantity
Standard Solution (40ng / ml)	0.5 ml × 1
Pre – coated ELISA plate	12 * 8 well strips × 1
Standard Diluted	3 ml × 1
Streptavidin – HRP	6ml × 1
Stop Solution	6ml × 1
Substrate Solution A	6ml × 1
Substrate Solution B	6ml × 1
Wash Buffer Concentrate (25×)	20 ml × 1
Biotinylated human BK Antibody	1 ml × 1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

2.2.2. Membrane attack complex (MAC) Kit

Kit component

Table (2.5) Kit component of MAC

Components	Quantity
Standard Solution (3200 ng / ml)	0.5 ml × 1
Pre – coated ELISA plate	12 * 8 well strips × 1
Standard Diluent	3 ml × 1
Streptavidin – HRP	6 ml × 1
Stop Solution	6 ml × 1
Substrate Solution A	6 ml × 1
Substrate Solution B	6 ml × 1
Wash Buffer concentrate (25 ×)	20 ml × 1
Biotinylated human MAC Antibody	1 ml × 1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pics

2.2.3. C-Reactive Protein kit

Kit composition

Table (2.6) Kit component of CRP

Component	Quantity
Diluent (R 1)	Tris buffer 20 mmol/L , PH 8.2
Latex (R 2)	Latex particles coated with goat anti-human CRP , PH 7.3
Calibrator (CAL)	Human serum. CRP concentration is stated on the label vial and it is traceable to the certified reference material ERM-DA470(IRMM)

2.2.4. Serum ferritin kit

Kit composition

Table (2.7) Kit compositions of serum ferritin

Composition of kit		
60 FER reagent strips	STR	Ready to use.
60 FER SPRs (2 ×30)	SPR	Ready to use. SPRs coated with mouse monoclonal anti-ferritin antibodies
FER control (liquid) (1×2 ml)	C1	Ready to use. Tris buffer (0.1mol/l , PH 7.4) with human spleen ferritin and protein and chemical stabilizers

FER calibrator (liquid) (1×2 ml)	S1	Ready to use. Tris buffer (0.1mol/l , PH 7.4) with human spleen ferritin and protein and chemical stabilizers
FER dilution buffer (liquid) (1×2 ml)	R1	Ready to use. Tris buffer (0.1mol/l , PH 7.4) and protein and chemical stabilizers

Table (2.8) FRE Reagent Strip of serum ferritin

Well	Reagent
1	Sample
2-3-4	Empty
5	Conjugate: mouse monoclonal anti-ferritin anti-bodies conjugated to alkaline phosphatase with 1g/L sodium azide (600µl).
6-7	Wash buffer: sodium phosphate (0.01mol/l, PH 7.4) with 1g/l sodium azide (600µl).
8	Wash buffer: diethanolamine (1.1 mol/l, or 11.5%, PH 9.8) with 1g/l sodium azide (600µl).
9	Empty
10	Reading cuvette with substrate : 4-methylumbelliferyl phosphate(0.6 mmol/l) with diethanolamine (0.62mol/l or 6.6% , PH 9.2)+ 1g/l sodium azide (300 µl)

2.2.5. D – Dimer kit

Kit composition

Table (2.9) kit compositions of D- dimer

Composition of kit		
60DEX2 strips	STR	Ready to use
60 DEX2 SPRs 2×30	SPR	Ready to use. Interior of SPRs coated with anti-FbDP monoclonal immunoglobulins (mouse)
DEX2 controls: C1 control 2×2 ml (lyophilized) C2 control 2×2 ml (lyophilized)	 C1 C2	Reconstitute with 2 ml of distilled water. Waite for 5 minutes and then mix. After reconstitution, the controls are stable for 28 days at 2 – 8 ºC Or until the expiration date of the kit at - 25± 6 ºC (freeze immediately after reconstitution). 5 freeze/thaw cycles are possible. FbDP obtained from human plasma diluted in glycine-albumin bovine buffer + preservatives.
DEX2 calibrator S1 calibrator 2×2 ml (lyophilized)	S1	Reconstitute with 2 ml of distilled water. Waite for 5 minutes and then mix. After reconstitution, the controls are stable for 28 days at 2 – 8 ºC Or until the expiration date of the kit at - 25± 6 ºC (freeze immediately after reconstitution). 5 freeze/thaw cycles are possible. FbDP obtained from human plasma diluted in glycine-albumin bovine buffer + preservatives.
DEX2 diluent 1×5 ml (liquid)	R1	Ready to use. Tris buffer (0.05 mol/l, PH 7.4) + calf serum + preservatives.

Table (2.10) DEX2 Strip of D - dimer

Wells	Reagents
1	Sample well
2-3-4	Empty wells
5	Conjugate: alkaline phosphatase-labeled anti-FbDP monoclonal immunoglobulins (mouse) in TRIS buffer (0.05mol/l, PH 6.5) + horse serum +preservatives (400 µl).
6-7-9	Wash solution : TRIS buffer (0.05 mol/l , PH 7.3) + chemical stabilizers + preservatives (600 µl)
8	Diluent :TRIS buffer (0.05 mol/l , PH 7.4) + calf serum + protein and chemical stabilizers + preservatives (600 µl)
10	Reading cuvette with substrate: 4-mathyl-ymbelliferyi phosphate (0.6mmol/l) + diethanolamine(DEA)(0.62mol/l or 6.6%) PH 9.2+1 g/l sodium azide (300 µl).

2.3. Methods:

Principle and procedures of kits used in this study

2.3.1. Bradykinin (BK) Kit.

Specimen collection

Serum: allow serum to clot for 10 – 20 minutes in gel tube at room temperature. Centrifuge at 2000 – 3000 RPM for 20 minutes

Principles of the Assay

The human bradykinin kit is an Enzyme – Linked Immunosorbent Assay (ELISA). The plate has been pre – coated with human BK antibody. BK present in the sample is added and binds to antibodies coated on the wells. And then Biotinylated human BK antibody is added and bind to BK in the sample. Then streptavidin – HPR is added and binds to the Biotinylated BK 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 BK. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagent Preparation

- All reagents should be brought to room temperature before use
- Standard reconstitute the 120 µl of the standard (40 ng / ml) with 120 µl of standard diluent to generate a 20 ng / ml standard stock solution . Allow the standard to sit for 15 minutes with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (20 ng /ml) 1:2 with standard diluent to produce 10 ng/ ml, 5ng /ml, 2.5ng/ml and 1.25 ng/ml solutions. Standard diluent serves as the zero standards (0 ng/ml). Any remaining solution should be frozen at – 20 ºC and used within one month.

Table (2.11) Human BK Standard Preparation

Standard	Added	Into
20ng/ml	Standard No.5	120µl Original standard +120µl Standard Diluent
10ng/ml	Standard No.4	120µl Standard No.5 +120µl Standard Diluent
5ng/ml	Standard No.3	120µl Standard No.4+120µl Standard Diluent
2.5ng/ml	Standard No.2	120µl Standard No.3+120µl Standard Diluent
1.25ng/ml	Standard No.1	120µl Standard No.2+120µl Standard Diluent

Standard concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
40ng/ml	20ng/ml	10ng/ml	5ng/ ml	2.5ng/ml	1.25ng/ml

- **Wash buffer:** 20ml of wash buffer concentrate 25× was diluted into deionized or distilled water to yield 500ml of 1× wash buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

Assay procedure

1- All reagents, standard solutions and samples was Prepared as instructed. All reagents were brought to room temperature before use. The assay is performed at room temperature.
2- The number of strips requires for the assay was determined. Inserted the strips in the frames for use. The unused strips should be stored at 2 – 8 ºC
3- Standard 50µl was added to standard well. Note :Don't add antibody to standard well because the standard solution contains Biotinylated antibody
4- Sample 40µ was added to sample wells and then 10µl anti – BK antibody added to sample wells, then 50µl streptavidin – HRP added to sample wells and standard wells (not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37ºC
5- The sealer Removed and the plate was washed 5 times with buffer. Soak wells with at 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and washed 5 times with wash buffer, overfilling wells with wash buffer. The plate was blotted onto paper towels or other absorbent material.
6- Substrate solution 50µ was added to each well and then 50µl substrate solution B added to each well. Incubate plate covered with a new sealer for 10 minutes at 37 ºC in the dark.
7- Stop solution 50µl was added to each well, the blue color will change into yellow immediately.
8- The optical density (OD value) of each well immediately determined by using microplate reader set to 450 nm within 10 minutes after the stop solution.

Calculation of result

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best

fit curve through the points on the graph. This calculation can be best performed with computer – based curve – fitting software and the best fit line can be determined by regression analysis.

2.3.2. Membrane attack complex (MAC) Kit

Specimen collection

Serum: allow serum to clot for 10 – 20 minutes at room temperature. Centrifuge at 2000 – 3000 RPM for 20 minutes

Principles of the Assay

Human membrane attack complex kit is an Enzyme – linked Immunosorbent Assay (ELISA). The plate has been pre – coated with human MAC antibody. MAC present in the sample is added and binds to antibodies coated on the wells. And then Biotinylated human MAC antibody is added and binds to MAC in the sample. Then Streptavidin – HRP is added and bind to the Biotinylated MAC 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 MAC. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagent Preparation

- All reagent should be brought to room temperature before use
- Standard reconstitute the 120µl of the standard (3200ng/ml) with 120µl of standard diluent to generate a 1600ng/ml standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (1600ng/ml) 1:2 with standard diluent to produce 800ng/ml, 400ng/ml, 200ng/ml and 100ng/ml solution. Standard diluent serves as the

zero standards (0ng/ml). Any remaining solution should be frozen at – 20ºC and used within one month.

Table (2.12) Human MAC Standard Preparation

Standard	Added	Into
1600ng/ml	Standard No.5	120µl Original standard +120µl Standard Diluent
800ng/ml	Standard No.4	120µl Standard No.5 +120µl Standard Diluent
400ng/ml	Standard No.3	120µl Standard No.4+120µl Standard Diluent
200ng/ml	Standard No.2	120µl Standard No.3+120µl Standard Diluent
1.00ng/ml	Standard No.1	120µl Standard No.2+120µl Standard Diluent

Standard concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
3200ng/ml	1600ng/ml	800ng/ml	400ng/ml	200ng/ml	100ng/ml

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

Assay procedure

1- All reagents, standard solutions and samples was Prepared as instructed. All reagents were brought to room temperature before use. The assay is performed at room temperature.
2- Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2 - 8ºC.
3- Standard 50µl was added to standard well. Note: Don't add antibody to standard well because the standard solution contains Biotinylated antibody.
4- Sample 40µl was added to sample wells then 10µl anti – MAC antibody added to sample wells, then 50µl streptavidin – HRP added to sample wells and standard wells (not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37 ºC.
5- The sealer removed and then the plate washed 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to I minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.
6- Substrate solution A 50µl was added to each well and then 50µl substrate solution B was added to each well. Incubate plate covered with a new sealer for 10 minutes at 37 ºC in the dark
7- Stop solution 50µl was added to each well, the blue color will change into yellow immediately.
8- The optical density (OD value) of each well immediately determined by using a microplate reader set to 450nm within 10 minutes after adding the stop solution.

Calculation of result

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. This calculation can be best performed with computer – based curve – fitting software and the best fit line can be determined by regression analysis.

2.3.3. C-reactive protein kit

Principle

The latex particles coated with anti- CRP are agglutinated when they react with samples that contain C-reactive protein (CRP). The latex particles agglutination is proportional to the concentration of the CRP in the sample and can be measured by turbidimetry.

Reagents preparation

All reagents are ready to use.

Sample collection

Fresh serum stable 7 days at 2-8ºC or 3 months at -20ºC. Samples with presence of fibrin should be centrifuged before testing. Hemolyzed or contaminated samples are not suitable for testing.

Analytic Procedure

1- Distilled water used to zero the instrument at 450nm
2- 5µl of sample, calibrator add to 1ml working reagent was pipette into a cuvette

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

Calculation

$$\frac{(A2 - A1s)}{(A2 - A1c)} \times \text{CAL concen.} = \text{CRP value}$$

2.3.4. S. Ferritin kit

Principle of the procedure

The VIDAS ferritin (FER) assay is an enzyme-linked fluorescent immunoassay (ELFA) performed in an automated instrument. All assay steps and assay temperature are controlled by the instrument. A pipette tip-like disposable device, the solid phase receptacle (SPR), serves as a solid phase for the assay as well as a pipetting device. The SPR is coated at the time of manufacture with mouse monoclonal anti-ferritin antibodies.

The VIDAS ferritin assay configuration prevents nonspecific reactions with the SPR. Reagents for the assay is located in the sealed reagent strips. The sample is transferred into well containing the anti-ferritin antibody conjugated with alkaline phosphatase. The sample/conjugate mixture is cycled in and out of the SPR and the ferritin will bind to antibodies coated on the SPR and to the conjugate forming a sandwich. Wash steps removed unbound conjugate. A fluorescent substrate, 4-methlumbelliferyl phosphate is cycled through the SPR. Enzyme remaining on the SPR wall catalyzed the conversion of the substrate to the fluorescent product 4-methylumbelliferone. The intensity of fluorescence is measured by the optical scanner in the instrument.

Sample collection

Acceptable specimens include serum or plasma (with EDTA or heparin anticoagulant). The use of heat inactivated sera has not been established, do not heat sera.

2.3.5. D – Dimer kit

Principle

The assay principle combines a two - step enzyme immunoassay sandwich method and final fluorescent detection (ELFA). The solid phase receptacle (SPR), serves as the solid phase with an anti-FbDP monoclonal antibody adsorbed on it is surface as well as the pipetting device. Reagents for the assay are ready to use and pre-dispensed in the sealed single use reagent strips. All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times

First the sample is taken by the SPR, diluted and then cycled in and out of the SPR several times. The antigen binds to the anti-FbDP immunoglobulins coated on the SPR. Unbound components are eliminated during a washing step.

During the second step, the conjugate which contains an alkaline phosphatase-labeled anti-FbDP monoclonal antibody, binds to the antigen coated on the SPR to form a sandwich. Unbound components are eliminated during the washing step.

A detection step is then performed. The substrate (4-methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-methyl-umbellifrone), the fluorescence is measured at 450nm. The intensity of the fluorescence is proportional to the concentration of antigen present in the sample. At the end of the assay, results are automatically calculated by the instrument.

Specimen type and collection

Collect blood by clean venipuncture in tri-sodium citrate (0.109 mol/l 3.2% or o.129 mol/l 3.8%) or CTAD (sodium citrate, heophylline, adenosine and dipyriamole) .

2.4. Waste disposal:

Remnants of procedure which is involve syringe, gloves, tubes, cotton, biological materials, chemicals and microplate consider as a biohazardous material dispose according to protocol followed by Al-Hussein medical city lab-disposal approach.

2.5. Statistical Analysis

Statistical analyses were performed using SPSS statistical package for Social Sciences (version 20.0 for windows, SPSS, Chicago, IL, USA). Quantitative data are represented as mean, standard deviation and range. Qualitative data are represented as count and percentage. Correlation test was done to test the relation of BK and MAC with other parameters. Mann-Whitney U test was used to test differences. ANOVA test was used to test differences among groups. P value of <0.05 was considered statistically significant.

Chapter Three

Results

3. Results

The study describes results of the data analysis in a series of tables corresponding to the objectives of this study as following:

3.1. Demographic data of the studied groups:

The baseline characteristics of the studied samples according to age and gender with comparison of significance.

Ninety patients with COVID-19 (range from 19 to 88) years were classified according to total lung involvement diagnosed by pulmonary computed tomography score (CT. Scan) into three groups 44severe, 29 moderate and 17 mild. The study included 56 males at 62.2 % and 34 females at 37.8 %. (40 male, 5 females) are smoker and (16 males, 29 females) are non- smokers. The general mean age in studied groups (48.86±15.73), mean age within studied groups in severe, moderate and mild groups (50.30± 15.884, 49.52±17.778, 44±10.677). The severity covid -19 was noticed among the elderly patient more than youngest patient. According to the gender the male appeared to be more susceptible to infection with covid – 19 than the female.as seen in table (3.1).

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

Total number	**90 Patients**	
Gender	Male No. (%) Female No. (%)	56 (62.2%) 34(37.8%)
Smoker	Male No. (%)	40 (88.9%)
	Female No. (%)	5 (11.1%)
Non- Smoker	Male No. (%)	16 (35.65)
	Female No. (%)	29 (64.4 %)
Age Mean ±SD **Range**	48.86±15.73 (19-88)	
Mean and SD within groups	Mild group	44±10.677
	Moderate group	49.52±17.778
	Severe group	50.30± 15.884

3.2. Comparisons of hematological, CRP, D-dimer and serum ferritin parameters between studied groups (severe moderate and mild).

The hematological, CRP, D-dimer and serum ferritin parameters distribution according to diseases severity (mild, moderate and severe). According to hematological parameters the result showed that there was no significant relationship between diseases severity and studied parameter.

Regarding the CRP, there was a significant difference between studied groups. Moreover, the results showed high levels CRP in severe group (131.37±137.87), than moderate and mild groups (P=0.0005).

As for serum ferritin , the results showed a high levels of S. ferritin in severe patients (897.27±404.007) which is highly significant when compere to other patients groups (moderate and mild) (P=0.0005). Moreover, the results showed a high levels of D. dimer in moderate patients (1238.24±1239.62) which is highly significant when compere to other patients groups (severe and mild) (P=0.009), as in table (3.2).

Table (3.2) Comparison of hematological, CRP, D- dimer and serum ferritin parameters between studied groups (severe, moderate and mild)

Parameter	Patients			P. Value
	Mild No:17 Mean±SD	Moderate No:29 Mean±SD	Severe No:44 Mean±SD	
PCV %	38.24 ±5.00	39.03 ± 4.76	38.70 ± 4.79	P=0.863
Hb g/l	12.31±1.75	12.98±1.57	12.71 ± 1.60	P=0.409

Parameter	Patients			P. Value
	Mild No:17 Mean±SD	Moderate No:29 Mean±SD	Severe No:44 Mean±SD	
WBCx10^9/L	7.18±2.08	10.82±6.083	9.07 ± 8.75	P=0.063
Platelets x10^9/L	234.76±74.94	239.86±103.98	232.59±78.05	P=0.940
D-dimer ng/ml	473.00±198.73	1238.24±1239.62	900.70±527.39	P=.009
Ferritin ng/ml	421.65±166.59	795.03±204.35	897.27±404.00	P=0.0005
CRP mg/ml	19.39±14.83	56.24±24.42	131.37±137.87	P=0.0005

NS. No significant difference at (P>0.05). HS: Highly Sig. at P=0.001

3.3 Comparison in the Mean of Serum Bradykinin Levels in the studied groups:

The Mean± SD was (3.58±1.5 ng/ml) in mild patients, and in moderate group the Mean± SD (8.77 ± 1.74 ng /ml). In severe patients the Mean± SD was (20.01±5.72ng /ml). According to analysis of serum levels of Bradykinin, there was a highly significant difference correlation among the covid – 19 patient groups. The highly concentration of serum levels of BK it noticed in the severe covid – 19 patients when compared with the other groups. Descriptive statistics of serum level of BK were listed in table (3.3).

Table (3.3) Comparison in the Mean of Serum Bradykinin Levels in the studied groups:

Studied parameter	Studied groups		
	Mild N. 17 Mean± SD	**Moderate N. 29 Mean± SD**	**Severe N. 44 Mean± SD**
BK	3.58±1.5	8.77 ± 1.74	20.01±5.72
P value	**P=0.0005**		

3.4 Comparison in the Mean of Serum MAC Levels in the studied groups:

Serum levels of MAC shows the Mean± SD was (218.75±130.37 ng /ml) in mild patients and in moderate group the Mean± SD (734.45±466.07 ng /ml). In severe patients the Mean± SD was (1130.14±648.78 ng/ml). Moreover analysis of serum levels of MAC, showed a different significant correlation statistically detected in the mean value of membrane attack complex with each patients groups (P=0.0005) as in table (3.4)

Table (3.4) Comparison in the Mean of Serum MAC Levels in the studied groups:

Studied parameter	Studied groups		
	Mild N. 17 Mean± SD	**Moderate N. 29 Mean± SD**	**Severe N. 44 Mean± SD**
MAC	218.75±130.37	734.45±466.07	1130.14±648.78
P value	**P=0.0005**		

3.5 Comparison in the Mean of total lung involvement in the studied groups:

The total lung involvement shows the Mean± SD was (4.94 ± 2.436) in mild patients, and in moderate group the Mean± SD (12.59 ± 1.680). In severe patients the Mean± SD was (19.98 ± 2.063). Moreover analysis of total lung involvement showed a highly significant difference in the mean value between each patients groups (P=0.0005) as in table (3.5).

Table (3.5) Comparison in the Mean of total lung involvement in the studied groups:

Studied parameter	Studied groups		
	Mild **N. 17** **Mean± SD**	**Moderate** **N. 29** **Mean± SD**	**Severe** **N. 44** **Mean± SD**
Total lung involvement	4.94 ± 2.436	12.59 ± 1.680	19.98 ± 2.063
P value	**P=0.0005**		

3.6 Pearson's Correlation Coefficients among BK with total lung involvement in studied groups:

A significant positive correlation was observed between BK ng/ml with total lung involvement in mild group (r=0.687, P=0.002). Moreover, a positive significant correlation notably among total lung involvement with BK (P<0.005) in moderate and severe groups (r=0.950, P=0.0005, r=0. 1, P=0.0005 respectively), as showed in table (3.6).

Table (3.6) Pearson's Correlation Coefficients among BK with total lung involvement in studied groups.

		Correlations BK		
	PC & P-value	**Mild No:17**	**Moderate No:29**	**Severe No:44**
Total lung involvement	**r**	.687	.950	1
	P-value	0.002**	0.0005**	0.0005

**. Correlation is significant at the 0.01 level (2-tailed).

3.7. Pearson's Correlation Coefficients among MAC with total lung involvement in studied groups.

A significant positive correlation was observed between MAC ng/ml with total lung involvement in mild (r=0.511, P=0.036). Moreover, non-significant correlation notably is seen among total lung involvement with MAC (P>0.05) in moderate and severe groups (r=0. .003, P=0.989, r=0.052, P=0. 739), as showed in table (3.7).

Table (3.7) Pearson's Correlation Coefficients among MAC with total lung involvement in studied groups.

		Correlations MAC		
	PC & P-value	**Mild No:17**	**Moderate No:29**	**Severe No:44**
Total lung involvement	**r**	.511	.003	.052
	P-value	.036*	.989	.739

*. Correlation is significant at the 0.05 level (2-tailed).

3.8 Pearson's Correlation Coefficients among BK with hematological parameters, CRP, S. Ferritin and D. dimer:

The study showed non-significant correlation between bradykinin and hematological parameters. According to the D. dimer there was a significant positive correlation with BK in severe group (r=0. 310, P=0. 040*), Moreover there was negative correlation notably between BK and D. dimer in moderate group, BK with s. ferritin in severe group (r= -0.008, p=0.965, r= -0.119, p=0.441). On the other hand there was non-significant correlation of bradykinin with other parameters in the groups. As in table (3.8).

Table (3.8) Pearson's Correlation Coefficients among BK with hematological parameters, CRP, S. Ferritin and D. dimer:

		Correlation BK		
Parameters	**PC & P-value**	**Mild N. 17**	**Moderate N. 29**	**Severe N. 44**
PCV	r	-.361	-.184	.063
	P	.154	.339	.685
HB	r	-.437	-.219	-.050
	P	.079	.253	.746
WBCs	r	.120	.154	-.254
	P	.645	.426	.096
Platelets	r	.348	.309	-.024
	P	.171	.103	.876
CRP	r	.322	.323	.056
	P	.208	.087	.718
D. dimer	r	.387	-.008	.310
	P	.124	.965	.040*
S. ferritin	r	.347	.319	-.119
	P	.172	.092	.441

*. Correlation is significant at the 0.05 level (2-tailed).

3.9 Pearson's Correlation Coefficients among MAC with hematological parameters CRP, S. Ferritin and D. dimer:

The study showed non-significant correlation among MAC and hematological parameters. According to D. dimer there was positive significant correlation with MAC in mild group (r=0. 559, P=0.020). Moreover, there was positive significant correlation of MAC with S. ferritin in severe and mild groups (r= 0.304, p= 0.045, r=0. 490, p=0.046). On the other hand there was negative correlation among the CRP in severer group and D. dimer in moderate group with MAC. As in table (3.9).

Table (3.9) Pearson's Correlation Coefficients among MAC with hematological parameters CRP, S. Ferritin and D. dimer:

		Correlation MAC		
Parameters	**PC & P-value**	**Mild N. 17**	**Moderate N. 29**	**Severe N. 44**
PCV	r	-.104	-.386	-.104
	P	.690	.038*	.501
HB	r	-.174	-.215	-.052
	P	.505	.262	.737
WBCs	r	.347	.225	.068
	P	.173	.240	.660
Platelets	r	-.363	.068	.291
	P	.152	.725	.055

CRP	r	.054	.178	-.145
	P	.836	.355	.349
D. dimer	r	.559	-.105	.267
	P	.020*	.586	.079
S. ferritin	r	.490	.257	.304
	P	.046*	.179	.045*

*. Correlation is significant at the 0.05 level (2-tailed).

3.10 Pearson's Correlation Coefficients among total lung involvement with hematological parameters:

The study showed that there was negative correlation notably in total lung involvement with hemoglobin, packed cells volume in mild and moderate covid – 19 patients. Moreover there was non-significant correlation notably among the total lung involvement diagnosed by pulmonary CT. Scores with the other hematological parameters as in the table (3.10)

Table (3.10) Pearson's Correlation Coefficients among total lung involvement with hematological parameters:

		Correlations total lung involvement		
Parameters	**PC & P-value**	**Mild N. 17**	**Moderate N. 29**	**Severe N. 44**
PCV	r	-.486	-.172	.143
	P	.048*	.372	.355
Hb	r	-.521	-.212	.079
	P	.032*	.269	.611
WBC	r	.101	.037	-.152
	P	.700	.851	.326
Platelets	r	.134	-.331	-.077
	P	.608	.080	.620

3.11Pearson's Correlation Coefficients among total lung involvement with CRP, D. dimer and S. ferritin:

The study showed positive significant correlation between total lung involvement with D. dimer and S. ferritin in the mild group (P=0. .000**, P=0.003). According to the with D. dimer in moderate group and s. ferritin in severe group, there was negative correlation notably with total lung involvement. Moreover there was non-significant correlation notably among total lung involvement with other parameters as in table (3.11)

Table (3.11) Pearson's Correlation Coefficients among total lung involvement with CRP, D. dimer and S. ferritin:

		Correlations total lung involvement		
Parameters	**PC & P-value**	**Mild N. 17**	**Moderate N. 29**	**Severe N. 44**
CRP	r	.401	.293	.116
	P	.110	.123	.454
D. dimer	r	.779	-.074	.281
	P	.000**	.703	.065
S. ferritin	r	.673	.319	-.197-
	P	.003**	.092	.199

**. Correlation is significant at the 0.01 level (2-tailed)

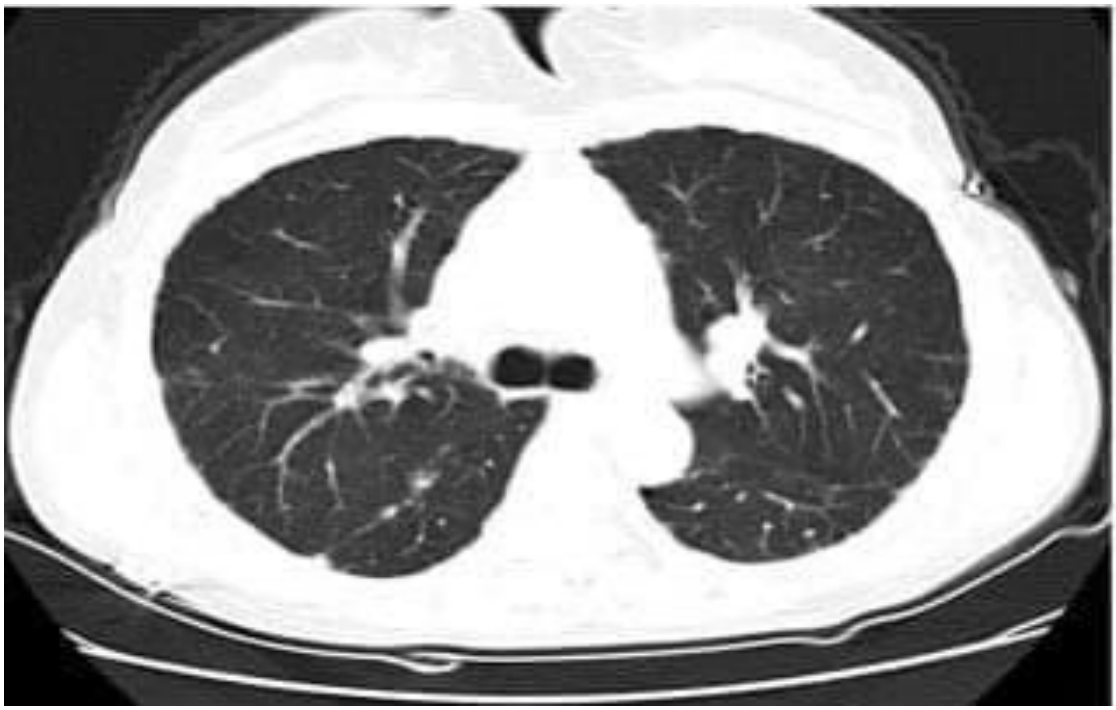

Figure 3.1: a- Chest CT findings of mild COVID-19.

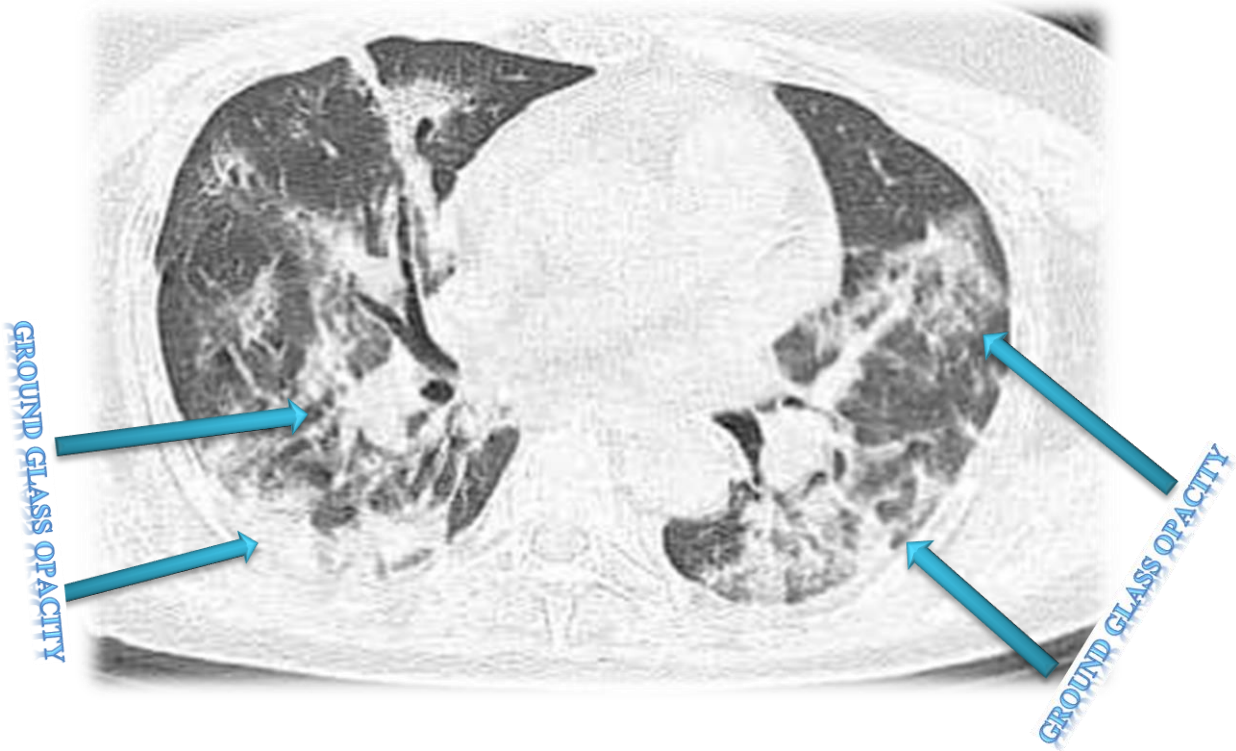

Figure 3.1: b- Chest CT findings of severe COVID-19 with Ground glass opacity.

Chapter Four

Discussion

4. Discussion

4.1 Demographic data of the studied groups:

4.1.1 Age:

The age was considered as a significant effector in the occurrence of the disease severity. The study show the age of the sample was found to be correlated with the disease severity.

The general mean of age groups was 48.86±15.73range from (19 -88) years, as in the table (3.1). In a study conducted by (Guan et al., 2020) the mean age was 47 and 52.1% of the patients were male. Another study by (Li *et al*., 2020) revealed that 56% of all patients were male and the mean age was 59. Furthermore, another study conducted by (Xu et al., 2020) showed a mean age of 41 and 56% of the patients were male (Usul *et al*., 2020).

The mean age in the studied covid – 19 patient groups, showed elderly patients present the highest risk to Covid-19, even greater than having any comorbidity. On the other hand, it seems that younger adult patients present some protection, same results was found by (Ahmadzadeh. *et al*., 2020).

The severity of COVID-19 correlated significantly with older age the results were found by (Hirashima *et al*., 2021).

Advanced age was a risk factor for viral infection and poor prognosis. In agreement with the study (Wang *et al*., 2020) suggested that age and comorbidity can be risk factors for poor outcomes (Wang *et al*., 2020). The possible reason for the association between infection-related mortality, particularly viral infections with age, may be due to impaired cellular immune function and a longer duration of inflammation in the elderly (Zhou *et al*., 2020).

In another study increasing age was related to increased severity of the disease (Li *et al*., 2020). The most severe disease and the highest mortality rates were found in the 50- to 59-year age group were found by (Mallapaty 2020).

Cumulative studies confirmed that older age was associated with poor outcomes in COVID-19 patients. In the study BY (Zhang *et al*., 2020), Older patients were prone to have severe COVID-19 symptoms and unimprovement, and were more likely to die in hospital

4.1.2 Gender:

The study included 56 males and 34 females , gender was also found to be associated with the disease severity as in the table (3.1) , Men tended to develop more serious cases than women, according to the clinical classification of severity (Jin *et al*., 2020). The study showed that a male predominance in the incidence of COVID-19 has been noted, similarly to that of SARS-CoV, indicating males are more susceptible to SARS-CoV-2 infection than females, the reduced susceptibility of females to viral infections could be attributed to the protection from X chromosome and sex hormones, which play an important role in innate and adaptive immunity same results were found by (Chen *et al*., 2020).

Another study that suggest the women are, in general, more likely to be infected by SARS-CoV-2, especially in some specific age groups. The infection of SARS-CoV-2 occurs primarily through the angiotensin-converting enzyme 2 (ACE2) receptor, which serves as a gateway for the virus's entry into tissues the results was found by (Batlle *et al*., 2020).

The age and gender are risk factors for higher severity and mortality in patients with COVID-19.The current literature suggests that men tend to have a higher risk of severe infection and mortality related to COVID-19. Regard to gender, we observed a large prevalence of male patients ,but the mortality risk was

not different, and this may be in part explained by the stronger effect of older age in population.

The age difference of the subjects between literature and the current Study have many explanations including one prominent explanation is that the sample was selected from a public health hospital that is designated for adults. One other reason is that the patients may be referred to private hospitals and/or clinics for diagnosis and treatment and not public health hospitals, the reporting of the disease may be associated with the active status as seen in our results, explaining the elevated threshold of the age groups of patients.

Many difficulties faced the researcher during this study and affected to the number of sample and period of the study these obstacles it was most the people with medications followed by the pandemic of corona virus and longtime quarantine of people.

4.1.3 Smoker:

The study involved 40 males and 5 female smokers and it is implicated as trigger for the disease development in the covid-19 patients. The smoking is a risk factor for progression of COVID-19, with smokers having 1.91 times the odds of progression in COVID-19 severity than never smokers was found by (Patanavanich & Glantz 2020).

Smoking considering risk of severe disease indicates that there is a significant association between COVID-19 and current or ever smoking. Smoking is associated with increased disease severity in COVID-19; this result was found by (Grundy *et al*., 2020, Reddy *et al*., 2021).

Vardavas and Nikitara and Lippi and Henry concluded that did not provide clear evidence of an association between smoking and poorer COVID-19 (Grundy *et al*., 2020).

Smoking is associated with up-regulation of ACE2, the receptor for the SARS-CoV-2 virus in the lung, with up-regulation in epithelial cells and downregulation in alveolar type 2 cells. The situation is further complicated by the possibility that internalization of ACE2 due to viral infection leads to unopposed ACE inhibitor activity and high angiotensin II levels, contributing to endothelial damage and the coagulopathy and micro thrombosis seen in severe COVID-19.Smoking itself causes vascular endothelial damage, a prominent feature in the pathophysiology of severe COVID-19, smokers are exposed to higher SARS-CoV-2 loads as a result of increased expression of ACE-2, which may provide a mechanistic explanation for the increased risk of severe disease and mortality associated with smoking in COVID-19 patients (R. K. *et al*., 2021).

4.2 Comparisons of hematological, CRP, D-dimer and serum ferritin parameters between patient groups (severe moderate and mild):

In the study there is no significant difference between hematological parameters in the cases as in table (3.2). The results are similar to what was found by (Liu *et al*., 2020). Henry et al. also concluded in COVID-19 positive patients with moderate and fatal disease had significantly increased WBC, compared to mild or non-severe disease (Henry *et al*., 2020).

Huang et al. reported that the percentage of severe COVID-19 patients who had increased WBC counts was significantly higher than non-severe counterparts (54% vs 19%), further highlighting the fact that the extent of deviation from normal white blood cell counts correlates with disease severity (Huang *et al*., 2020).

Another study found the level of white blood cells decreased (Li *et al*., 2020, Li *et al*., 2020). Also WBC counts of COVID-19 patients were significantly higher

in the severe cases as compared to the non-severe group. Monitoring the patients' WBC counts during hospitalization also disclosed a simple method of predicting COVID-19 prognosis (Wang *et al*., 2020).

Regarding the platelets, among the covid- 19 studied patient groups there is no significant difference as in the table (3.2).

Several studies suggest that the number of platelets was reported to be significantly reduced in COVID-19 patients (Yang, *et al*., 2020, Ganji, *et al*., 2020). There were several reasons why the platelets of COVID-19 patients declined in the early stages. On the one hand, viral infection induced lung tissue damage, resulting in activation, aggregation, and entrapment of the platelet. This led to thrombosis at the lung injury site, which increased the consumption of platelet. On the other hand, mature megakaryocytes may release platelets in the lungs. Therefore, when the damaged lungs caused pulmonary fibrosis and pathological changes, production of platelet might be affected (Zhao *et al*., 2020).

Xu *et al.* revealed in their study that thrombocyte counts are significantly low in pneumonia patients and that this decrease is directly proportional to the patients' clinical status (Xu *et al*., 2015). In a study by Fan *et al.* mild thrombocytopenia and leukopenia was observed in some patients at first admission who were COVID-19 positive. The effects of viral pneumonia on the immune system show a decrease in thrombocyte, leukocyte counts (Fan 2020).

As for the hemoglobin there is no significant difference among the studied covid – 19 patient groups as in table (3.2). Hemoglobin levels in COVID-19 positive patients were found to be significantly higher than in COVID-19-negative patients. While no significant difference was observed among females regarding hemoglobin, higher hemoglobin levels were seen in COVID-19 positive male patients. It is possible that these results are also affected by other reasons, such as the presence of comorbidities or anemia, and habits such as cigarette smoking. The

patient files used for this study did not include a detailed patient history, and thus, their effect on hemoglobin levels were not accounted for. Also, the normal hemoglobin level in the female population is lower than that of males (Dirican *et al*., 2016).

Other studies that show lower hemoglobin level in more severe cases hemoglobin in patients with covid - 19 are significantly lower compared with those without covid – 19 (Ghahramani *et al*., 2020 **,** Mahallawi *et al*., 2018).

Routine blood tests refer to the examination of blood condition and disease by observing the quantity change and shape distribution in blood cells, including white blood cells (WBCs), red blood cell count (RBC), hemoglobin (Hb) and platelets (PLTs). Routine blood test indicators are sensitive to many pathological changes, which may assist in diagnosis when the cause of the disease is unknown. In addition, routine blood tests are a common indicator for the evaluation of medication or discontinuation and disease recurrence or recovery.

A significant limitation of this data is that most of this information was collected from as a single time point per patient and, when defined, was most often at initial presentation. We now know that automated hematology parameters may be dynamic over the course of disease and convalescence.

Regarding to the CRP, S. Ferritin and D – dimer in the study there was significant difference between studied covid – 19 patient groups. As for serum ferritin there was a significant difference between studied groups and show high levels in severe group. Moreover, the results showed a high levels of D. dimer in moderate patients which is highly significant when compere to other covid – 19 patients groups

The serum D-dimer concentrations in patients with severe forms of the disease were significantly higher than those in patients with milder forms, the same result was found by (Paliogiannis *et al*., 2020)

Huang et al. reported that those with severe covid -19 had D-dimer values 5 times higher than those of the other patients (Huang *et al*., 2020) In agreement with study by, Tang et al. concluded that the D-dimer level was approximately 3.5 times higher in patients with severe covid -19 than moderate and mild (Tang *et al*., 2020). In a study by Yao et al. reported that D-dimer levels were significantly higher in patients with severe conditions than in other patients (Yao *et al*., 2020). In agreement with study by, Ozen et al. concluded that D-dimer levels were significantly elevated in patients with severe conditions were reported to be significantly higher than those of mild and moderate patients(Ozen *et al*., 2021).

In the study by Gao et al. 2020 revealed that D-dimer was significantly elevated in D-dimer levels in COVID-19 patients with severe disease have been reported in China (Gao *et al*., 2020). Other study that found a significant difference in D-dimer level between patients with severe and mild COVID-19, there suggests that elevated D-dimer levels have a significant association with the COVID-19 severity. The results revealed that COVID-19 patients with ARDS would have abnormal coagulation, and the acute lung inflammatory response may have been associated with increased thrombotic activity (Ortega-Paz *et al*., 2021)

Regarding to the serum ferritin there was a significant difference between the covid – 19 patient groups as in the table (3.2).

This study showed that the ferritin level was elevated in ARDS-COVID-19 patient groups, the data confirm that increased ferritin level was directly associated

with the disease severity, this result was in agreement with Lin et al. Who reported an increase of ferritin level in Chinese patients with severe COVID-19 than patients with non-severe COVID-19 disease (Lin *et al*., 2020). Raised ferritin levels in circulation could indicate severe inflammatory reaction in ARDS-COVID-19, and elevated ferritin levels in circulation may play an important role by contributing to cytokine storm development resulting in pulmonary edema and ARDS (Kernan & Carcillo 2017).

A significant increase in ferritin levels was demonstrated in patients with moderate and severe disease, compared to patients with mild disease. Severe patients had significantly higher levels of ferritin the same result was found by (Dahan *et al.*, 2020)

Regarding the CRP levels in the patient groups was significantly higher in severe COVID-19 patients compared with that in moderate and mild COVID-19, same results was found by (Shi *et al*., 2021). CRP levels were significantly higher in severe patients than in non-severe patients, and the same results were found by (Wu *et al*., 2020).

Higher levels of CRP were proposed to be highly associated with severe COVID19. CRP can be used to predict the prognosis of COVID-19 infection; the higher CRP, the great risk of mortality (Bakr *et al*., 2021).

A significantly high level of CRP was observed in the severe COVID-19 group compared to non-severe COVID-19 cases, confirming previous reports of the clinical utility of CRP levels as an indicator for severe disease and progressive inflammation (Soraya & Ulhaq 2020).

4.3 Comparison in the Mean of Serum Bradykinin Levels in the studied groups:

In this study was revealed different significant correlation among the covid – 19 patient groups, the levels of bradykinin elevated in severe group than the moderate and mild groups, and higher in moderate group when compared with mild this results are agreement with Ulrich et al., 2018 and Kontos. *et al*., 2013, those found that the levels of BK are elevated (Ulrich *et al*., 2018 , Kontos *et al*., 2013).

In patients with HA and AA examined during an acute attack, the bradykinin concentrations were two to 12 times the upper limit of normal, and in individual patients bradykinin reached up to 35 times the concentrations measured during remission . Measured bradykinin in many such patients and compared the concentrations both during acute attacks and in with those of healthy controls. Bradykinin concentrations were higher in patients with HA or AA in acute attack than in the healthy controls (Nussberger *et al*., 1998). Another study results showed a very high significant difference among the patient groups and health group, (P value ≤ 0.05), where it noted that in the respiratory subjects which were virally infected had decreased levels of the bradykinin compared to healthy group (Al-Kaif *et al*., 2020).

In the study of serum bradykinin in covid-19 was considered as a new marker to comparison and differentiation between covid-19 patient severities.

4.4Comparison in the Mean of Serum MAC Levels in the studied groups

In comparison between covid-19 patient groups (severe, moderate and mild), the sMAC showed high levels in severe groups than the moderate and mild groups, and high in moderate than mild groups, as a results shown in table (3.4).

Several studies that show the serum levels of sC5b-9 were significantly high in the patients with moderate disease than those with mild disease and significantly higher in the patients with severe disease than in those with moderate and mild. SC5b-9 levels were increased in patients with mild, moderate or severe disease, these results are found by (Guan *et al*., 2020, Chen *et al*.,2020 , Cugno *et al*.,2020).

Complement activation, formation of C5b-9 or membrane attack complex (MAC) or terminal complement complex (TCC) is important to be evaluated. In the study by Kumari et al. 2021 shown that C5b-9 was significantly higher in COVID19 patients with moderate disease than mild and those with severe disease than mild, and also C5b-9 was significantly higher in patients with severe disease than those with moderate disease. Thus, C5b-9 levels probably are an index of severity of disease as C5b-9 also has a role in neutrophil activation and inflammation that leads to endothelial damage (Kumari *et al*., 2021).

Consequently, measuring complement activation products (particularly SC5b-9) may provide a sensitive and useful biomarker of COVID-19 severity and activity.

4.5 Comparison in the Mean of total lung involvement in the studied groups:

The comparison between covid-19 patient groups in total lung involvement diagnosed by pulmonary CT. scores showed highly significant difference in the patients groups, as in table (3.5).

The CT scores in the progressive-stage group were significantly greater than those in the early-stage group. The CT scores were positively correlated with the total lung involvement in severe, moderate and mild groups , Moreover CT scores showed a significant positive correlation with ages in all patients, the same results was found by (Zhou *et al.*, 2020.) In the mild and moderate types of patients, only few pulmonary lobes were involved according to the previous studies (1–3) lobes involvement, the critically ill patients, involvement of all 5 lobes was observed in 98% of the patients.(Chung *et al.*, 2020). A total of the lung severity scores of mild and moderate patients were calculated and a summation of each lobe score (with similar 0–4 scales) was performed to determine the degree of involvement of the lung field. Their results showed that the mean total lung severity score was 9.9 in a range of 0–20 (Zhang *et al.*, 2020). The severity score of lung involvement in patients who severe from COVID-19 was also significantly greater than that in patients with mild to moderate COVID-19 (12.97±5.87 vs. 7±4) the results was found by(Hu *et al.*, 2020).

4.6 Pearson's Correlation Coefficients among BK with total lung involvement in studied groups:

We investigated the serum levels of BK as markers in patients with COVID-19. There is a significant correlation between total lung involvements with the serum levels of BK in patient groups, as in table (3.6).

The pro-inflammatory activity of BK involves the stimulation of cytokines, like IL-6 and IL-8, through the MAPK/AP-1 signaling axis, whereas the

hypotensive effects are due to vasodilation and increased vascular permeability (Gomez-Gutierrez & Perez 2021). Bradykinin is a very potent non-peptide vasoactive, capable of dilating venules through the release of nitric oxide (NO) and increasing vascular permeability through its action on B1 and B2 receptors (Florêncio *et al*., 2020).

The Angiotensin-Converting Enzyme 2 (ACE 2), also known as Kininase II, is one of those responsible for degrading kinins, including bradykinin SARS-CoV-2 binds to ACE 2 receptors to enter the cells (Colarusso *et al.*, 2020). Thus, in COVID-19, because this virus is bound to this receptor, there may be an infra-regulation of ACE 2 receptors, which may result in decreased kinin degradation, thus generating a cascade that may be partially responsible for the emergence of some of the clinical manifestations of COVID-19, among them, angioedema (Cohen *et al*., 2020).

The downregulation of ACE2 not only increases the levels of Ang II, but it also leads to increased levels of des-Arg(9)-bradykinin (DABK) which is associated with acute lung damage and inflammation. The subsequent dysregulation allows for a bradykinin storm to occur; with the lack of breakdown of DABK *via* ACE2, there will be high levels of free BK available to act on target cells. DABK is known to bind not only to bradykinin-1 receptors (B_1Rs), but also to bradykinin-2 receptors (B_2Rs) (Roche & Roche 2020).IL-1 and IL-6 are upregulated by SARS–CoV-2 infection, and their effects are added to the actions induced by the enhanced generation of BK in the lungs and throughout the body, IL-1 and IL-6 also stimulate the expression of B_1Rs and can subsequently lead to the bradykinin storm that may be critical in the severity of COVID-19 (Wilczynski *et al.*, 2021).

4.7 Pearson's Correlation Coefficients among MAC with total lung involvement in studied groups.

The current study investigated the serum levels of sC5b-9 as markers of complement activation in patients with COVID-19. There is a significant correlation between total lung involvement with the levels of sC5b-9 in mild group (P=0.036) as in table (3.7).

Complement activation contributes to the induction and amplification of the inflammatory process as a result of the ability of C5a and SC5b-9 to recruit phagocytic cells in the lung and other infected organs, activate endothelial cells, and stimulate vascular permeability (Bossi *et al*., 2004). The anaphylatoxin produced by the activated complement pathway, C4a, C3a, and C5a, have important immunostimulatory roles in vascular permeability and inflammatory cell recruitment ,C3a and C5a in particular are noted for their roles in causing mast cell degranulation, initiating a cytokine storm, promoting vascular permeability, and contributing to acute lung injury . Activation has been observed severe respiratory infections, including coronaviruses **(**Gralinski *et al*., 2018)

Complement activation will lead to cleavage of C5 into the split products C5a and C5b. In turn, TCC is composed of the C5b subunit together with C6, C7, C8, and several C9 molecules (Nilsson *et al*., 2017). C5a is, next to C3a, a potent anaphylatoxin and acts as a strong activator of neutrophils, monocytes, and macrophages, leading to pro-inflammatory cytokine release and induction of inflammation (Prohászka *et al*., 2016).

The current study found that changes in the levels of individual components and activation products of the complement and kallikrein/kinin systems were associated with survival/death, organ function, and scores of illness severity. The kallikrein/kinin system was correlated with organ failure, reflected by injury to receive mechanical ventilation, indicating that the lungs, kidneys and heart were

affected. Logistic regression analysis of prekallikrein strongly suggested that the association with death was due to is respiratory failure (Lipcsey *et al*., 2021).

4.8 Pearson's Correlation Coefficients among BK with hematological parameters, CRP, S. Ferritin and D. dimer:

In the study there is no significant correlation between bradykinin with hematological parameters, Moreover there was correlation between bradykinin with D. dimer in severe group, and there was no correlation between BK with s. Ferritin and CRP, as a result in the table (3.8).

Bradykinin is a vasoactive pro-inflammatory neuropeptide that induces relaxation of vascular smooth muscle in arteries and arterioles and promotes adhesion molecule expression, leukocyte sequestration, and the formation of interendothelial gaps and protein extravasation in postcapillary venules (Abbott 2000).

While the mechanisms underlying the vasorelaxant properties of this peptide have been extensively studied, the cell signaling pathways that mediate the powerful pro-inflammatory effects of bradykinin in postcapillary venules have received less attention. Indeed, the effect of the neuropeptide to induce leukocyte infiltration has only recently been recognized (Schuschke *et al*., 2001).

The role of BK in platelet activation is very limited. In the present study, an intracarotid infusion of BK resulted in increased numbers of rolling platelets. This platelet activation was B2 receptor-dependent, as application of the B2 receptor antagonist attenuated platelet rolling. An explanation for these results is provided by data supporting not only the well-known importance of platelets in hemostasis, but also in inflammation found by (Waldner *et al*., 2012).

CRP exerts pro-inflammatory biological activities which may contribute to the development and maintenance of ACEI-induced angioedema. This includes the

release of cytokines such as interleukin-6 and the activation of phagocytic cells as well. Since interleukin-6 is a strong inducer of acute-phase proteins, we speculate that CRP-induced interleukin-6 increases fibrinogen release. At the same time, CRP-induced interleukin-6 might increase the release of kininogens which stimulates the generation of endogenous kinins from kininogen such as bradykinin (Bas *et al*., 2005).

The study show no significant correlation between the bradykinin and CRP ($P>0.05$), the results are similar to that was found by (Stoves *et al*., 2001).

The kallikrein–kinin system is activated and increased bradykinin may cause detrimental effects such as enhanced stimulation of t-PA release, with resulting fibrinolysis and bleeding. The endothelium is the principal site of t-PA synthesis and storage, whereas PAI-1 is synthesized and secreted from several sources including endothelium, adipose tissue, liver, and platelets. Various agonists, including bradykinin and factors related to the coagulation cascade (for example, thrombin), stimulate the release of t-PA through G protein–-coupled receptors. Once released, active t-PA catalyzes the conversion of plasminogen to plasmin that in turn degrades fibrin to fibrin degradation products (Balaguer *et al*., 2013).

Although bradykinin is a potent stimulus of endothelial t-PA release through its B_2 receptor, bradykinin B_2 receptor antagonism is not expected to affect alternative pathways of endothelial t-PA release by agonists such as thrombin (Shariat-Madar *et al.,* 2006). Therefore, bradykinin B_2 receptor antagonism does not appear to be an effective strategy in reducing fibrinolysis as measured by D-dimer formation ((Balaguer *et al*., 2013).

Bradykinin levels were not correlated with the parameters of activation of coagulation, fibrinolysis, complement, and cytokines found by (Cugno *et al*., 2001).

4.9 Pearson's Correlation Coefficients among MAC with hematological parameters, CRP, S. Ferritin and D. dimer:

In this study there was a positive correlation between MAC and D. dimer in mild group, positive correlation between MAC s. Ferritin in severe and mild group. Moreover there is no correlation with D. dimer and no significant association between membrane attack complexes with hematological parameters as the results in the table (3.9).

Increased sMAC reflects increased complement activation. Therefore, the increased plasma values in the patients is likely to represent a discreet complement activation as part of such patients' frequent systemic low-grade inflammation, confirmed by elevated CRP levels in all patients (Bjerre *et al*., 2010). Another study found significant correlation between CRP and sC5b-9 (Hoffmeister *et al*., 2002).

CRP is multifunctional proteins that can activate the classical pathway through its interaction with C1q. Studies implicating CRP in the classical pathway suggest that activation entails C2 and C4 consumption that is only rarely accompanied by terminal pathway activation or generation of the MAC. The primary role of CRP may be to promote a cellular immune response rather than complement attack. CRP could perform this function by binding to apoptotic cellular debris such as membrane components, histones and recruit phagocytic cells through Fc receptor-mediated interactions (Johnson *et al*., 2006).

Regarding to the D – dimer. Positive correlation between TCC and D-dimer is noticed. The relationship between the complement and coagulation pathways. Both the end-product of the complement pathway, the C5b-9 complex (or TCC), and the anaphylatoxins C3a and C5a can stimulate the coagulation cascade via several processes. They may activate platelets and promote secretion of von

Willebrand factor and P-selectin via activation of endothelial cells, and they may increase tissue factor activity(de Nooijer *et al*., 2021).

According to Gular et al, the mechanism of the rise of D-dimer in COVID19 is related to virus life cycle (Guler *et al*., 2020). The virus multiplies itself by infecting the host's endothelial cells and lyses these cells for their release. The processes involved in destruction of these endothelial cells are either direct disruption or through inflammatory mechanisms. This leads to the activation of both, intrinsic and extrinsic coagulation pathways. Also, complement pathways do contribute in activation of coagulation factors. Thus, triggered coagulation processes lead to increased levels of D-dimer (Kumari *et al*., 2021).

4.10 Pearson's Correlation Coefficients among total lung involvement with hematological parameters:

There was no significant correlation between lung involvement and hematological parameters in study groups as the results as in the table (3.10).

Another study found the chest CT score was positively correlated with WBC (Zhang. *et al*., 2020). Only a poor correlation was found between WBC count and disease severity, as determined by CT findings found by (Kim *et al*., 2011).

In agreement with study by Yilmaz et al. 2021 did not find a correlation between WBC and platelet count and CT severity. Among these laboratory parameters, we did not find a correlation between WBC and platelet count and CT severity. In this respect, our study came up with a different conclusion from the literature data, and we hold the view that these two parameters are not suggestive of CT severity but of CT findings. Based on these findings, we can conclude that the low scores in these parameters can help to predict the severity of CT (Yilmaz *et al*., 2021).

Chest CT score was positively correlated with laboratory parameters in the early and progressive stages, the results was found by (Zhang *et al*., 2020).

4.11. Pearson's Correlation Coefficients among total lung involvement with CRP, D. dimer and S. ferritin:

In this study there was positive correlation between total lung involvement diagnosed by pulmonary CT. Scores with S. ferritin and D. dimer in mild group. Moreover there is no significant correlation between total lung involvement diagnosed by pulmonary CT. Scores with CRP, S. ferritin and D. dimer in moderate and severe groups, as in table (3.11).

Several studies that show the levels of serum CRP in the CT imaging score ≥ 11 group were higher compared with the < 11 group the results was found by (Badawi & Ryoo 2016). The CRP showed significant positive correlation with the severity of pneumonia quantified on initial CT score was found by (Xiong *et al*., 2020).

The serum CRP level had significant correlation with CT severity was found by (Saeed *et al*., 2020).

Another study reporting that CRP levels and CT findings are positively correlated was found by (Tan, *et al*., 2020).

Another study found that CRP increased significantly at the initial stage in severe COVID-19 patients; while still no significant difference in the CT scores were found between the severe and mild groups. CRP was an early biomarker for predicting the severity of COVID-19 with good performance. These results suggested that CRP could be used to early identify patients who might become severely ill and before the CT finding (Tan *et al*., 2020).

In another study where the relationship between CT lesions scores CRP levels were found to have moderate positive correlation with CT scores. CRP was also moderately correlated with CT was found by (Sun *et al*., 2020, Francone *et al*., 2020).

The serum CRP levels and CT findings of severe patients were consistent with the newly reported pathological manifestations, which were diffuse alveolar lesion under microscope, a large amount of exudative monocytes, lymphocytes and plasma cells in the alveolar cavity and pulmonary interstitium, and extensive pulmonary interstitial fibrosis(Luo *et al*., 2020).

In this study revealed a positive association between CRP level and lung CT. Severity in COVID-19 patients. This result was in agreement with Wang 2020. Who reported an increase in CRP level in Chinese patients with critical and severe COVID-19 than patients with moderate and mild COVID-19 (Wang, L. 2020).

Regarding D – dimer there was significant correlation between total lung involvement diagnosis by pulmonary CT. Scores and D. dimer in mild, as in table (3.11).

Many studies found CT imaging score ≥ 11 group had dramatically increased levels of D-dimer in the blood. Another study show moderate correlation between CT scores D – dimer. The D-dimer levels were significantly increased in severe patients, and the level of increase was consistent with the aggravation degree of chest CT lesions, the results were found by (Badawi & Ryoo 2016, Zhu *et al*., 2020, Wei *et al*., 2020).

There is a significant correlation between D-dimer levels and disease severity stratified by the area of affected lungs on chest CT (Wu *et al*., 2020). The pathological features of COVID-19 include diffuse alveolar damage with cellular fibromyxoid exudates, desquamation of pneumocytes and hyaline membrane

formation, pulmonary edema with hyaline membrane formation, and interstitial mononuclear inflammatory infiltrates, dominated by lymphocytes, which greatly resemble those seen in SARS and MERS coronavirus infection. The observed D-dimer elevation signifies a hyperfibrinolysis state and increased inflammatory burden induced in SARS-COV-2 infection (Yao *et al*,. 2020).

Serum ferritin levels have moderate positive correlation with CT score (Yilmaz *et al*., 2021). There was a statistically high significance between increased levels of serum ferritin in correlation with CT staging, where ferritin was increased in 18.4% in the mild stage, 63% in the moderate, and 100% in the severe stage; these findings were in agreement with Lin et al. who reported that patients with severe COVID-19 pneumonia showed higher levels of serum ferritin than the non-severe patients using a multivariate logistic regression analysis showing that the serum ferritin level was an independent risk factor for disease severity (Lin *et al*., 2020).

In the study serum ferritin levels were observed to be high in the CT-positive group and have moderate positive correlation with CT severity. This situation indicates that serum ferritin level is closely related to the radiological severity as well as to the clinical severity of the subjects. In a similar vein, the high level of D-dimer in the CT-positive group and its positive correlation with CT severity, though not as high as ferritin, indicate that the serum D-dimer level is associated with clinical and radiological severity (Yilmaz *et al*., 2021).

Conclusion And Recommendation

Conclusion:

The recent study concludes the following:

1- There was a significant correlation of serum BK levels with the severity of covid – 19. In addition there is positive correlation of serum BK levels with total lung involvement in covid – 19 patients.

2- This study reported a significant correlation between C - reactive protein, D-dimer and serum ferritin in severe covid – 19 patients. In addition Serum level of BK positive correlated with D – dimer in severe group, while negative correlated with D. dimer in moderate group and S. ferritin in severe group.

3- Serum level of MAC significant correlated with mild lung involvement, while non- significant correlated with moderate and severe lung involvement. In addition serum level of MAC significant positive correlated with D. dimer in mild group and with S. ferritin in severe and mild groups, while non-significant correlated with CRP, platelets, PCV and WBCs

4- Total lung involvement positively correlated with D. dimer and S. ferritin in the mild group, while non-significant correlated with CRP, platelets, PCV and WBCs

Recommendations:

1- Study the BK, MAC in a large sample size of covid – 19 patients.

2- Study the genetic polymorphism of bradykinin and its receptors in covid – 19 infection

3- Further studies of Anti-bradykinin effects on the cytokines storm in covid – 19 patients.

4- Further studies of others markers with covid - 19

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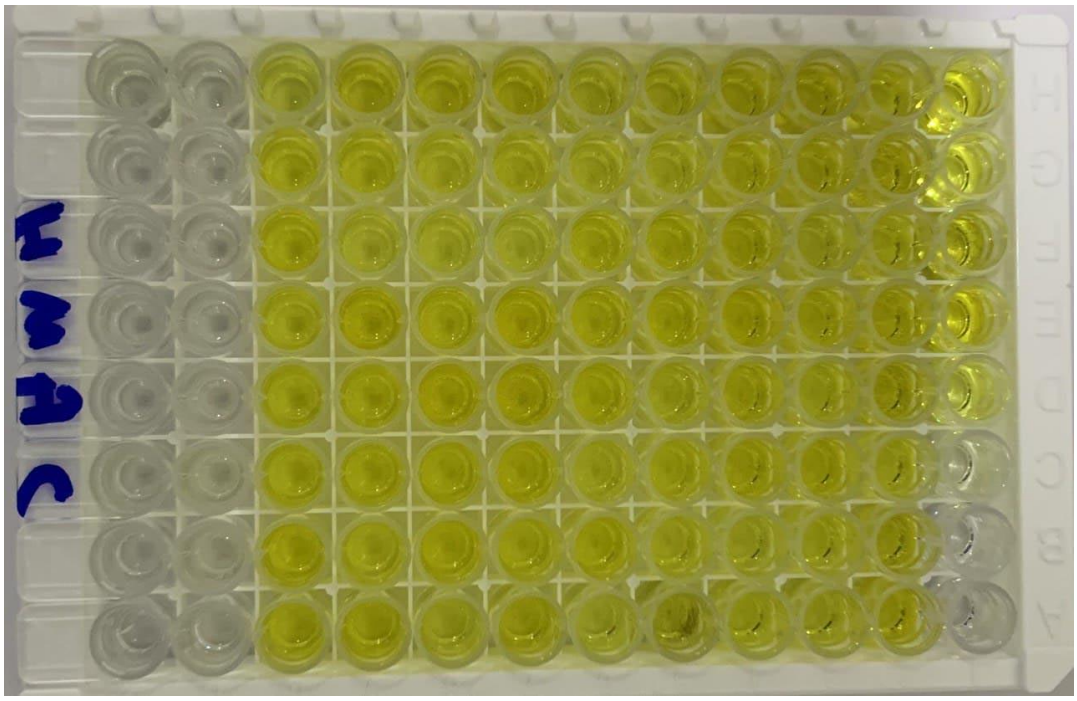
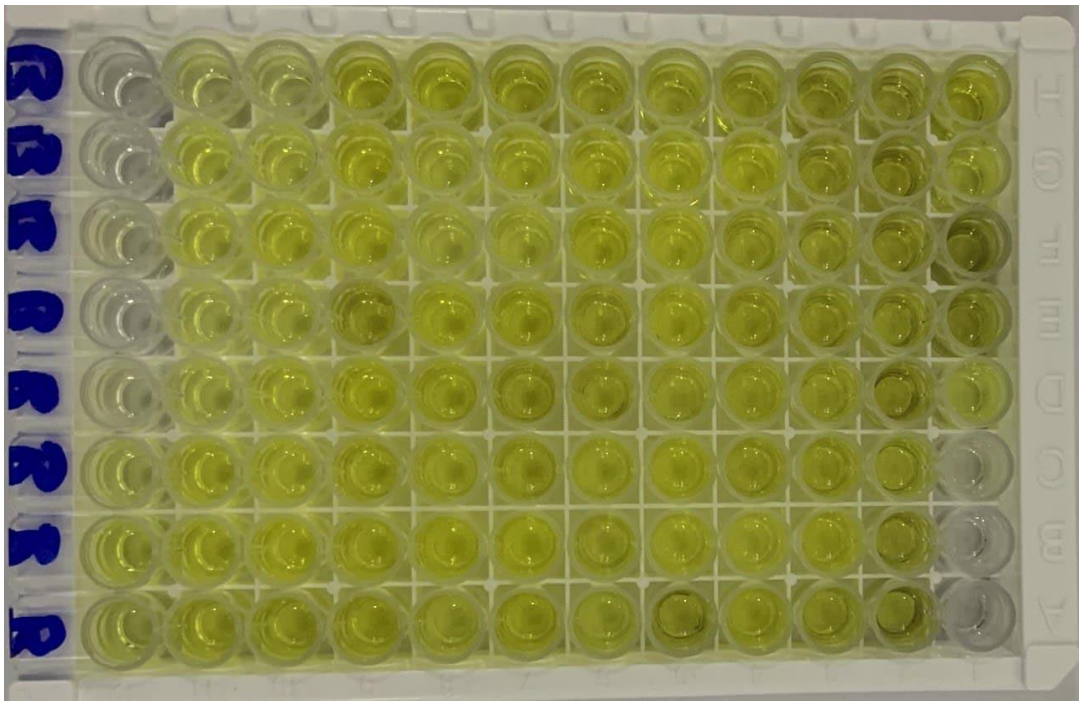
Appendix

Appendix 1

ELISA system with plate



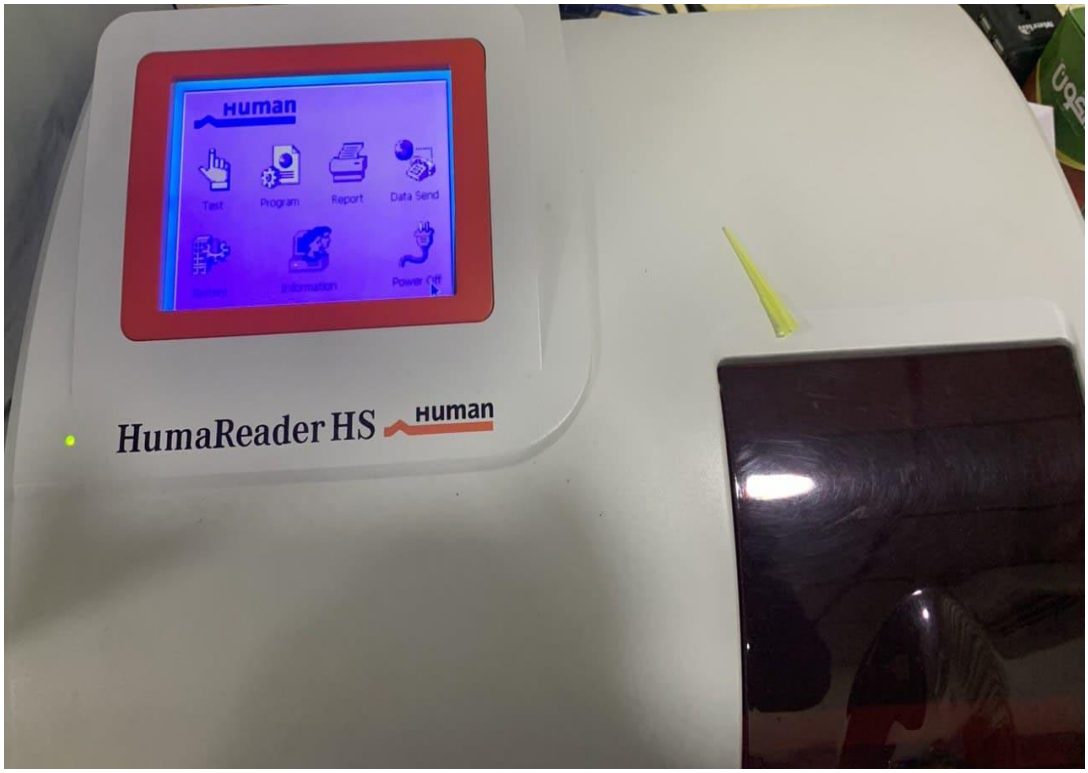
Membrane attack complex plate



Bradykinin plate

Appendix

ELIZA system



Appendix 2

Questionnaire

a- Dimorphic data :

Name		Age	
Gender			
Smoker			
Family history			

b- **Laboratory data** :

PCV	Hb	WBCs	PLT	CRP	S. ferritin	D. dimer

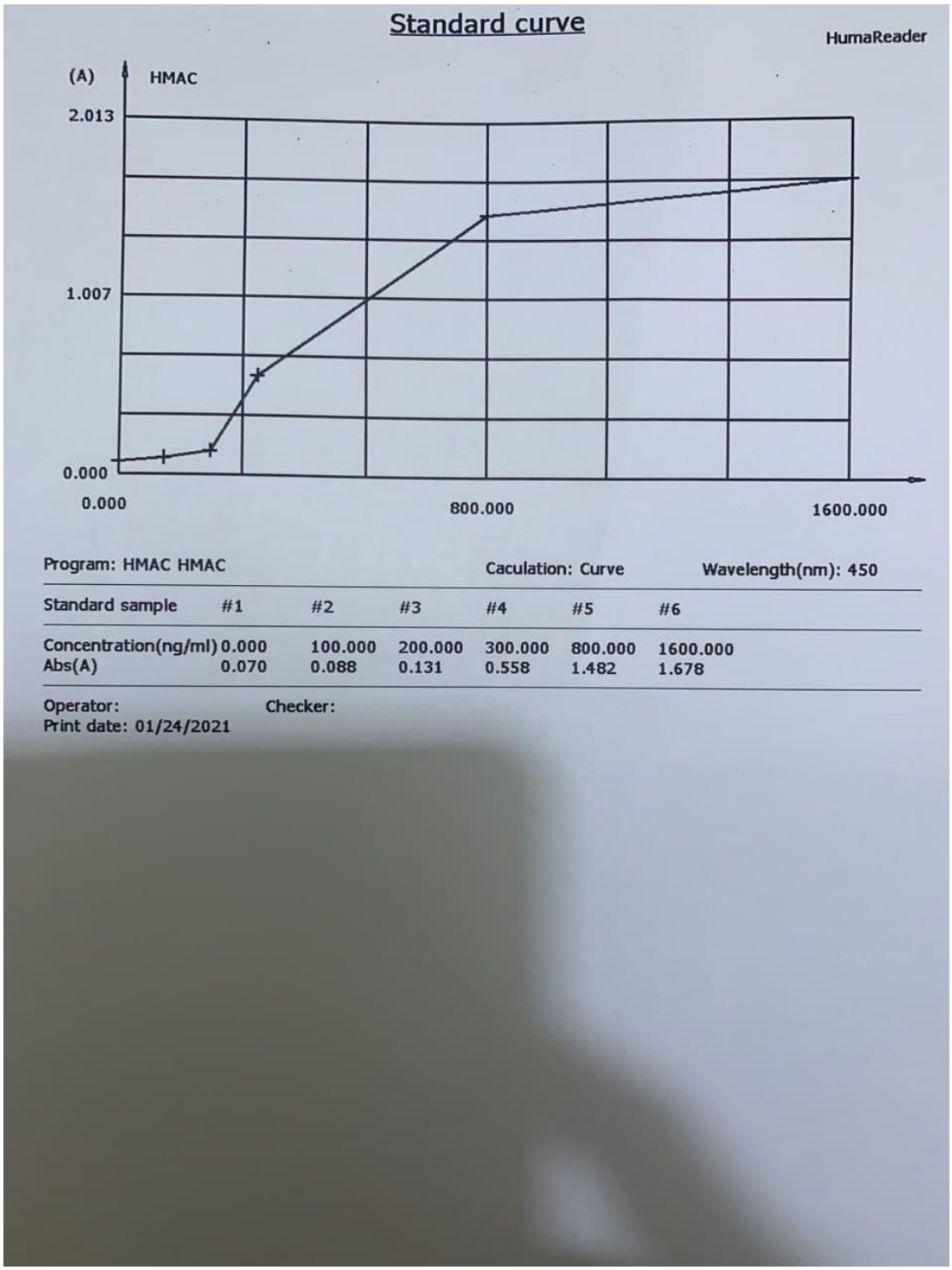
C.T score:

C.T severity	**%involvement**	**Score**
Right upper lobe		
Right middle lobe		
Right lower lobe		
Left upper lobe		
Left lower lobe		
	Total	/25

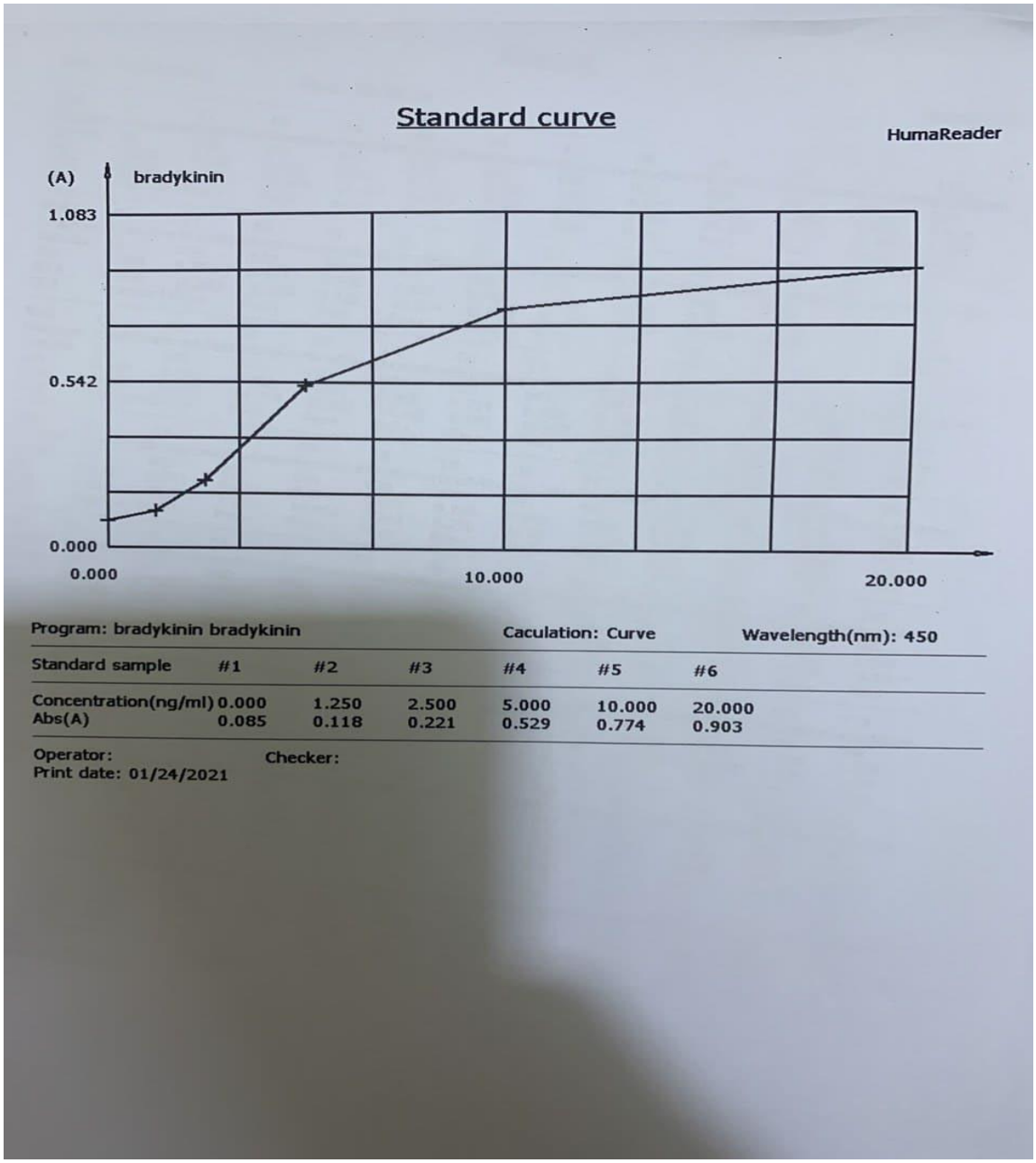
Appendix 3: Curve of Immunological Markers

a- Bradykinin

b- Membrane attack complex



Appendix



Program: bradykinin bradykinin Caculation: Curve Wavelength(nm): 450

Standard sample	#1	#2	#3	#4	#5	#6
Concentration(ng/ml)	0.000	1.250	2.500	5.000	10.000	20.000
Abs(A)	0.085	0.118	0.221	0.529	0.774	0.903

Operator: Checker:
Print date: 01/24/2021

الخلاصة :

مرض فيروس كورونا 2019 (COVID-19) هو مرض معد ناجم عن الفيروس التاجي للمتلازمة التنفسية الحادة الوخيمة 2. وهو يسبب أمراضا تنفسية خطيرة مثل الالتهاب الرئوي وفشل الرئة. وتهدف الدراسة إلى تقييم ارتباط مستويات مصل bradykinin و membrane attack complex و التصوير المقطعي الرئة في المرضى الذين يعانون من الفيروسات التاجية

الدراسة شملت 90 مشاركا مريضا يتألفون من 44 شديدا و29 معتدلا و17 معتدلا كانوا يحضرون في مدينة الحسين الطبية ، وحدة الحياة في كربلاء بالعراق للفترة من أكتوبر 2020 إلى يناير 2021. تم تصنيف المواد المسجلة في الدراسة إلى ثلاث مجموعات من المرضى شديدة ومعتدلة وخفيفة وفقا لشدة الضرر في الرئة و التي تم تشخيصها من خلال درجات التصوير المقطعي الرئوي تحت إشراف أخصائي الأشعة.

تم إجراء الاختبارات المخبرية بواسطة التقنيات ELISA للكشف عن مستوى bradykinin and membrane attack complex

كشفت نتيجة الدراسة أن متوسط العمر العام في المجموعات المدروسة (48.86±15.73) (19-88) سنة، وكان من بين 90 مريضا 56 ذكرا بنسبة 62.2٪ و34 أنثى بنسبة 37.8٪. (40 ذكرا و 5 إناث) مدخنون و(16 ذكرا و29 أنثى) غير مدخنين.

وفقا لمتوسط مستويات المصل من bradykinin بين مجموعات المرضى كان (3.58±1.5) في الاصابة الخفيفة ، و الاصابة معتدلة (8.77 ± 1.74)بينما الاصابة الشديدة (20.01±5.72)، وبنسبة ارتباط كبيرة جدا (P = 0.0005) مع مرضى covid – 19 .

وكما أظهر مستوى المصل MAC ارتباط كبير (P = 0.0001) ، حيث كان متوسط مستويات المصل (130.37±218.75) في الاصابة الخفيفة ،وفي معتدلة (734.45±466.07). بينما اظهرت الاصابة الشديدة (1130.14±648.78) لدى مرضى covid – 19 المريض، وقد لوحظ تركيز عال من مستويات المصل من bradykinin, MAC في مجموعة شديدة بالمقارنة مع المجموعات المعتدلة وخفيفة وفقا لشدة الضرر في الرئة و التي تم تشخيصها من خلال درجات التصوير المقطعي الرئوي تحت إشراف أخصائي الأشعة.

كما واظهرت الدراسة ان متوسط Total lung involvement في الاصابة الخفيفة (4.94 ± 2.436)، معتدلة (12.59 ± 1.680) وفي شديدا (19.98 ± 2.063)، وأظهرت وجود ارتباط معنوي كبير للغاية (P = 0.0005) مع مجموعات المرضى covid – 19

بالإضافة إلى ذلك، زادت مستويات البروتين التفاعلي (C. (CRP، D. dimer و S. ferritin في مجموعات معتدلة وشديدة بالمقارنة مع مجموعة خفيفة وأعطيت علاقة كبيرة (P = 0.0005، P =.009، P = 0.0005).

وقد أظهرت نتائج هذه الدراسة مستويات مصل مرتفعة من BK وMAC في مجموعة شديدة مقارنة مع مجموعات معتدلة وخفيفة من 19 مريضا covid ، وبالتالي ، قد يكون لهذه العلامات دورا هاما في تشخيص مرضى covid – 19 .

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء / كلية الطب

فرع الاحياء المجهرية

ارتباط مستوى مصل من برديكنين و هجوم غشاء معقد مع التصوير المقطعي المحوسب للرئة في المرضى Covid-19.

رسالة مقدمة الى

مجلس كلية الطب / جامعة كربلاء

كجزء من متطلبات نيل شهادة الماجستير في الاحياء المجهرية

من قبل

عقيل سلمان عبد السلام

بكالوريوس تقنيات صحية وطبية / كلية التقنيات الصحية والطبية بغداد 2016

بأشراف

أ. د. الاء سعد العتابي
كلية الطب / جامعة كربلاء
أ. د. ضمياء مكي حمزه
كلية الطب / جامعة كربلاء

محرم/ 1443/هـ ايلول /2021 / م