Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Kerbela

College of Medicine

Department of Chemistry and Biochemistry

Serum amyloid A protein as marker of severity of COVID-19 infection in comparison with other acute phase reactant mediators

A Thesis

Submitted to the Council of the College of Medicine, University of Kerbala, as a Partial Fulfillment in the Requirements for the Degree of Master in Clinical Chemistry

By

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2021 A.D. **1443 A.H.**

بسم الله الرحمن الرحيم

﴿قَالَ رَبِّ اشْرَحْ لِي صَدْرِي (٢٥) وَيَسِّرْ لِي أَمْرِي (٢٦)﴾

سورة طه

Acknowledgements

This Master thesis would not have finished without help of many people within Imam Hussein medical city and department of Chemistry and Biochemistry of the College of Medicine/University of Kerbala.

First of all, I would like to express my deepest gratitude to **Dr. Rana Majeed Hameed** and **Dr. Riyadh Abd-Alrasool Hnewa** for providing me with the opportunity to carry out this study. I also thank them for invaluable advice, patience and inspiring guidance throughout this work.

I would like to extend my warmest thanks with my highest appreciation to all staff of the **Al-Hayat Unit and the staff in Laboratory of AL-Hussein Teaching Hospital.** Also, I would like to express my sincere thanks to all **COVID-19 patients** for their donation of blood.

Last but not least, I would like to extend my thanks, gratitude and love to my dear freinds **Aliaa Ahmed, Lina Abbas Hasan,** and **Hawraa Fadhil Abbas** for helping me get the thesis done.

Thanks for all…

Suhaila Rayhaan

Summary

Background: Global health resources have faced huge challenges from the pandemic of coronavirus disease (COVID-19) since December 2019. As on March 1, 2021, the world health organization reported that there were 113,467,303 confirmed cases worldwide with 2,520,550 confirmed deaths. Therefore, it is important to know the best biomarkers which might be associated with disease severity in order to avoid more death cases. The biomarkers that studied in COVID-19 were varied and they had significant role in the diagnosis, treatment, and prediction of the clinical outcomes of patients. Recently, it has been reported that Acute phase reactants have important role for the early diagnosis, treatment, and for monitoring the progression of COVID-19. Currently, there are few reports about the relationship between serum amyloid A and COVID-19 severity. Serum amyloid A is a plasma component and the precursor of amyloid. It is an acute phase protein mainly produced by the liver in response to proinflammatory cytokines that are secreted by the activated monocytes. Therefore, this study aimed to examine the feasibility of employed serum amyloid A protein levels towards COVID-19 severity since it had an ability to promote inflammatory response through activating chemokine and inducing chemotaxis even at a very low concentration.

Material and method: The study was a cross sectional study. It started in 3rd of October 2020 and finished in 15th of September 2021. The medical data of (91) COVID-19 patients admitted to Al-Hayat unit at Imam Hussein Medical City/ Kerbala were collected. COVID-19 cases were divided into three groups based on disease severity (moderate, severe, and critical). The levels of biomarkers were measured for the following parameter: Serum amyloid A was performed using

ELISA Technique; Quantitative measurement of C-Reactive protein was determined through photometric measurement of immunocomplex; D-Dimer in plasma and serum ferritin were performed by sandwich chemiluminescence immunoassay; Complete blood count was done by XP-300™ Automated hematology analyzer Sysmex. The association between biochemical markers and disease severity was evaluated. The efficiency of the predicting value was assessed using receiver operating characteristic curve.

Results: Levels of hematological parameters (Red cell distribution width-coefficient of variation, White blood cells count, Neutrophils percentage, Lymphocytes percentage, and Neutrophils to lymphocytes ratio) were varied based on the severity of the disease. In critical COVID-19 patients, C-reactive protein has shown a significant correlation with neutrophils percentage, lymphocytes percentage, and neutrophils to lymphocytes ratio. Serum ferritin level was significantly higher in critical cases. In severe COVID-19 patients, serum amyloid A level had shown a significant negative correlation with red cell distribution width-coefficient of variation, neutrophils percentage, and neutrophils to lymphocytes ratio, while it had shown a positive correlation with lymphocytes percentage. Ratio of predictive value for the inflammatory indicators (serum amyloid A to C-reactive protein ratio) had shown statistically difference between the patient groups. Serum amyloid A to C-reactive protein ratio was significantly higher in severe and critical groups than those of moderate patients. Receiver operating characteristics curves indicated that the diagnostic performance of serum amyloid A to C-reactive protein ratio in both COVID-19 groups exhibited much better predictive value than other tests.

Conclusion: Levels of both C-reactive protein and serum amyloid A were statistically significant among groups of COVID-19 patients. The combination of these levels were assessed. In both groups COVID-19 patients (severe and critical), serum amyloid A to C-reactive protein ratio demonstrated a high prognosis value of the combined inflammatory indicators.

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List of Abbreviations	
ACE 2: Angiotensin 2 Converting Enzyme	MIG: Monokine induced by gamma interferon
ALT: Alanine transaminase	min: Minute
AP-1: Activator protein 1	mL: Milliliter
ARDS: Acute respiratory distress syndrome	NF-κB: Nuclear factor Kb
AST: Aspartate transaminase	ng: Nanogram
AUC: Area under the roc curve	NK: Natural killers
AUP: Area under PR-curve	NLR: Neutrophils to lymphocytes ratio
B cells: Bone marrow cells	OR: Odds ratio
BNP: Brain natriuretic peptide	PCT: Procalcitonin
BUN: Blood urea nitrogen	PLT: Platelets count
CI: Confidence interval	PSP: Presepsin
CK: Creatine kinase	PT: Prothrombin time
COVID-19: Coronavirus disease 2019	RNA: Ribonucleic acid
CRP: C-Reactive protein	ROC: Receiver operating characteristics
c-Tn-I: Cardiac troponin I	rpm: Rotation per minute
CXCL:C-X-C Motif Chemokine Ligand	rs: Pearson's correlation
ESR : Erythrocyte sedimentation rate	RT-PCR: Reverse transcription polymerase chain reaction
FDP : Fibrin degradation product	SAA : Serum Amyloid A
ICU: Intensive care unit	SARS-COV: Severe acute respiratory syndrome by coronavirus
IFN: Interferon	sCD14-ST : Soluble CD14 subtype
IL: Interleukins	T cells: Thymus cells
IL-2R : Interleukin-2 receptor	TNF-α: Tumor necrosis factor-alpha
IP-10: Inducible protein-10	WBC: White blood cells
LDH: Lactate dehydrogenase	WHO: World health organization
MCP: Monocyte chemoattractant protein	µg: Microgram
MERS-COV: Middle East respiratory syndrome–related coronavirus	µL: Microliter

CHAPTER ONE

Introduction and literature review

1. Introduction

1.1. The 2019 novel coronavirus disease (COVID-19)

The continuing pandemic of severe acute respiratory syndrome by coronavirus 2 (SARS-COV-2) is still to have vary diagnostic and therapeutic problems. In December 2019, SARS-COV-2 was first reported from Wuhan in China, the World Health Organization (WHO) on February 11, 2020 officially named this illness as coronavirus disease 2019 (COVID-19) and the causative virus as SARS-COV-2. It was stated as a pandemic on March 11, 2020 [(1)]. As on March 1, 2021, the WHO reported that there were 113,467,303 confirmed cases worldwide with 2,520,550 confirmed deaths [(2)].

SARS-COV-2 is a member from the family of Coronaviridae, which involves a great number of species able to infect several wild animals, a number of which also infect humans [(3)]. Most coronavirus infections result in mild respiratory infections and may cause roughly 20–30% of common colds in human [(4)]. However, both SARS-COV and Middle East respiratory syndrome–related coronavirus (MERS-COV), which arised in the last two decades, were capable to cause epidemics of severe respiratory diseases. Beta-CoV has three coronaviruses causing more dangerous pathologies. They are significantly different in epidemiology but their genomic and structural are similar. SARS-COV and MERS-COV have a decreased transmissibility but a high mortality, while SARS-COV-2 has a significant high transmissibility and a grade of mortality not yet determined globally [(5)].

Coronavirus has a single-stranded positive RNA, a spheral shape, of roughly 30 Kbp, and a diameter of 80–120 nm. Their envelope comprises the spike –S-, envelope –E, and membrane -M- proteins, and the nucleocapsid -N- inside the virion that coats the nucleic material (RNA) [5], as shown in figure (1-1).

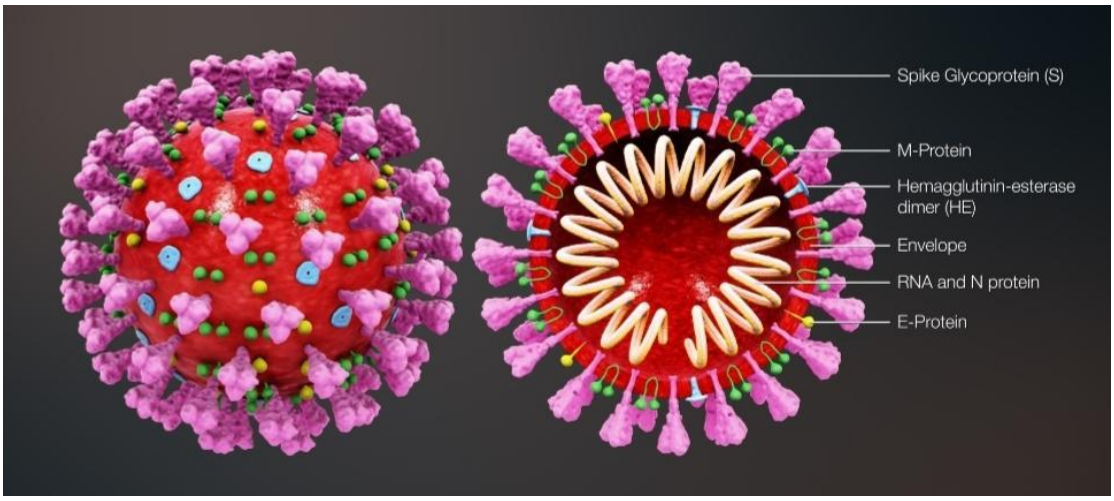

Figure (1-1): Overview of the SARS-COV-2 structure (6).

The genes for the replicases, open reading frame (ORF) 1a,b are located on the genome, from 5' to 3', which take two thirds of the genome and code for the polyproteins pp1a and pp1b [7]. The genes for structural proteins *S, E, M,* and *N* are located toward the 3' end [8]. The best studied of the coronaviruses proteins is *S*- protein, since it includes the receptor binding domain (RBD) for the ligand on the host cell membrane, and also has epitopes recognized by T and B cells, which promote the formation of neutralizing antibodies [9]. The structural *S*- protein is a type I trimeric glycoprotein that protrudes from the virion membrane, giving it the appearance of a crown. *S*- protein is formed by two subunits: S1, or bulb, that contains the RBD [10]; and S2, or stalk, responsible for the fusion of the virion with the host cell membrane [11].

The main receptor for SARS-COV and SARS-COV-2 on the membrane of the target cells is the Angiotensin 2 Converting Enzyme (ACE 2), a metallopeptidase present on the membrane of many cells, including type-I and -II pneumocytes, small intestine enterocytes, kidney proximal tubules cells, the endothelial cells of arteries and veins, and the arterial smooth muscle, among other tissues [(12)]. RBD-ACE 2 binding induces conformational changes on *S*- protein that lead to cleavage of S1 and S2, a process mediated by the transmembrane protease serine 2 (TMPRSS2), allowing S2 to facilitate the fusion of the virus envelope with the cell membrane, thus permitting viral RNA entrance into the cytoplasm of the target cells [(13)]. Thereafter, viral RNA serves as a template for the translation of the polyproteins pp1a and pp1b that are cleaved into 5–16 non-structural proteins (nsp2-nsp9), which in turn induce rearrangement of the membranes to form the vesicles where viral replication and transcription complexes are anchored. The virions are assembled in the ER-Golgi and mature virions are subsequently released by the secretory pathway [(5)].

1.2. Infection Process

The wide spectrum of clinical manifestations found in COVID-19 patients has been associated with risk factors such as gender and age. Diabetes, cardiovascular disease, or diseases, or treatments affecting the immune system result in the highest risk of severe disease and death [(14)]. It is, however, estimated that nearly 80% of all infections remain undocumented, either because patients are asymptomatic or present with very mild symptoms [(15)]. From the epidemiological point of view, these inapparently infected persons may have low viral loads, while still disseminating the virus and can therefore be responsible either for silent

epidemics, leading to infection in more susceptible people who will eventually develop a clinical disease, or for contributing to the establishment of herd immunity [16].

SARS-COV-2 is acquired by exposure to microdroplets present in the exhalates of infected individuals or by contact with viral particles present in contaminated fomites. Once the virus reaches the bronchioles and alveolar spaces, the main targets are the cells of the bronchial epithelium and the type-II ACE 2^+ pneumocytes of the alveolar epithelium. SARS-COV infection induces autophagy [17], detachment of the basal membrane, and inhibition of ACE 2 expression [18], hence allowing angiotensin II to bind the AT1aR receptor, resulting in acute lung damage [19]. Importantly, the main early defence mechanism of the infected cell is the production of type-I and type-III interferon (IFN) and, although coronaviruses are sensitive to their anti-viral effects, they are able to inhibit its induction [18]. The release of large number of virions leads to both infection of neighbouring target cells and viremia, the latter resulting in systemic infection since ACE 2^+ cells are widely distributed in many tissues [12].

1.3. Pathophysiology of SARS-COV-2

The pathophysiology is not yet fully understood; however, the available details are shown in figure (1-2).

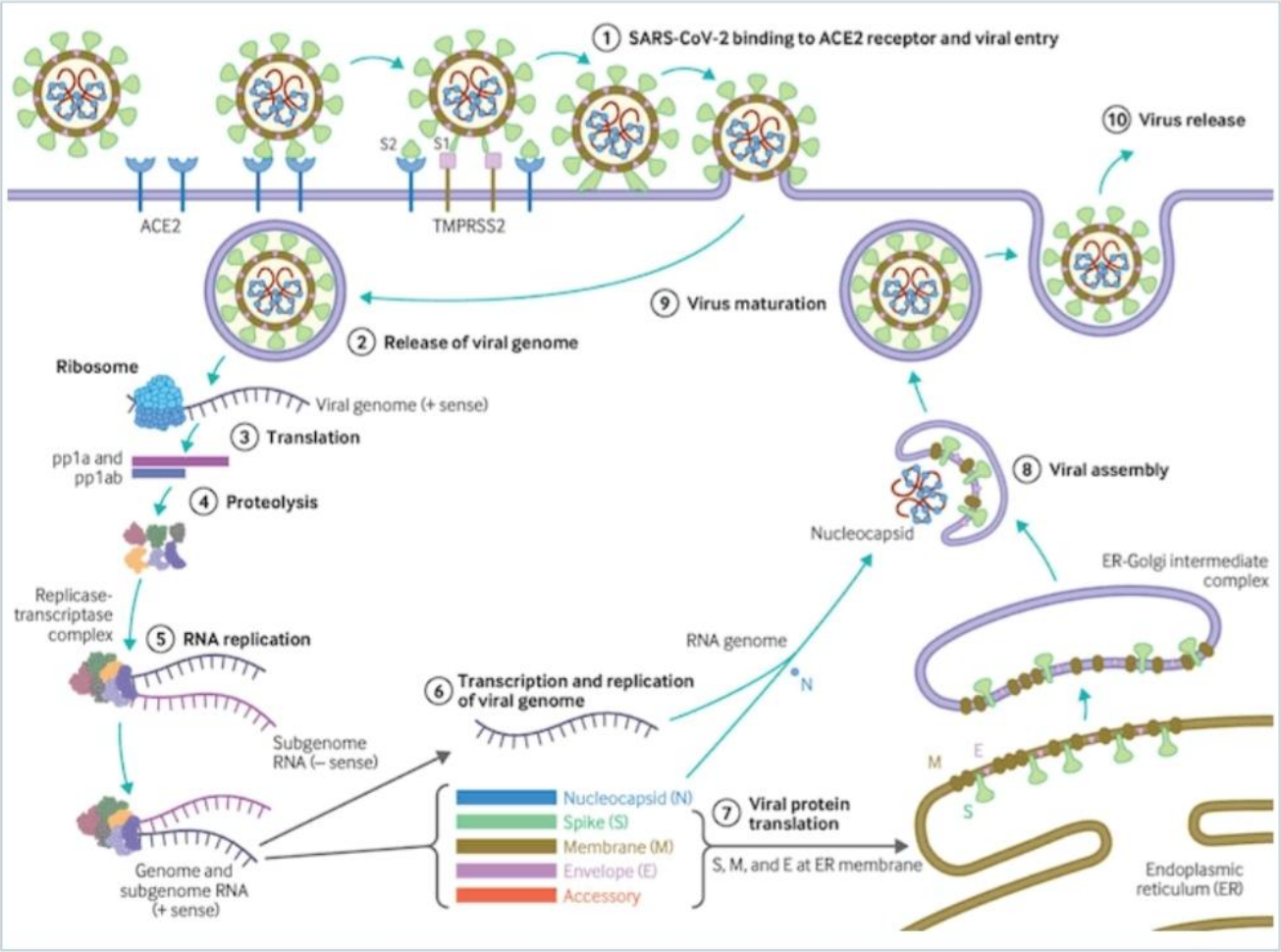

Figure (1-2): Coronavirus replication cycle (20).

Following viral transmission, SARS-COV-2 attaches to the surface of the epithelial membrane of the oral cavity, the mucosal membranes of the conjunctiva or the otic canal. ACE 2 protein, which is highly expressed on multiple human cells including type II alveolar cells (AT2), oral, esophageal, ileal epithelial cells, myocardial cells, proximal tubule cells of the kidneys as well as urothelial cells of the bladder is believed to mediate the internalization of SARS-COV-2 [(12)].

The spike *S*- protein of SARS-COV-2 is cleaved by a cellular enzyme named furin at the S1/S2 site. This cleavage is essential for viral entry to the lung cells [(21)].

The activated *S*- protein is primed by the TMPRSS2 and finally attaches ACE 2 receptors to enter the host cells. The genetic sequence of SARS-COV-2 is homologous with the SARS-COV, and the structure of *S*- protein of these viruses is highly similar. They both use the same receptor to enter the host cell; however, SARS-COV-2 binds ACE 2 receptors with tenfold higher affinity [(22)] .

Experimental studies suggest that the ACE 2/angiotensin (1–7) has a fundamental role in inflammation and signalling pathways contributing to tissue injury [(23)]. The physiological role of ACE 2 is the degradation of angiotensin II and the production of angiotensin (1–7), which counteracts ACE 2 [(24)]. Following the viral replication in the host cell, downregulation of ACE 2 inhibits breakdown of angiotensin II into angiotensin (1–7). Disturbance in ACE 2/angiotensin (1–7) axis explains particular clinical features of COVID-19, such as hypokalemia, vasoconstriction [(25)], and development of acute respiratory distress syndrome (ARDS) [(26)]. Interestingly, the extent of ACE 2 expression in the gastrointestinal tract (GIT), cardiovascular, genitourinary, endocrine (pancreas), and genitourinary (testis) systems is extremely higher than that in the predominant target of the virus, the respiratory system [(12)]. Evidence has not shown the presence of SARS-COV-2 in some organs enriched with ACE 2 receptors, such as expressed prostatic secretion of COVID-19 patients [(27)]. Hence, there is no correlation between the virus infectivity and the level of ACE 2 expression.

1.4. Immune response in COVID-19

Accumulating evidence has suggested that inflammatory responses play a critical role in the progression of COVID-19 [(28)]. The strength of the immune system determines the disease status and prognosis [(29)].

1.4.1. The Innate Immune Response:

During viral infections, after viruses enter the host cells, they are recognized by pattern recognition receptors (PRRs) such as toll-like receptors 7 (TLR7) and toll-like receptors 8 (TLR8) in the case of single-stranded RNA viruses, retinoic acid-inducible gene-I like receptors “RIG-I-like” (RLRs), and nucleotide binding and oligomerization domain “NOD-like" receptors (NLRs), all expressed by epithelial cells as well as by local cells of the innate immune response, such as alveolar macrophages [(5)]. Upon ligand binding, PRRs recruit adaptor proteins which activate crucial down-stream transcription factors, including interferon regulatory factor (IRF), nuclear factor κB (NF-κB), and activator protein 1 (AP-1), resulting in production of the Type-I and -III antiviral Interferons and different chemokines [(30)]. These chemokines attract more innate response cells [polymorphonuclear leukocytes, monocytes, natural killer (NK) cells, and dendritic cells (DCs)], which also produce chemokines, such as monokine induced by gamma interferon (MIG), inducible protein 10 (IP-10), and monocyte chemoattractant protein 1 (MCP-1), capable of recruiting lymphocytes, which in turn, will recognize the viral antigens presented by DCs [(31)].

Recent publications highlight the initial phases of the SARS-COV-2 infection, compared to other coronavirus, and their effects on subsequent immune and inflammatory responses. Chu *et al,* [(32)] compared the *in vitro* infection of human lung explants with SARS-COV and SARS-COV-2 and demonstrated that both viruses can equally infect type-I and -II pneumocytes, plus alveolar macrophages, although SARS-COV-2 had a better capacity to replicate in pulmonary tissues. Interestingly, while SARS-COV induced the expression of IFN-I, IFN-II, and IFN-III, SARS-COV-2 failed to induce any such immune mediators and was also less

efficient in inducting other cytokines. SARS-COV induced the production of the 11 cytokines studied, while SARS-COV-2 induced only five (IL-6, MCP1, CXCL1, CXCL5, and CXCL10/IP10).

1.4.2. Adaptive Immune Response:

The transition between innate and adaptive immune responses is critical for the clinical progress of SARS-COV-2 infection. It is at this crucial moment when immune regulatory events, still poorly understood, will lead to the development of either a protective immune response or an exacerbated inflammatory response[33]. The protective response is T cell dependent, with CD4 helping B cells, geared toward the production of specific neutralizing antibodies, and cytotoxic CD8 cells capable of eliminating infected cells. It is worth noting that 80% of the infiltrating cells in COVID-19 are CD8 [29].

Contrariwise, a dysfunctional response which is unable to inhibit viral replication and elimination of the infected cells, may result in an exacerbated inflammatory response leading possibly to a cytokine storm, manifested clinically by severe ARDS and systemic consequences, such as disseminated intravascular coagulation (DIC).In a SARS-COV primate model of infection, Clay *et al,* [34] showed that the virus replicated in the lungs until Day 10 post-infection; but, surprisingly, lung inflammation was more intense after virus clearance, reaching its peak at Day 14 and remaining so until Day 28. These results suggest that an early phase dependent on virus replication does occur, while a later viral-independent, immune-dependent phase seems to be accompanied by an exacerbated inflammatory component. The viral-independent phase has been explained by the inflammatory reaction secondary to ACE 2 inhibition or by an autoimmune

phenomenon due to the epitope spreading caused by prolonged tissue destruction [35]. It remains to be demonstrated whether a similar two-phase course also occurs in COVID-19. Although T and B cells, macrophages, and DCs do not express ACE 2, some reports suggest that DC-SIGN may serve as a trans receptor for SARS-COV on DCs, which even when not infected may transfer the virus to other susceptible cells [36].

Recently, Vandakari and Wilce [37] reported that CD26, an aminopeptidase involved in T cell activation, may bind to the *S* protein of SARS-COV-2, resulting in a non-productive T cell infection. Wang et al, [38] reported that CD147, a protein of the immunoglobulins superfamily that induces the metalloproteinases of the extracellular matrix, binds to the S1 domain and facilitates viral entrance into host cells. The significance of non-productive T cells infection is not clear; however, it is tempting to speculate that it may be related to the lymphopenia found in patients with SARS, MERS, and COVID-19 [39]. The binding of SARS-COV-2 *S*- protein to molecules like CD26 and CD147, which participate in T cell activation, would suggest that a non-productive T cell infection may result in activation-induced cell death (AICD). MERS-COV has been reported to induce T cells apoptosis [40], and there is evidence that T cells are functionally exhausted in patients with severe COVID-19 [41].

1.4.3. The Antibody Response:

Multiple evidences support that the humoral response, mainly antibodies against the *S*- protein, blocks virus attachment to susceptible ACE 2+ cells [42]. However, there are still many questions regarding the significance of antibodies against the different viral proteins, and the cross reactivity of antibodies against

other highly prevalent alpha- and beta-coronavirus, although it seems that cross reactivity occurs mostly within the beta-coronaviridae [43], particularly between SARS-COV and SARS-COV-2 that share 90% of the amino acid sequence in S1 [9].

IgM and IgA antibodies can be detected early during the first week of symptom onset, whereas IgG can be detected at around 14 days after the initiation of symptoms [44]. An intriguing phenomenon that worries many clinicians and researchers is the Antibody-Dependent Enhancement (ADE), which could be linked to the severity of coronavirus infections and could possibly create difficulties with new vaccines [45].

Ho et al, [46] studied the antibody response in SARS, found that patients with more severe clinical courses had earlier and higher antibody responses, and hypothesized that earlier responders may have had, during the acute phase, cross-reacting antibodies with non-SARS coronaviruses. Jaume et al,[47] and Yip et al, [48] demonstrated that anti-S antibodies, while inhibiting viral entrance in permissive cells, potentiated the infection by binding to IgG Fc receptor-II positive (FcγRII+) cells, like B cells and macrophages. Thus, IgG anti-S antibodies bound to FcγRII on mononuclear phagocyte membranes enhance viral entrance through canonical viral-receptor pathways, as recently shown for MERS-CoV, thereby activating these cells and inducing the production of proinflammatory cytokines [49].

1.5. Severity of disease

WHO determined the clinical features for each stage of disease (mild, moderate, severe, and critical). The features of each stage are summarised in table (1-1):

Table(1-1):World Health Organization: COVID-19 disease severity (50)

Mild Cases

- Symptomatic patients: COVID-19 without evidence of hypoxia or pneumonia.
- Common symptoms: fever, cough, fatigue, anorexia, dyspnoea, and myalgia.
- Non-specific symptoms: sore throat, nasal congestion, headache, diarrhoea, nausea/ vomiting, and loss of smell/taste.
- Older patients and immunosuppressed patients may have atypical symptoms (e.g., fatigue, reduced alertness, reduced mobility, diarrhoea, loss of appetite, delirium, absence of fever.
- Symptoms due to physiological adaptations of pregnancy or adverse pregnancy events (e.g., dyspnoea, fever, gastrointestinal symptoms, fatigue) or other diseases (e.g., malaria) may overlap with COVID-19 symptoms.

Moderate Cases

- Adolescent or adult: clinical signs of pneumonia (i.e., fever, cough, dyspnoea, fast breathing) but no signs of severe pneumonia, including blood oxygen saturation levels (SpO_2) ≥90% on room air.
- Children: clinical signs of non-severe pneumonia (i.e., cough or difficulty breathing plus fast breathing and/or chest indrawing) and no signs of severe pneumonia. Fast breathing is defined as:
  1. <2 months of age: ≥60 breaths/min.
  2. 2-11 months of age: ≥50 breaths/min.
  3. 1-5 years of age: ≥40 breaths/min.

Severe Cases

- Adolescent or adult: clinical signs of pneumonia (i.e., fever, cough, dyspnoea, fast breathing) plus one of the following:
  1. Respiratory rate >30 breaths/min.
  2. Severe respiratory distress.
  3. SpO_2 <90% on room air.
- Children: clinical signs of pneumonia (i.e., cough or difficulty in breathing) plus at least one of the following:
  1. Central cyanosis or SpO_2 <90%
  2. Severe respiratory distress (e.g., fast breathing, grunting, very severe chest indrawing)
  3. General danger signs: inability to breastfeed or drink, lethargy or unconsciousness, or convulsions
  4. Fast breathing (<2 months: ≥60 breaths per minute; 2-11 months: ≥50 breaths per minute; 1-5 years: ≥40 breaths per minute).
- While the diagnosis can be made on clinical grounds, chest imaging may assist in diagnosis and identify or exclude pulmonary complications.

Critical Cases

- Presence of acute respiratory distress syndrome (ARDS), sepsis, or septic shock.
- Other complications include acute pulmonary embolism, acute coronary syndrome, acute stroke, and delirium.

1.6. Risk factors for COVID-19

Characterisation of people at high risk of COVID-19 mortality is important for prioritisation of interventions. Several risk factors are summarised in table (1-2):

Table 1-2: Risk factors for COVID-19

Strong Risk factors	
• Residence/work/travel in location with high risk of transmission [51] • Older age [52] • Residence in a long-term care facility [53] • Male sex [54] • Ethnicity [55] • Presence of comorbidities [56] • Autoimmune disease [57] • Hypertension [58] • Obesity [59]	• Diabetes [60] • Chronic respiratory disease [61] • Chronic kidney disease [62] • Malignancy [63] • Sickle cell disease [64] • Solid organ transplant [65] • Cerebrovascular disease [66] • Dementia [67] • Chronic liver disease [68] • Metabolic dysfunction-associated fatty liver disease [69] • Surgery [70]
Weak Risk factors	
• Vitamin D deficiency [71] • Air pollution [72] • Climate and latitude [73] • ACE inhibitor/angiotensin-II receptor antagonist use [74] • Statin use [75] • Proton-pump inhibitor use [76] • HIV infection [77] • Down's syndrome [78] • Blood group A [79] • Gut dysbiosis [80]	

1.7. Diagnosis of COVID-19 Disease

1.7.1. Molecular Testing

World health organization recommended real-time reverse transcription polymerase chain reaction (RT-PCR) for SARS-COV-2 in patients with suspected infection whenever possible. A positive RT-PCR result confirms SARS-COV-2 infection. If the result is negative, and there is still a clinical suspicion of infection (e.g., an epidemiological link, typical x-ray findings, or absence of another aetiology), the test is repeated. If the second test is negative, serological testing is considered [(81)]. However, RT-PCR may generate false-negative results due to insufficient swab samples, which are collected from the oropharynx or nasopharynx [(82)].

False negative RT-PCR results may delay the diagnosis and the early treatment of patients, especially those with COVID-19 pneumonia, increasing the risk of community transmission [(82)]. Therefore, clinicians have begun to request chest CT scans to evaluate the lungs, especially in patients with suspected pneumonia, for the early diagnosis and treatment of the patients [(82)].

1.7.2. Serological tests

Commercial and non-commercial tests measuring binding antibodies [Total immunoglobulins (Ig), IgG, IgM, and/or IgA in different combinations] utilizing various techniques including lateral flow immunoassay (LFI), enzyme-linked immunosorbent assay (ELISA), and chemiluminescence immunoassay (CLIA) have become available. A number of validations and systematic reviews on these assays have been published [(83)].

The performance of serologic assays varies widely in different testing groups (such as in patients with mild versus moderate-to-severe disease as well as in young versus old), timing of testing and the target viral protein. Understanding these performance variations will require further study. Antibody detection tests for coronavirus may also cross-react with other pathogens, including other human coronaviruses or with pre-existing conditions (e.g. pregnancy, autoimmune diseases) and thus yield false-positive results [(84)].

1.8. Biomarkers that used in follow-up of COVID-19 patients

1.8.1. Hematological parameters:

Around the seventh to the fourteenth day of infection, the COVID-19 begins to impact organs with higher SARS-COV-2 cell receptor expression, the ACE 2 [(85)], with characteristic clinical symptoms and expressive elevation in the concentrations of cytokines and inflammatory mediators [(29)], more expressive hematological alterations are notable, specifically a significant decrease in the lymphocytes count. A decreased lymphocyte to leukocyte count ratio has already been reported indicating severe disease and/or fatal outcomes [(86)]. Similarly, increased neutrophil to lymphocyte and neutrophil to platelets ratio may be indicative of myocardial injury and increased mortality [(87)]. Therefore, it is important to monitor the hematological parameters in order to try to assess the progression and prognosis of COVID-19. The useful hematological parameters that helped in monitoring COVID-19 patients are:

A. **Hemoglobin:** Higher mortality was associated with anemia, and the need for intensive care unit (ICU) admission and mechanical ventilation were predicted by a higher (ferritin to transferrin) ratio [(88)].

B. Lymphocytes: Lymphopenia on admission is associated with three-fold risk of worse outcome, in younger as compared to older patients (Lymphopenia was defined as lymphocyte number less or equal to 1,100 cells/µl) [(89)]. Severe infection was distinguished by marked decrease in the absolute number of circulating CD4+ cells, CD8+ cells, B cells and NK cells. Plasma cells are remarkably elevated [(90)]. The highest levels of inflammatory biomarkers promptly associated with the reduction in CD8 T-cells, an effect that was not noted with CD4 cells [(91)].

C. Neutrophils: Patients with greater percentage and absolute count of neutrophils required admission to the ICU [(91)].

D. Eosinophils: Serum eosinophil-derived neurotoxin (EDN-1) and airway and a low percentage of eosinophils can be a probable biomarker of COVID-19 pneumonia [(92)].

E. Platelets: Thrombocytopenia was linked with other coagulation biomarkers and high risk of mortality [(93)] .

F. Composite Hematological Markers: IL-2R levels correlated positively with the other cytokines and negatively with lymphocyte number. An elevated IL-2R to lymphocytes ratio was discriminative of severe and critical disease. In fact this ratio was superior to other markers for differentiation of critical infection. The ratio was significantly decreased in recovered patients, but further increased in patients who deteriorated, thus correlating with the outcome [(94)]. Zheng *et al,* devised a score based on the neutrophil, lymphocyte and platelet counts, with an "NLP score" of >6, predicting progression to severe disease [(95)]. A high neutrophil to lymphocyte ratio (NLR) at admission can be a good surrogate marker for diagnosis of COVID-19. A rising NLR can also be used as a

prognostic marker for predicting poor outcomes [96]. Another prognostic marker the lymphocyte to CRP ratio (LCR), used in several types of cancers, may also be helpful. A meta-analysis on six studies concluded that a rise in the NLR and decline in LCR correlates with the severity of COVID-19 [97]. Specifically, a low LCR at presentation was seen to predict ICU admission and need for invasive ventilation.

1.8.2. Inflammatory biomarkers:

In critical infection, patients have elevated levels of inflammatory biomarkers, indicating a probable immune dysregulation [98]. In cases with severe COVID-19 illness compared with cases with mild infection, higher serum levels of chemokines (IL-8) and proinflammatory cytokines (IL-1,TNF-α, and IL-6) were reported, analogous to conclusions seen in MERS and SARS and suggest a function for hyperinflammatory responses in pathogenesis of COVID-19 [86]. A cytokine storm, the excessive immune response emerges from overproduction of early response proinflammatory cytokines such as TNF-α, IL-6, and IL-1β. It can cause ARDS, multi-organ failure, and finally death.

United Kingdom researchers recommend that all patients with severe COVID-19 should be screened for hyperinflammation using laboratory trends (e.g. Elevated ferritin, low platelet counts, or elevated erythrocyte sedimentation rate) to identify patients that can be treated with immunosuppressive therapies such as steroids, intravenous immunoglobulin, selective cytokine blockade or JAK inhibition [28]. Based on a results of study of 4,000 cases hospitalized in New York City, CRP, D-dimer, and ferritin were strongly correlated with critical disease. NYU Grossman School of Medicine researchers recommend routine measure of

inflammatory indicators during COVID-19 hospitalization [99]. On admission, Procalcitonin (PCT) is usually normal as in other viral diseases, but it may elevate in COVID-19 cases who admitted to ICU [100]. Elevated PCT was correlated with a nearly five-fold greater risk of severe COVID-19 infection [101].

1.8.3. Kidney injury biomarkers:

Renal impairment state was common reported in COVID-19 patients [102], and acute kidney injury (AKI) frequently progressed during hospitalization for the infection and was correlated with in-hospital mortality. Monitoring kidney function in COVID-19 is recommended especially in patients with elevated plasma creatinine [103] .

1.8.4. Cardiac biomarkers:

COVID-19 infections are associated with increased levels of cardiac biomarkers due to myocardial injury probably associated with infection-induced myocarditis and ischemia [104]. In multivariable adjusted models, cardiac injury is significantly and independently associated with mortality. Similarly, elevated troponin levels due to cardiac injury are associated with significantly higher mortality [87]. Severe COVID-19 infections are also potentially associated with cardiac arrhythmias at least in part due to infection-related myocarditis. Myoglobin, CK-MB, c-Tn-I, and NT-proBNP are the most common myocardial injury specific biomarkers and elevated to varying stages, particularly in severe and critical COVID-19 infection. Additionally, the elevated levels were correlated with greater mortality [105]. To predict mortality, cut-offs of these parameters have been found to be much lower than for ordinary cardiac disease [106]. Natriuretic

peptides and troponin have been studied for risk stratification, to help in report making with respect to rationalistic employment of echocardiography (ECG) and aggressive treatments, and prognostic [107].

1.8.5. Coagulation parameters:

D-dimer level associates with disease severity and is dependable predictive biomarker for in-hospital mortality [108]. High D-dimer (Almost ≥0.5 mg/L) was reported in 46% of patients, 43% with non-severe, but 60% with severe infection. Prothrombin time (PT) was slightly prolonged in non-survivors at admission in comparison to survivors and in those who required critical care support in comparison to the non-ICU group [109].

The International Society of Thrombosis and Haemostasis (ISTH) recommends measurement of D-dimers, PT, and platelet count in all patients with COVID-19 infection [98]. Patients who have elevated D-dimers (3-4 fold elevation) should be admitted to hospital even in the absence of other severity determinants. Furthermore, the ISTH shows that serum fibrinogen biomarker may be valuable for diagnosis of DIC [110].

1.9. Biomarkers that associated with disease severity

The dynamic changes in levels of biomarkers may assist in predicting disease course, prognosis, and clinical outcomes. Table (1-3) illustrates a different uses of biomarkers in COVID-19. Higher disease severity [111] and hospitalization [112] in COVID-19 patients have been associated with lymphopenia and elevated levels of CRP, LDH, ferritin, and neutrophils. In severe and fatal illness compared to those with non-severe illness and survivors, there were significantly increased WBC

count, and lower platelet and lymphocyte counts [113]. The elevated CRP and WBC levels in severe COVID-19 cases may suggest accompanying bacterial infection. In severe infection of COVID-19, high concentrations of AST, ALT, and creatinine have been shown a higher risk for impaired kidney and liver function. Inflammatory biomarkers such as IL-6 and IL-10, of kidney, liver function, coagulation measurements and of heart injury were also significantly increased in severe and fatal COVID-19 cases. Lymphocyte count, WBC count, platelet count, serum ferritin and IL-6 should be closely monitor as biomarkers for probable progression to critical disease [114].

A retrospective comparison of the hematological parameters between mild and severe cases showed that IL-6 and D-dimer were closely related to the occurrence of severe COVID-19 in adults, and their combined detection had the highest specificity and sensitivity for early prediction of the severity of COVID-19 [113]. A similar result was reported from a pooled analysis of nine studies involving 1,779 COVID-19 patients, in which low platelet count was associated with increased risk of severe disease and mortality in patients and served as a clinical indicator of worsening illness during hospitalization, the researchers said [115].

Mortality in several studies has been associated with lymphopenia and elevated D-dimer [116]. In patients who died compared with those who recovered, there were elevated levels of CK, creatinine, NT-proBNP, c-Tn-I, LDH, ALT, AST, and D-dimer [99].

Table (1-3): Types of biomarkers in COVID-19.

1)	Predictors of SARS-COV-2 RT-PCR results	Low WBC count; High [CRP, Neutrophils count, LDH, AST, and ALT]; Hypoalbuminaemia (117).
2)	Hematologic and coagulation biomarkers	WBC: variable, mostly ↑ (118); ↓ Lymphocyte count and/or % (117); ↓CD3+/CD4+/CD8+ T-lymphocyte count and/or % (119); Neutrophil count and/or %:mostly ↑, rarely ↓ (120); ↑NLR (121); Monocyte count and/or % : variable (122); ↓ Eosinophil count and/or % (123); ↓ Platelet count (124); ↑ PT (125); ↑ D-dimer (126);↑ Fibrinogen and/or FDP(127).
3)	Inflammatory and biochemical biomarkers	High: CRP (128); Ferritin (119); SAA (129); Procalcitonin (130); ESR (131); PSP or sCD14-ST (132); Il-2 and/or IL-2R (133); IL-6 (134); IL-8 (135); IL-10 (133); TNF-α (136); IP-10 and MCP (134); LDH (120); CK and/or CK-MB (137); c-Tn-I (138); BNP (139); Myoglobin (140); Bilirubin (total and/or direct) (141); BUN (117); Creatinine (142); Cortisol (143). Low: Albumin and/or prealbumin (144) and IFN-γ (136).
4)	Predictors of disease severity	*Severe: High [CRP, ferritin, LDH, and ALT] (136). *Severe or critical: – ↓ [Lymphocytes, prealbumin, and albumin]; ↑ [WBC count, neutrophil count, CRP, and LDH] (145). – ↑ [SAA, NLR, PT, D-dimer, FDP, and inflammatory cytokines IL-2R, TNF-α, and IL-10] (146). *Critical: lymphopenia, ↓ eosinophil count, thrombocytopenia, ↑ CRP, ↑ procalcitonin, ↑ IL-6 and IL-10, ↑ liver enzymes, ↑ total bilirubin, ↓ renal function, hypercoagulable state higher, and higher incidence of complications (118). *Poor recovery group (147): – Long viral shedding which detect by serial RT-PCR test and anti–SARS-COV-2 IgM test . – ↑ [ESR, CRP, ferritin, and IL-4]. *Lymphocytopenia and age reported to have the most significant role in determination of disease severity (135).
5)	Predictors of case mortality	– ↑ [AST, ALT, CK-MB, myoglobin, BUN, and creatinine] (148). – Multiple biomarkers: CRP, N-terminus pro-Brain natriuretic peptide, myoglobin, D-dimer, procalcitonin, CK, and c-Tn-I (140).

1.9.1. Acute phase reactants (APRs)

The acute-phase response to inflammation and a tissue injury is accompanied by an elevation in the hepatic biosynthesis of plasma proteins known as acute phase reactants (APRs) [149]. Acute phase response takes place, by changes in a heterogeneous group of proteins which consists of around 30 proteins in response to bacterial infection, trauma, myocardial infarction, collagen tissue disorders which result in the production of IL-1, IL-6, TNF-α [150]. Despite its name, the acute phase response accompanies chronic as well as acute inflammatory states and is associated with a wide variety of disorders, including infection, trauma, infarction, inflammatory arthritides, other systemic autoimmune and inflammatory diseases, and various neoplasms. Less marked changes may occur in response to metabolic stresses [151].

Acute phase reactants are defined as those proteins whose serum concentrations increase or decrease by at least 25 percent during inflammatory states [152]. Changes in the levels of APRs largely reflect altered production by hepatocytes, resulting primarily from the effects of cytokines produced during the inflammatory process by macrophages, monocytes, and a variety of other cells.

The major inducer of most APRs is IL-6 [153]. Some of the other major cytokines relevant to the acute phase response are IL-1β, TNF-α, and IFN-γ. These cytokines also suppress the synthesis of albumin, termed a "negative APR" because its levels decrease with inflammation [154]. Combinations of cytokines can have additive, inhibitory, or synergistic effects, and patterns of cytokine production differ under various inflammatory conditions [155].

Acute phase reactants have significant role for the precocious diagnosis, treatment, and monitoring of COVID-19 progression. The acute phase response to

a tissue injury and inflammation is accompanied by a dramatic increase in the hepatic synthesis of plasma proteins known as APRs. The most important APRs are the ESR, CRP, ferritin, PCT, LDH, fibrinogen, and troponin [156]. The acute phase reactants that studied in COVID-19 are:

1.9.1.1. C-reactive protein (CRP)

C-reactive protein and many other APRs can influence multiple stages of inflammation, and CRP has both proinflammatory and antiinflammatory actions, although the primary effect may be anti-inflammatory [157]. CRP can promote the recognition and elimination of pathogens and enhance the clearance of necrotic and apoptotic cells [158]. Proinflammatory effects of CRP include activation of the complement system and the induction in monocytes of inflammatory cytokines and tissue factor [159] and shedding of the IL-6 receptor [160]. As a result, the CRP response to tissue injury may worsen tissue damage in some settings [161].

CRP levels are positively-correlated with computed tomography (CT) scan severity scores in COVID-19 patients [112]. Zhu *et al,* [162] and Young *et al,* [163] reported that CRP was higher than the normal levels range, this elevation of CRP was related to COVID-19 infection associated with acute inflammatory pathogenesis during which released of multiple cytokines and there was associated with severity of disease COVID-19 patients. When inflammation and/or tissue injury is resolved, CRP level falls up to 86%, making it a good biomarker in severe infection of COVID-19 patients. In severe infection, CRP level increased more than non-severe or mild infection [109].

1.9.1.2. Ferritin

Ferritin is an ubiquitous protein which represents not only a crucial element of iron homeostasis regulation but also the most used biomarker of iron deficiency [(164)]. Moreover, serum ferritin is a well-known acute-phase protein reflecting the degree of acute and chronic inflammation and compelling evidence suggest a potential active role of ferritin in chronic inflammatory diseases [(165)]. Accordingly, several studies report a direct association between serum ferritin levels and chronic inflammation of mild degree [(166)]. Serum ferritin levels elevated more than the normal range in patients SARS COVID-19 [(167)]. Pro-inflammatory effects immune dysregulation, suggesting that ferritin levels increased and non-stop, due to contributing to the cytokine storm [(168)] hyperferritinemia might be a critical factor influencing the severity of COVID-19.

1.9.1.3. Tumor necrosis factor-alpha (TNF-α)

TNF-α is a highly pleiotropic cytokine that affects practically any type of cell. It triggers cellular responses reaching from the induction of inflammatory gene expression programs, over the stimulation of cellular proliferation and differentiation to the activation of cellular suicide programs such as apoptosis and necroptosis [(169)]. The mean of TNF-α was elevated in COVID-19 patients compared to the healthy people [(167)]. There were highly statistically significant differences and it was agreed with Huang *et al,*[(100)] that showed elevated serum TNF-α in severe infection of COVID-19 patients. A poor prognosis in patients with COVID-19 was associated with overproduction of TNF-α [(170)].

1.9.1.4. Interleukin-6 (IL-6)

Interleukin-6 is a pleotropic cytokine produced in response to tissue damage and infections [171]. Multiple cell types including fibroblasts, keratinocytes, mesangial cells, vascular endothelial cells, mast cells, macrophages, dendritic cells, and T and B cells are associated with the production of this cytokine [172]. Biological activities affected by production of IL-6 include: Control of the differentiation of monocytes into macrophages by regulating the expression of macrophage colony-stimulating factor[173], increasing B-cell IgG production by regulating the expression of IL-21 [174], negative regulation of dendritic cell maturation by activation of the STAT3 signaling pathway [175], as well as the promotion of the T helper type 2 (Th2) cells response by inhibiting Th1 polarization [176]. IL-6 is the most common type of cytokines that released by activated macrophages elevated sharply in severe infection of COVID-19 [177]. Recent study reported that the increase of IL-6 was in relationship with symptoms severity [178]. Cytokine storm and progressive disease correlated with high expression of IL-6 in COVID-19 patients [179].

1.9.1.5. Serum amyloid A (SAA)

SAA proteins are a family of apolipoproteins associated with high-density lipoprotein (HDL) in plasma. Different isoforms of SAA are expressed constitutively (constitutive SAAs) at different levels or in response to inflammatory stimuli (acute phase SAAs). These proteins are produced predominantly by the liver [180]. SAA was also found as one of the major acute phase proteins that are produced in large quantities by hepatocytes and released to blood circulation in response to trauma, infection, late-stage malignancy and

severe stress [149]. Extending from these early findings, increased levels of SAA were found both in plasma and in injured and inflammatory tissues. A large body of literature reports SAA as a biomarker in a variety of diseases ranging from acute inflammation, chronic inflammation, type 2 diabetes, malignancy and postsurgical complications [181].

Several forms of SAA have been identified in serum. These include acute phase (SAA 1 and SAA 2) and constitutive (SAA 4) isoforms, allelic variants, and posttranslational modifications of these gene products [182]. Acute phase SAA proteins (SAA 1 and SAA 2) are apolipoproteins, primarily associated with specific high-density lipoprotein (HDL), and are also expressed extrahepatically (eg, synovial membrane) in the absence of HDL [183]. Expression of SAA 1 and SAA 2 is induced by a number of factors, particularly IL-6, but also IL-1, TNF, lipopolysaccharide (LPS), and several transcription factors, notably including SAA-activating factor (SAF)-1 [184].

1.9.1.5.A. SAA structure:

SAA 1 and SAA 2 proteins are 104 amino acids in length and their sequences are for more than 90% identical to each other. Within SAA 1, the variants differ in only a few amino acids: SAA 1α contains valine (V) and alanine (A) at positions 52 and 57, respectively, whereas SAA 1β is characterized by alanine (A) at position 52 and valine (V) at position 57 [185]. Both positions 52 and 57 are occupied by alanine (A) in SAA 1γ [186]. Beach *et al,* [187] described the same SAA 1β variant as Parmelee *et al* [185], but reported aspartic acid (D) at position 72 instead of glycine (G), as was already observed by Kluve-Beckerman *et al* [188].

Since the other SAA 1 and SAA 2 variants all contain glycine (G) at position 72, the SAA 1β protein described by Parmelee and co-workers was adopted. The two allelic variants of SAA 2 are identical, except for the substitution of histidine (H) for arginine (R) at position 71 in SAA 2β. SAA 2α and SAA 2β have seven and eight amino acid substitutions, compared with SAA 1α, resulting from the 12 and 13 nucleotide differences in the α- and ß-allelic variants of the SAA 2 gene. Besides the same two amino acid substitutions at positions 52 and 57 as in SAA 1β, SAA 2α and -β both contain the following amino acid substitutions: aspartic acid (D) for asparagine (N) at position 60, phenylalanine (F) for leucine (L) and threonine (T) at positions 68 and 69, respectively, glutamic acid (E) for lysine (K) at position 84 and lysine (K) for arginine (R) at position 90. SAA 2β also contains arginine (R) instead of histidine (H) at position 71. SAA 1 and SAA 2 isoforms lacking their amino terminal arginine (R) and/or serine (S), coexisting with the full length mature proteins, are described [(189)]. These processed forms originate from proteolytic cleavage of the proteins, which occurs mainly extracellularly [(190)].

Tomita *et al,* [(191)] reported for the first time the detection of the protein SAA 3 via a specific sandwich ELISA in conditioned media from one of the human mammary gland epithelial cell lines, previously used by Larson *et al* [(192)]. Indeed, SAA 3 is the main SAA form being expressed extrahepatically in non-human mammals [(193)].

SAA protein is constitutively present in the blood and differs thereby from SAA 1 and SAA 2, which are mainly induced when inflammation occurs. Therefore, SAA 4 is also denominated “constitutive SAA” or “C-SAA”, whereas SAA 1 and SAA 2 are named “acute phase SAA” or “A-SAA”. The last two amino acids from the inserted octapeptide in SAA4 are part of an N-linked glycosylation site, consisting

of the sequon asparagine (N) – serine (S) – serine (S) [194]. This tripeptide is responsible for the coexistence of unglycosylated (14 kDa) and glycosylated (19 kDa) forms of SAA 4 in serum.

1.9.1.5.B. Functions of SAA:

In humans, SAA is one of the most dynamic components with a potential increase in circulating concentration of more than 1000-fold, implying that it has important functions in the inflammatory response. Nonetheless, the exact contributions SAA proteins play in host defence remain somewhat opaque [195]. SAA proteins may increase the affinity of HDLs for macrophages and adipocytes during the acute phase response, a property termed "reverse cholesterol metabolism" [196]. Other properties include extracellular matrix binding [197]; opsonization of Gram-negative bacteria [198]; chemoattractant activity for monocytes, neutrophils, and lymphocytes [199]; induction of the release of proinflammatory cytokines from neutrophils [200]; and platelet effects [201].

Pro- and antiinflammatory effects of SAA vary with regard to the blood pool of hepatically derived acute phase SAA; local effects of tissue- and macrophage-derived SAA; the use of recombinant SAA for many in vitro studies; and whether SAA is bound to HDL or delipidated [202] .

1.10. SAA as predictor for COVID-19 severity

Clinical symptoms in patients with COVID-19 usually appear after 36 to 48 hour of infection. SAA gradually elevates and at 3–4 day post infection, the elevation reaches to peak. At the recovery phase, SAA level was continuously diminished and the rate of diminution was faster than that of CRP [203]. The CRP

and SAA levels were higher in the severe group, and SAA was more efficient in predicting severe COVID-19 than CRP. For recovered patients, the CRP and SAA levels were negatively correlated with treatment days, and SAA had a high prediction efficiency for the recovery of COVID-19. These results indicated that SAA may be considered to be a biomarker for predicting the severity and recovery of COVID-19. Therefore, SAA can be used for early warning of a poor outcome from COVID-19, as well as monitoring the recovery process, which has important clinical value [(204)].

Patients with severe COVID-19 had significantly elevated level of SAA, suggesting SAA could be used as a good biomarker for monitoring of the respiratory diseases progression [(205)]. Huang *et al,*[(100)] suggested that patients infected with COVID-19 had a large amount of IL-1β, IFN-γ, IP-10, MCP-1 and other cytokines present in system, leading to the activation of Th1 cell. Critically ill COVID-19 patients may have more IL-6, IL-1β, MIP-1, MCP-1, TNF-α, and other cytokines expressed in compared with mild patients, which boost hepatic cells to produce SAA. Li *et al,*[(206)] analysed the dynamic changes of SAA in patients with COVID-19, studied the correlation between SAA in different groups, before and after treatment, and drawn ROC curves to focus on the prognosis of SAA at different time points in COVID-19 patients.

In the acute infections , SAA has a significant predictive role for the final clinical outcome of COVID-19 [(207)]. This role may be due to its mechanism that can activate inflammatory cells such as neutrophils, promote inflammatory factors release, and trigger inflammation of the body. At the same time, SAA can be incorporated with high-density lipoprotein (HDL) to yield an SAA/HDL complex, which chemoattracts inflammatory cells; in addition, SAA may effect on the

lipoxin signalling pathway, which can enhance the increase of the survival time of neutrophils, and aggravate the stage of inflammation and infection, leading to complicate patient's condition.

At the acute phase, there was a significant elevation in SAA levels thus strengthening the correlation [208]. The elevated SAA levels, which are related to the mechanism of inflammation and induction of inflammatory cells, could reflect the continuing inflammation at the infection site. There was pulmonary fibrosis in the COVID-19 patients [209]. Additionally, the increase of SAA was correlated with disease severity and fever. Some studies that linked between COVID-19 and SAA are summarised in table (1-4).

Table (1-4): Studies that linked between COVID-19 and SAA

Titles	Authors	Aims	Markers	Results
1) Serum Amyloid A is a biomarker of severe Coronavirus Disease and poor prognosis/2020. (210)	Huan Li, Xiaochen Xiang, Hongwei Rena, Lingli Xu, Lisha Zhao, Xiaoqiong Chen, Hui Long, Qiang Wang, and Qingming Wua.	To evaluate the clinical roles of the dynamic alterations of inflammation biomarkers in prediction the disease severity and prognosis of COVID-19 patients .	SAA, CRP, PCT, WBC, Lymphocytes , and Platelets.	↑ SAA
2) Associations between serum amyloid A, interleukin-6, and COVID-19: A cross-sectional study/2020. (211)	Qian Liu, Yaping Dai, Meimei Feng, Xu Wang, Wei Liang, and Fumeng Yang.	To provide probable laboratory basis for auxiliary distinguishing COVID-19 by determining inflammation-related biomarkers of SAA and IL-6.	SAA, IL-6, WBC, Neutrophils, Lymphocytes, RBC, Platelets, Hemoglobin , and CRP.	↑ SAA
3) Prognostic value of serum amyloid A in patients with COVID -19 /2020. (206)	Li Cheng, Jian‐Zhong Yang, Wen‐Hui Bai, Zhuan‐Yun Li,Li‐Fang Sun, Juan‐Juan Yan, Chen‐Liang Zhou, and Bao‐Peng Tang.	To investigate the prognostic role of SAA in the patients with COVID-19.	Blood routine, Blood biochemistry, Coagulation function, D-dimer, and SAA.	SAA has ↑ sensitivity
4) Serum Amyloid A Protein as a Potential Biomarker Useful in Monitoring the Course of COVID-19: A Retrospectively studied/2020. (208)	Ying Zhang, Donglian Wang, Minjie Lin, Tong Sun, Jiaxi Chen, Jiaqin Xu, Hongguo Zhu, Guangjun Zhu, Ruyue Lu, Luxiao Hong, Bo Shen, Xiaomai Wu, and Yufen Zheng.	To gradually detect the potential role of SAA in disease monitoring.	SAA	↑ SAA
5) The predictive value of serum amyloid A and C-reactive protein levels for the severity of COVID -19/ 2020. (212)	Meiqiao Chen, Yuanbo Wu, Wei Jia, Ming Yin, Zhe Hu, Rui Wang, Wenting Li, and Guoping Wang.	To investigate the prognostic role of inflammatory indicators such as SAA, CRP, and PCT in predicting COVID-19 severity.	SAA, CRP, and PCT.	SAA levels demonstrate a prognostic value for predicting the severity of COVID-19.
6) The value of serum amyloid A for predicting the severity and recovery of COVID-19/2020. (204)	Jun Fu, Pian-Pian Huang, Shuang Zhang, Qing-Dong Yao, Rui Han, Hai-Feng Liu, Yi Yang, and Dong-You Zhang.	To evaluate the role of SAA in COVID-19 and compared the value of SAA and CRP in predicting the severity and improvement of COVID-19.	CRP and SAA.	SAA was an independent factor for predicting the recovery of COVID-19.

7) Lymphocyte Subset Counts in COVID-19 Patients: A Meta-Analysis /2020. (213)	Wei Huang, Julie Berube, Michelle McNamara, Suraj Saksena, Marsha Hartman, Tariq Arshad, Scott J.Bornheimer, and Maurice O'Gorman.	To make a meta-analysis of studies that included measures of lymphocyte subset counts and illness severity in hospitalized patients of COVID-19.	PCT, LDH, D-dimer, CRP, Neutrophils, pro-inflammatory cytokines: such as IL-6, Platelets, lymphocyte subset counts, such as CD4 and CD8 T cells, B cells, and NK cells.	↑ SAA
8) Characteristics of disease progress in patients with coronavirus disease 2019 in Wuhan, China/2020. (145)	Mengyao Ji, Lei Yuan, Wei Shen, Junwei Lv, Yong Li, Ming Li, Xuefang Lu, Lanhua Hu, and Weiguo Dong.	To investigate the correlation between prognosis and the variation trend of the clinical, laboratory information, and radiological characteristics of confirmed COVID-19 cases.	Blood urea, PCT, D-dimer, PT, NEU, Lymphocytes, Prealbumin , Albumin, WBC, CRP, LDH, and SAA.	↑ SAA
9) Clinical characteristics of COVID-19 patients are changing at admission/2020 (214).	Zhaowei Chen, Jijia Hu,Zongwei Zhang, Shan Jiang, Tao Wang, Zhengli Shi and Zhan Zhang.	To describe clinical and the laboratory characteristics of COVID-19 patients from the perspective of clinical doctors, and to compare the initial clinical features between patients infected in different periods.	Blood routine, CRP, SAA and CD3, CD4, CD8 cells.	↑ SAA
10) Analysis clinical features of COVID-19 infection in secondary epidemic area and report potential biomarkers in evaluation /2020. (129)	Weiping Ji, Gautam Bishnu, Zhenzhai Cai, and Xian Shen.	To determine probable biomarkers for the evaluation of COVID-19 patients, guide the diagnosis and treatment of this disease in secondary epidemic areas and get a reference for the clinical prevention and control of this epidemic disease .	SAA and CRP.	↑ SAA

Table (1-5) : Other useful acute phase reactants that used in COVID-19

APRs	Significant clinical changes during SARS-COV-2 infection
1) Albumin	Decreased levels correlated with greater severity and mortality and lack of recovery [136].
2) ESR	Higher levels correlated with: ↑Mortality [14].
3) PCT	Elevated levels of procalcitonin associated with: ↑Severity, ↑Mortality, Bacterial superinfections [215] .
4) LDH	Elevated levels associated with: ↑Severity, ↑Mortality, Lack of recovery [100].
5) IL-1β	Elevated levels may be associated with: ↑Mortality, Undefined data for severity [114].
6) Fibrinogen	Increased in severe cases or non-survivors with COVID-19 [216].
7) Hs-TnI	Significantly elevated levels of high-sensitivity troponin I (Hs-TnI) in non-survivors compared to survivors [217].

1.11. Knowledge gap

A plenty of clinical and scientific researches focused on set laboratory indicators have been reported on SARS-COV-2, but the findings of this systematic reviewing regarding the prediction of disease severity in COVID-19 patients has not been entirely completed. A study was performed and considered a databases that published on a many considerable journals and it was focused on a broad extent of biomarkers and acute-phase reactants. The dynamic alteration in levels of a biomarker or an indicator of inflammation may help in prediction disease course, prognosis, and outcome.

This master work suggesting the probability of using SAA level as an suitable biomarker for the prediction of COVID-19 severity. The danger of AA amyloidosis induction in severe COVID-19 patients is quite great, so the verification of this hypothesis seems to be an urgent task as detection of the agents leading to cause systemic complications through SARS-COV-2 infection which might save many human beings.

1.12. Aims and objectives

The aims and objectives of this study were:

- To study the level of serum amyloid A protein towards COVID-19 severity.
- To compare the level of serum amyloid A protein with other acute phase reactant mediators such as ferritin and CRP.
- To evaluate the sensitivity and specificity of the markers (C-reactive protein, ferritin, and serum amyloid A).

CHAPTER TWO

Materials and Methods

2. Materials and Methods

2.1. Study Design

The present study was a cross sectional study for a group of (91) patient samples: (22) critical patient samples, (24) moderate patient samples and (45) severe patient samples. The work started in 3rd of October 2020 and finished in 15th of September 2021. Samples of COVID-19 patients were collected from Al-Hayat unit at Imam Hussein Medical City. They were also subjected to medical scan for signs and symptoms of COVID-19 by specialized physician. The accurate diagnosis of COVID-19 patient was determined by positive-result of RT-PCR test at the Laboratory of Public Health in Kerbala city.

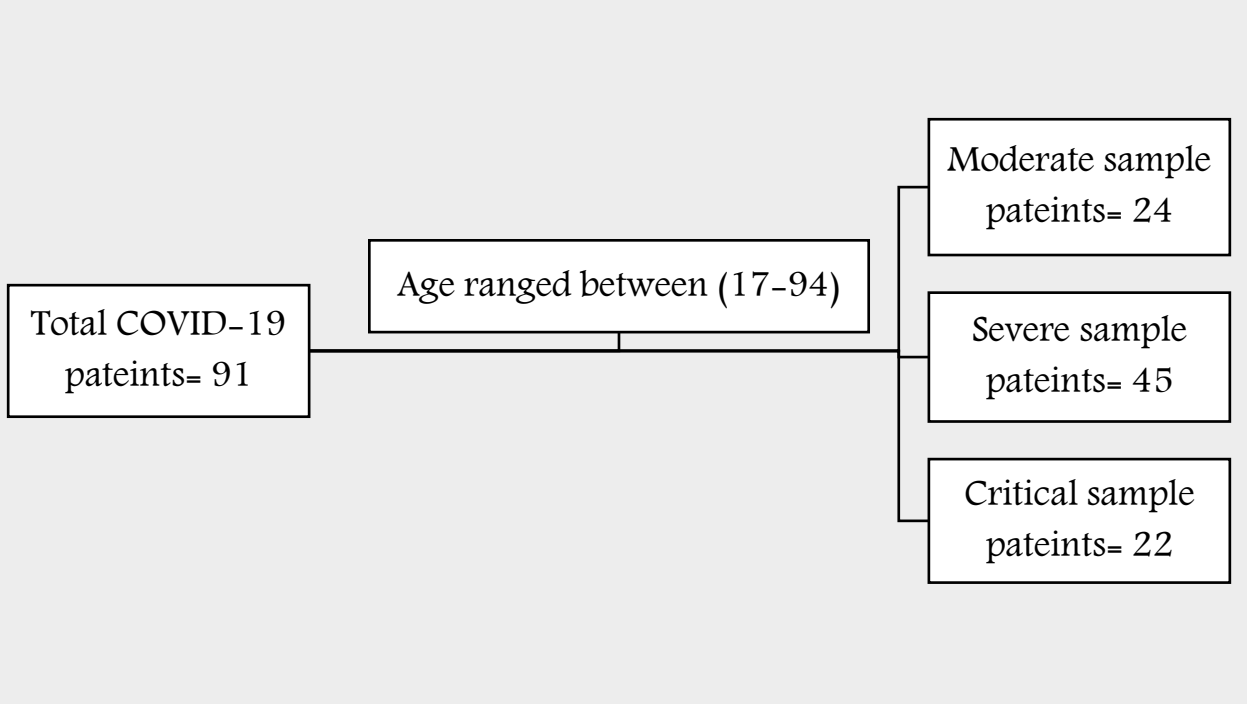

Figure (2-1): Schematic representation of sample groups.

2.2. Instruments and materials

Table (2-1): The instruments that used in the study

Instruments	Suppliers
1. Centrifuge	HETTICH/ Germany
2. Cobas c 311	Germany
3. Cobas e 411	Germany
4. Deep freezer	COOLTECH/ China
5. ELISA system	UNO/HUMAN/ Germany
6. Ruller Mixer	China
7. XP-300™ automated hematology analyzer Sysmex	Jaban

Table (2-2): The kits that used in the study

Materials	Suppliers
Kit SAA	Bioassay technology laboratory/ China
Kit Ferritin	Maglumi/Germany
Kit D-dimer	Snibe Maglumi 800/Germany
Kit C-Reactive protein	China
Kit CBC	Jaban

2.3. Inclusion and Exclusion criteria

2.3.1. Inclusion criteria:

All patients must be reported as confirmed case of COVID-19 (people with positive RT-PCR result in the hospital). They should undergo to the full clinical history, clinical scan, and pertinent laboratory analysis.

2.3.2. Exclusion criteria:

The study excluded non-hospitalized patients (mild cases of COVID-19).

2.4. Study variables

2.4.1. Dependent variable:

The dependent variables of this study are White blood cells (WBC) count, Neutrophils percentage (NEU%), Lymphocytes percentage (LYM%), Neutrophils to lymphocytes ratio (NLR), Coefficient of variation of red cell distribution width (RDW-CV), C-reactive protein (CRP), Ferritin, D-dimer, and Serum Amyloid A (SAA).

2.4.2. Independent variable:

The independent variables of this study are Age, gender, smoking state, body temperature, dry cough, Oxygen-level saturation and shortness of breath**,** and current chronic diseases (Hypertension, Diabetes, and Autoimmune diseases).

2.5. Approval of the Ethical Committee

The protocol of the research was confirmed by ethical council of college of medicine in university of Kerbala , and council of Al-Hayat unit in Imam Hussein Medical City. Samples were taken after approval from patients or the relatives of patients.

2.6. Measurement and Data collection

2.6.1. Data Collection:

A detailed questionnaire was precisely sketched to obtain information which aids to select people according to the selection criteria of the research. The sociodemographic side of the patients were taken through the self-reported method (student questionnaire) involving age, gender, and having any current chronic diseases (Hypertension, Diabetes, and Autoimmune diseases). Smoking

state, body temperature, dry cough, Oxygen-level saturation and shortness of breath were taken from each patient .

2.6.2. Blood Collection and Storage:

Five mL of blood sample was drown from patient by venipuncture using 5 mL disposable syringe. Each blood sample was partitioned to three parts :

1) Two ml of blood was left for 15 min at room temperature in gel tube. Serum was separated by centrifuging for 10 min at approximately 4000 rpmxg. Then serum sample was collected by eppendroff and stored at -20°C to avoiding multiple freezing-thawing cycles. Serum samples was used to measure the levels of CRP, SAA, and Ferritin.

2) One point five mL of blood was collected by sodium citrate anticoagulant tube. Plasma was separated by centrifuging for 10 min at approximately 4000 rpmxg. Plasma was used to measure the levels of D-dimer.

3) One point five ml of blood was collected by Ethylenediaminetetraacetic acid (EDTA) anticoagulant tube. The samples were left on mixruller at room temperature to avoid formation of clot. The samples were used for determination of CBC**.**

The tubes of blood collection were disposable, non-pyrogenic, and not liable for production of any endotoxin.

2.7. Methods

2.7.1. Determination of serum amyloid A using ELISA technique:

Enzyme Linked Immunosorbent Assay system (ELISA) was done using sandwich method to determine the concentrations of SAA .

Principle: The principle of SAA assay was an ELISA system. The plate has been pre-coated with human SAA antibody. SAA that presents in the sample was added and bound to antibodies coated on the wells, and then biotinylated human SAA antibody was added and bound to SAA in the sample. Then Streptavidin-HRP was added and bound to the Biotinylated SAA antibody. After incubation period, unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and color developed in proportion to the quantity of human SAA. The reaction was finished by addition of acidic stop solution and absorbance was determined at 450 nm.

Reagents: List of reagents were listed in table (2-3).

Table (2-3): List of reagents of SAA kit

Components	Quantity
Standard Solution (40µg/ml)	0.5 ml x 1
Pre-coated ELISA Plate	12* 6 well strips x 1
Standard Diluent	3 ml x 1
Streptavidin-HRP	6 ml x 1
Stop Solution	6 ml x 1
Substrate Solution A	6 ml x 1
Substrate Solution B	6 ml x 1
Wash Buffer Concentrate (25x)	20 ml x 1
Biotinylated human SAA Antibody	1 ml x 1

Preparation of reagents: Stock solutions were prepared according to the procedure of the kit. All reagents were prepared freshly at room temperature before using.

- **Standard solution:** The 120µl of the standard (40µg/ml) was reconstituted with 120µl of standard diluent to generate a 20µg/ml standard stock solution. The standard was allowed to sit for (15 min) with gentle agitation prior to making dilutions. The duplicate standard points were prepared by serially diluting the standard stock solution (20µg/ml) 1:2 with standard diluent to produce 10µg/ml, 5µg/ml, 2.5µg/ml and 1.25µg/ml solutions. Standard Diluent was served as the zero standard (Blank= 0 µg/ml). Dilution of standard solutions were shown as follows table (2-4) and figure (2-1):

Table (2-4): Dilution of standard solutions in SAA Kit

20 µg/Ml	Standard No.5	120µL Original Standard +120µL Standard Diluent
10 µg/mL	Standard No.4	120µL Standard No.5 +120µL Standard Diluent
5 µg/mL	Standard No.3	120µL Standard No.4 +120µL Standard Diluent
2.5 µg/mL	Standard No.2	120µL Standard No.3 +120µL Standard Diluent
1.25 µg/mL	Standard No.1	120µL Standard No.2 +120µL Standard Diluent

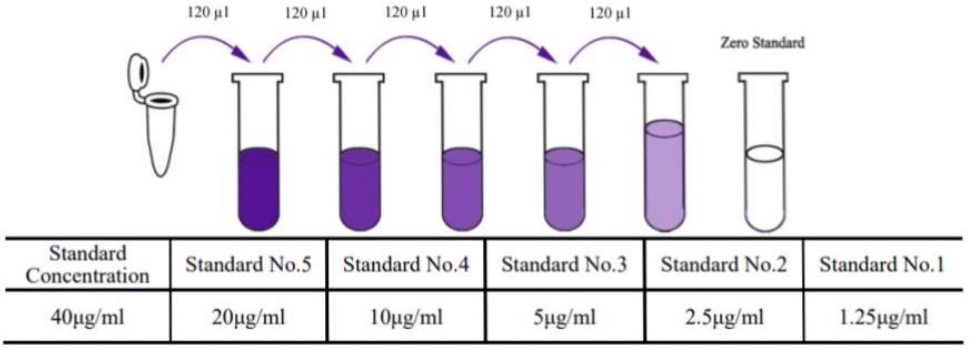

Figure (2-2): Dilution of standard solutions in SAA Kit

- **Wash Buffer:** 20ml of Wash Buffer Concentrate 25x was diluted into deionized or distilled water to yield 500 ml of 1x Wash Buffer. When crystals had formed in the concentrate, the solution was mixed gently until the crystals had completely dissolved.

Procedure: All reagents, standard solutions and samples were prepared as instructed. All reagents were brought to room temperature before use . The assay was performed at room temperature. The strips were inserted in the frames for use.

- (50µL) standard was added to standard well. (**Note:** Antibody did not added to standard well because the standard solution contained biotinylated antibody).
- (40µL) sample was added to sample wells and then (10µL) anti-SAA antibody was added to sample wells, then 50µl streptavidin-HRP was added to sample wells and standard wells (Not blank control well). They were mixed well. The plate was covered with a sealer and it was incubated 60 minutes at 37°C.The sealer was removed and the plate was washed 5 times with Wash Buffer.
- The washing was automated. All wells were aspirated and washed 5 times with Wash Buffer, wells were overfilled with wash buffer. Then the plate was blotted onto paper towels.
- (50µL) substrate solution A was added to each well and then (50µL) substrate solution B was added to each well. Plate was incubated and covered with a new sealer for 10 minutes at 37°C in the dark.
- (50µL) Stop Solution was added to each well, the blue color changed into yellow immediately.

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

Calculation of results : A standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and a best fit curve was drawn through the points on the graph. These calculations can be best done with computer-based curve-fitting software and the best fit line can be determined by regression analysis. The result was expressed in µg/mL.

2.7.2. Measurement of C-Reactive protein:

Method: Turbidimetry Method

Principle: Measure of C-Reactive protein level through photometric measurement of immunocomplex between antibodies of CRP and CRP that presents in the sample, the absorbency elevation was directly proportional to the CRP level .

Anti-human CRP antibody + CRP ↔ Immunocomplex (agglutination)

Preparation of reagents:

Table (2-5): Components and concentration of CRP Kit

R1:	**Tris buffer** **PEG** **Surfactant**	**100 mmol/L** **0.26 mmol/L** **<2% (m/v)**
R2:	Tris buffer anti-human CRP Antibody (goat)	100 mmol/L dependent on titer

Calculation: After calibration, The analyzer calculated CRP level of each sample automatically. Conversion factor: mg/dL x 0.1= mg/L ; Or: C sample= (ΔA sample/Δ A calibration) xC calibration

2.7.3. Measurement of D-dimer:

Principle: D-Dimer assay was a sandwich chemiluminescence immunoassay. The sample, ABEI labeled with anti-D-Dimer monoclonal antibody, buffer and magnetic microbeads coated with another anti-D-Dimer monoclonal antibody were mixed thoroughly and incubated to create a sandwich; after sedimentation in a magnetic field, the supernatant was poured , and a wash cycle was done . Subsequently, the Starter 1+2 were added to initiate a flashchemiluminescent reaction. The light signal was determined by a photomultiplier as relative light units (RLUs), which was proportional to D-Dimer level that presents in the sample.

Table (2-6): Kit components of D-dimer

Components	Contents	100 tests (REF:130606008M)	50 tests (REF:130606008M)
Magnetic microbeads	Magnetic microbeads coated with anti-D-Dimer monoclonal antibody, containing BSA, NaN_3 (<0.1%).	2.5Ml	2 mL
Calibrator Low	D-Dimer antigen containing BSA, NaN_3 (<0.1%).	2.5mL	2 mL
Calibrator High	D-Dimer antigen containing BSA, NaN_3 (<0.1%).	2.5mL	2 mL
Buffer	containing BSA, NaN_3 (<0.1%).	6.5 mL	4 mL
ABEI Label	Anti-D-Dimer monoclonal antibody labeled with ABEI, containing BSA, NaN_3 (<0.1%).	6.5 mL	4 mL
Internal Quality control	D-Dimer antigen containing BSA, NaN_3 (<0.1%).	2 mL	2 mL

***All reagents are provided ready-to-use.**

Prepration of the reagents: Resuspension of the magnetic microbeads took place automatically when the kit was loaded, the magnetic microbeads were ensured to be fully resuspended homogenous prior to usage.

Dilution: Sample dilution by analyzer was not provided in the reagent kit. Samples with quantities above the measuring range were diluted manually. After manual dilution, the result was multiplied by the dilution factor.

Calculation of results: The analyzer automatically calculated the D-dimer level in each sample by mean of a calibration curve which was generated by a 2-point calibration master curve method .The result was expressed in µg FEU/ mL.

2.7.4. Measurement of Ferritin:

Principle: Ferritin was a two-sandwich chemluminescence immunoassay. The sample and magnetic microbeads coated with anti-Ferritin monoclonal antibody were mixed thoroughly and incubated, and then a wash cycle was done. Then ABEI labeled was added with anti-Ferritin monoclonal antibody were mixed thoroughly and incubated to form sandwich complexes. After sedimentation in a magnetic field, the supernant was poured, and then another wash cycle was done. Subsequently, the starter 1+2 were added to initiate a chemiluminescent reaction. The light signal was measured by a photomultiplier as relative light unit (RLUs), which was proportional to the concentration of Ferritin present in the sample .

Table (2-7): Kit components of Ferritin

Components	Contents	100 tests (REF:130606008M)	50 tests (REF:130606008M)
Magnetic microbeads	Magnetic microbeads coated with anti- Ferritin monoclonal antibody, containing BSA, NaN_3 (<0.1%).	2.5mL	2 Ml
Calibrator Low	Ferritin antigen containing BSA, NaN_3 (<0.1%).	2.5mL	2 mL
Calibrator High	Ferritin antigen containing BSA, NaN_3 (<0.1%).	2.5mL	2 mL
Buffer	containing BSA, NaN_3 (<0.1%).	12.5 mL	7 mL
ABEI Label	Anti- Ferritin monoclonal antibody labeled with ABEI, containing BSA, NaN_3 (<0.1%).	22.5 mL	12.5 mL
Diluent	0.9%NaCl	25mL	15mL
Internal Quality control	Ferritin antigen, containing Bovine Serum, NaN_3 (<0.1%).	2 mL	2 mL

***All reagents are provided ready-to-use.**

Prepration of the reagents: Resuspension of the magnetic microbeads took place automatically when the kit was loaded, the magnetic microbeads were ensured to be fully resuspended homogenous prior to use.

Dilution: The automatic sample dilution was available after dilution settings were done in MAGLUMI series fully-auto chemiluminescence immunoassay analyzer user software.

Calculation of results: The analyzer automatically calculated the Ferritin level in each sample by mean of a calibration curve which was created by a 2-point calibration master curve method. The result was expressed in ng/mL.

2.7.5. Determination of Complete blood count (CBC):

The measures of CBC were done by XP-300™ Automated hematology analyzer Sysmex .

Principle:

- DC detection method for WBC .
- DC detection method for RBC/PLT .
- Non-cyanide haemoglobin analysis method for HGB .

Parameters: WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, LYM%, MXD%, NEU%, LYM#, MXD#, NEU# RDW-SD, RDW-CV, PDW, and MPV.

Sample volume:

- Whole blood (WB) mode : Approximately 50µL .
- Pre-diluted (PD) mode : Approximately 20µL .

2.8. Statistical Analysis

Information from the questionnaire and all test results from patients samples were organized in several data sheets. The data analysis for this work was generated using the Real Statistics Resource Pack software for Mac (Release 7.2) of the resource pack for Excel 2016. Copy-right (2013 – 2020).

Descriptive statistics was performed on the participants' data of each group. Values were presented by n (%) for categorical variables. The distribution of the data was checked for normality using the Box plot test.

ANOVA test was used to adjust other risk factors including: age and gender (male, female). The 95% confidence intervals (95%CI) were also determined for all variables. Significant differences in continuous variables among the parameters were confirmed through analytical statistical tests. Results of all hypothesis tests with P values <0.05 (two-side) were considered to be statistically significant. Receiver operating characteristics (ROC) curves was also used to test the markers' diagnostic performance in each group.

CHAPTER THREE

Results and discussion

3. Results and discussion

In the context of using biomarkers for disease severity and clinical progression, this study aimed to facilitate the identification of COVID-19 patients based on the role of inflammation and immunity markers such as acute phase protein regarding the clinical progress of COVID-19, markers that reflect the activation of these pathways might be particularly useful for risk stratification.

3.1. Demographic and clinical characteristics

The clinical demographic characteristics and laboratory parameters of different stages of COVID-19 groups were summarized in Table (3-1). Table illustrated the mean age of participants which was within the age group of (17 – 94) years old. Gender distribution among the studied groups were: 68% male, 32% female.

Several reports point to sex differences in COVID-19 resulting from male patients having higher rates of infection. These disparities in sex mainly relate to factors concerning social behaviour and human biology [(218)]. Among social factors, it is considered that men represent a higher proportion of smokers, and more often exhibit lifestyles that cause the main comorbidities associated with COVID-19 infection. In addition, men enact cultural practices that put them at greater risk of becoming ill, spreading the infection or seeking less medical attention [(218)]. The greater susceptibility of men can also be related to their greater amounts of ACE 2 receptors compared to women [(11)].

Smoking is a putative risk factor for Middle East respiratory syndrome coronavirus infection [(219)]. To date, there is no strong evidence that smokers are protected against SARS-COV-2 infection. Moreover, there is growing evidence

that smokers have worse outcomes after contracting the virus than non-smokers [220]. The available evidence suggests that smoking is associated with increased severity of disease and death in hospitalized COVID-19 patients.

Table (3-1): Demographic characteristics of the study participants

Characteristics			Patient group			Normal range
			Moderate	Severe	Critical	
Demographics	Age Mean(Median)		56.58(55.5)	58.29(56)	59.18(58.5)	
	Gender (Male/Female)no.		(18/6)	(29/16)	(15/7)	
Medical history No.	Autoimmune disease (Yes/No)		(0/24)	(0/45)	(0/22)	
	Smoking(Yes/No)		(0/24)	(3/42)	(2/20)	
	DM(Yes/No)		(7/17)	(11/34)	(7/15)	
	HT(Yes/No)		(5/19)	(19/16)	(9/13)	
Symptoms	Dry Cough (Yes/No)no.		(8/14)	(24/21)	(12/12)	
	SOB (Yes/No)no.		(0/22)	(45/0)	(24/0)	
	Temp. Fahrenheit Mean(Median)		100.83(100.4)	101.2(102.2)	100.49(100.4)	98.6 °F
	Sat. O_2 Mean(Median)		92(93)	67.31(62.5)	37.95(37)	95% or higher
Biochemical parameters Mean(Median)	CRP		132.03(81)	49.87(16.6)	31.41(23.7)	0-6 mg/L
	D-dimer		1576.3(702.7)	898.49(514.3)	3061.08(2100)	<500 ng/mL
	Ferritin		896.88(319.2)	713.37(616.25)	1510.2(1334.9)	20-350 ng/mL
	SAA		13.05(13.405)	13.43(13.675)	13.07(13.135)	3.64 - 6.74µg/mL
RBC Mean(Median)	RBC count		4.57(4.39)	4.45(4.68)	4.68(4.89)	4.5-6.5(10^12/L)
	MCV		84.84(85.3)	90.65(85.9)	85.12(86.3)	78-96 fL
	RDW	RDW-CV	13.33(14)	14.2(13.9)	13.89(13.7)	11.5-14.5%
		RDW-SD	48.4(48.7)	48.55(46)	47.58(44.6)	37-54 fL
	HCT		38.81(38.5)	38.81(39.4)	39.78(40.4)	40-52%
	HB		13(12.8)	13(12.6)	12.79(12.7)	13.5-17.5 g/dL
	MCH		28.49(28)	28.49(27.6)	27.40(27.8)	27-34 pg
	MCHC		33.6(32.5)	33.6(32)	32.19(32.4)	32-36 g/dL
WBC Mean(Median)	WBC count		12.46(9.9)	10.3(13.5)	17.57(17.3)	4-11(10^9/L)
	NEU#		10.83(8.5)	8.9(10.8)	15.44(15.1)	1.9-7.5 (10^9/L)
	NEU%		86.14(84.9)	86(84.6)	88.19(86.4)	39.3-73.7 %
	LYM#		0.94(1)	0.65(1.1)	1.13(1.5)	1.3-3.5(10^9/L)
	LYM%		8.89(11)	7.2(9.2)	6.74(9.2)	18-45.3 %
	NLR		10.4(7.87)	15.47(9.02)	17.18(9.39)	Roughly 1-3
	MXD#		0.69(0.7)	0.75(1)	0.77(0.85)	
	MXD%		5.4(5.4)	6.8(7.4)	4.1(4.75)	
Platelets Mean(Median)	PLT count		323.57(215)	243(274)	283.85(246)	155-450(10^3/uL)
	MPV		9.74(9.7)	10.25(9.9)	10.21(10.5)	6.9-10.6 fL
	PDW		12.74(12.65)	13.1(12.6)	14.2(13.6)	9-17 fL

3.2. Examination the distribution of data in the studied groups

A box plot was used to visually show the distribution of data through displaying the data quartiles (or percentiles) and averages. Box plots show the five-number summary of a set of data: including the minimum score, first (lower) quartile, median, third (upper) quartile, and maximum score. The median is the average value from a set of data and is shown by the line that divides the box into two parts. In statistics, dispersion (also called variability, scatter, or spread) is the extent to which a distribution is stretched or squeezed. The smallest value and largest value are found at the end of the 'whiskers' and are useful for providing a visual indicator regarding the spread of measurements. On the other hand, figures also indicated that the interquartile ranges of the boxes regarding patients groups have more dispersion of a data set with indicated more variability.

3.2.1 Distribution of hematological parameters

A) Blood cell parameters (RDW-CV, WBC, NEU%, LYM%, and NLR):

Figure (3-1) demonstrates the distribution of blood RDW-CV, WBC, NEU%, LYM% and NLR in different stages of COVID-19 patients. The levels of RDW-CV, WBC, NEU%, LYM% and NLR were varied based on severity of disease. The mean levels of moderate group for RDW-CV, WBC, NEU%, LYM% and NLR were 13.33, 12.46 (10^9/L), 86.14%, 8.89%, and 10.40, respectively as shown in Figure (3-1A). while the mean levels of RDW-CV, WBC, NEU%, LYM% and NLR in severe COVID-19 patients were 14.2, 10.3(10^9/L), 86%, 7.2%, and 15.47, respectively as shown in Figure (3-1B). Figure (3-1C) indicated the mean levels of RDW-CV, WBC, NEU%, LYM% and NLR in critical COVID-19 patients which were 13.89, 17.57(10^9/L), 88.19%, 6.74%, and 17.18, respectively.

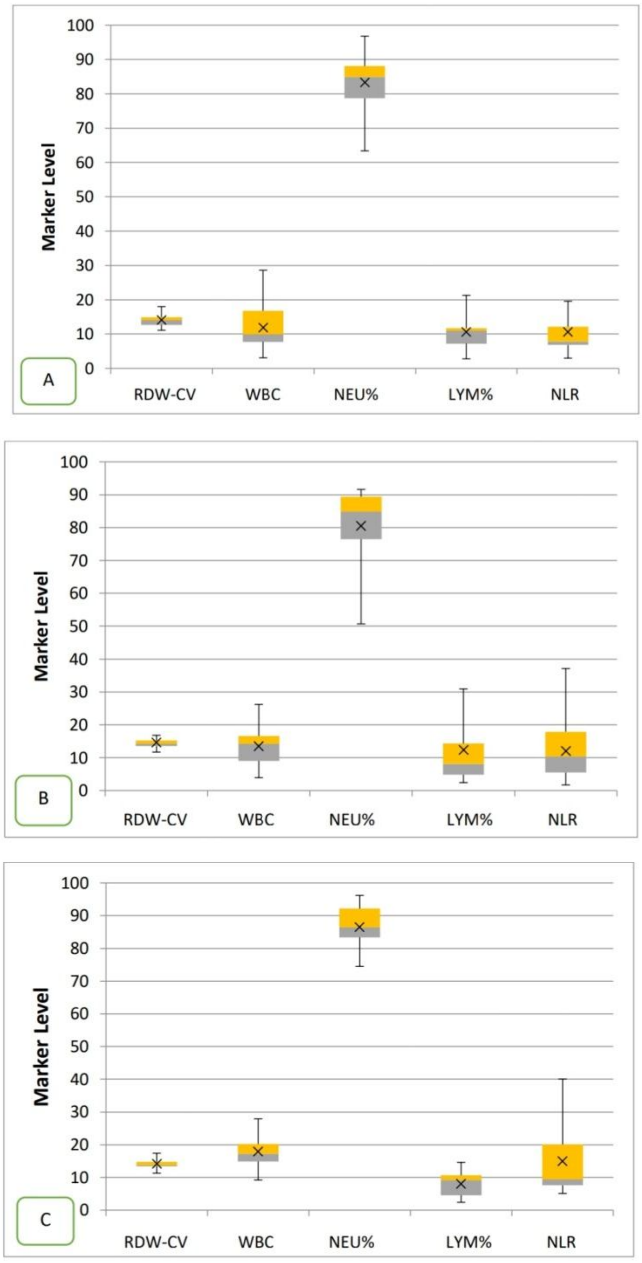

Figure (3-1): Boxplot of the Distribution of blood level of RDW-CV, WBC, NEU%, LYM%, and NLR in: (A) Moderate COVID-19 patients, (B) Severe COVID-19 patients (C) Critical COVID-19 patients.

Neutrophils percentage had shown a wide variability in moderate and severe COVID-19 patients that illustrated a wide variability range in the neutrophils percentage, lymphocytes percentage and neutrophils to lymphocytes ratio. Also the three groups of patients showed a significant increase in the WBC levels as observed in diseased individuals [(221)]. It has been previously reported that the WBC count increased with the severity of the COVID-19 disease [(117)]. With regard to leukocytosis (The increase in the WBC levels), both severe and critical patients were significantly associated with leukocytosis. Zhou *et al,*[(85)] also showed that non-survivors were significantly associated with having leukocytosis compared to survivors.

A decrease in the lymphocytes was observed in severe and critical patients. Based on our observation, it could be speculated that the lymphocytes count depletion is directly associated with the COVID-19 disease severity and the survival rate of the disease could be linked with the ability of T lymphocytes which are essential for the destruction of infected viral particles [(222)]. The decreased lymphocytes count was observed in the moderate and severe COVID-19 patients critical diseased individuals which could be attributed to increased inflammation and suppression of the immune system caused by COVID-19 infection [(221)]. Various studies have supported lymphocytopenia as a reliable and effective biomarker for the severity of COVID-19 disease [(223)].

Previous investigations have also reported neutrophil to lymphocytes ratio as important prognostic factor for disease progression [(121)]. A significant association was observed in NLR among various disease groups. As COVID-19 causes a systemic inflammatory response, neutrophils are activated by virus-

induced inflammatory markers IL-6 and IL-8, GCSF, IFN-Ɣ, TNF-α formed by lymphoid and endothelial cells. Conversely, the immune response is considerably depressed notably the helper T lymphocytes. Hence, NLR is elevated as a result and it is associated with disease progression [(221)].

Neutrophils to lymphocytes ratio was the highest in patients with critical disease. Liao *et al,* [(146)] also found elevated neutrophils to lymphocytes ratio as a useful predictor for severity and mortality of SARS-COV-2 infection. Elevated NLR on admission was considered an independent risk factor for severe disease and poor clinical outcomes in COVID-19 patients [(121)]. Neutrophilia was more prominent in moderate and critical than severe patients. Qin *et al,*[(86)] reported significantly higher neutrophil count in severe patients [(224)]. The presence of neutrophilia could be related to cytokine storm that characterizes COVID-19 disease [(148)].

B) Coagulation parameters(D-dimer):

The mean of D-dimer in moderate, severe, and critical groups were 1576.3, 898.49, 3061.08 ng/mL respectively, see table (3-1). Study also examined the level of D-dimer based on severity of the disease, D-dimer could be used to distinguish patients with moderate from severe stage [(126)]. Figure (3-2) shows that serum D-dimer levels was increased markedly in moderate and critical COVID-19 patients.

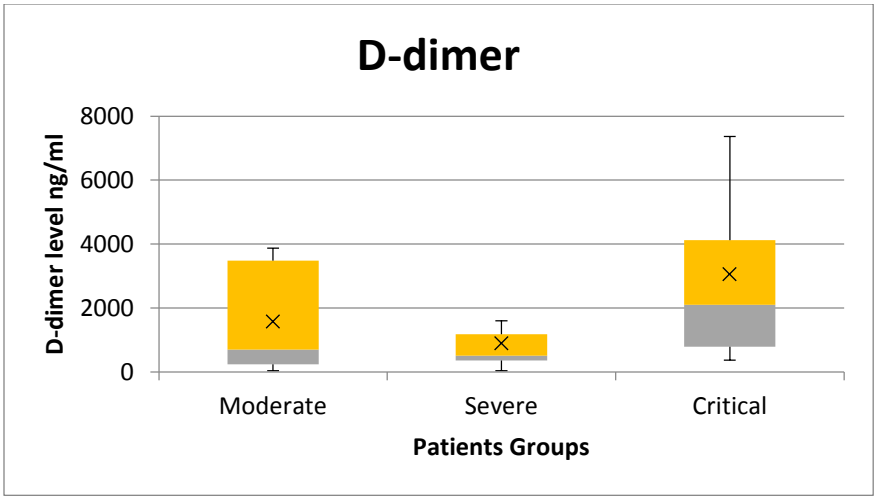

Figure (3-2): Boxplot of the Distribution of serum level of D-dimer ng/mL in COVID-19 patients based on their disease severity.

Table (3-2) shows the correlation between D-dimer and RDW-CV, WBC, NEU%, LYM%, and NLR in moderate, severe, and critical groups of COVID-19 patients. In severe group, there were a weakly significant negative-correlation between D-dimer and WBC [rs=-0.4, P value= 0.05], and D-dimer and NLR [rs=-0.4, P value= 0.02]. Furthermore, a weakly significant positive-correlation was demonstrated between D-dimer and LYM% [rs= 0.4, P value =0.03].

Table (3-2): Correlation coefficients by Spearman rank test between D-dimer and RDW-CV, WBC, NEU%, LYM%, and NLR in COVID-19 patients based on their disease severity.

	D-dimer in Moderate patients		D-dimer in Severe patients		D-dimer in Critical patients	
	Rs	P value	rs	P value	rs	P value
RDW-CV	0.1	0.8	-0.4	0.09	-0.4	0.1
WBC	0.2	0.2	-0.4	0.05	0.3	0.32
NEU%	0.2	0.5	-0.4	0.07	0.2	0.4
LYM%	0.1	0.6	0.4	0.03	-0.4	0.1
NLR	-0.4	0.8	-0.4	0.02	0.4	0.1

Even more, dynamic changes of D-dimer levels during the course of the disease was prognostic of poor outcome [225]. The Fibrinolytic system activates

when the coagulation cascade initiates and works to limit the clot. Fibrinolysis is an enzymatic procedure that breaks down the fibrin clot into D-dimer. D-dimer emerges with the dissolution of cross-linked fibrin and is one of the specific indicators of fibrinolysis used to estimate and diagnose pulmonary embolism, DIC, or deep vein thrombosis [(226)]. Also, D-dimer levels correlate with pneumonia. However, D-dimer is not a biomarker for viral pneumonia yet [(108)].

The high level of D-dimer in COVID-19 is triggered by excessive clots and hypoxemia. In addition, D-dimer elevation is frequently observed in COVID-19 patients with critical stage, and correlates significantly with mortality [(217)].

3.2.2 Distribution of acute phase reactants (CRP, Ferritin and SAA)

Figure (3-3) to figure (3-5) demonstrate the distribution of CRP, Ferritin, and SAA in different stage of COVID-19 patients groups. The levels of CRP, Ferritin, and SAA were varied based on severity of disease. The mean levels of CRP in moderate, severe, and critical groups were 132.03, 49.87, 31.41 mg/L respectively, while the mean of ferritin in moderate, severe, and critical groups were 896.88, 713.37, 1510.2 ng/mL respectively and the mean of SAA in moderate, severe, and critical groups were 13.05, 13.43, 13.07 µg/mL respectively, see table (3-1).

A) C-reactive protein: Elevated levels of CRP were observed up to 86% in severe COVID-19 patients which agreed with other studies [(177)]. Patients with moderate disease had a far elevated level of CRP than severe patients. CRP was found at increased levels in the initial stage than those in the critical group [(112)].

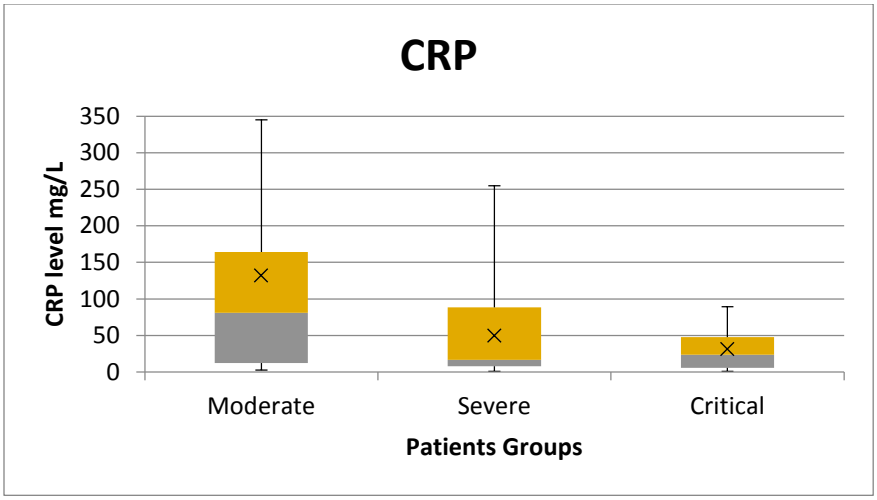

Figure (3-3): Boxplot of the Distribution of serum level of CRP mg/L in COVID-19 patients based on their disease severity.

Table (3-3) shows the correlation between CRP and RDW-CV, WBC, NEU%, LYM%, and NLR in moderate, severe, and critical groups of COVID-19 patients. There was a strongly significant positive-correlation between CRP and NEU% [rs= 0.7, P value= 0.0007]. Also, there were a moderately significant negative-correlation between CRP and LYM% [rs =-0.5, P value= 0.03] and a moderately significant positive-correlation between CRP and NLR [rs =0.5, P value = 0.02].

Table (3-3): Correlation coefficients by Spearman rank test between CRP and RDW-CV, WBC, NEU%, LYM%, and NLR in COVID-19 patients based on their disease severity.

	CRP in Moderate patients		CRP in Severe patients		CRP in Critical patients	
	Rs	P value	rs	P value	rs	P value
RDW-CV	-0.4	0.9	0.4	0.3	-0.4	0.2
WBC	0.3	0.1	0.1	0.9	0.4	0.1
NEU%	0.4	0.09	0.4	0.7	0.7	0.0007
LYM%	-0.4	0.1	0.1	0.6	-0.5	0.03
NLR	0.4	0.06	-0.4	0.6	0.5	0.02

Additionally, a significant association was observed between CRP concentrations and the aggravation of non-severe patients with COVID-19 [112],

and the researchers proposed CRP as a suitable marker for anticipating the aggravation probability of non-severe COVID-19 patients, with an optimal threshold value of 26.9 mg/L [227]. The researchers also noted that the risk of developing severe events is increased by 5% for every one-unit increase in CRP concentration in patients with COVID-19. The elevated levels of CRP might be linked to the overproduction of inflammatory cytokines in patients with COVID-19. Cytokines fight against the microbes, Thus, CRP production is induced by inflammatory cytokines and by tissue destruction in patients with COVID-19.

B) Ferritin: Serum ferritin level was significantly higher in critical cases [228]. Elevated ferritin levels were observed in non-survivors [229]. Zhou *et al,*[217] concluded that hyperferritinemia is an independent risk factor in COVID-19 patients and that can also predict disease severity. Figure (3-5) shows the serum ferritin levels which were increased markedly in moderated and severe COVID-19 patients.

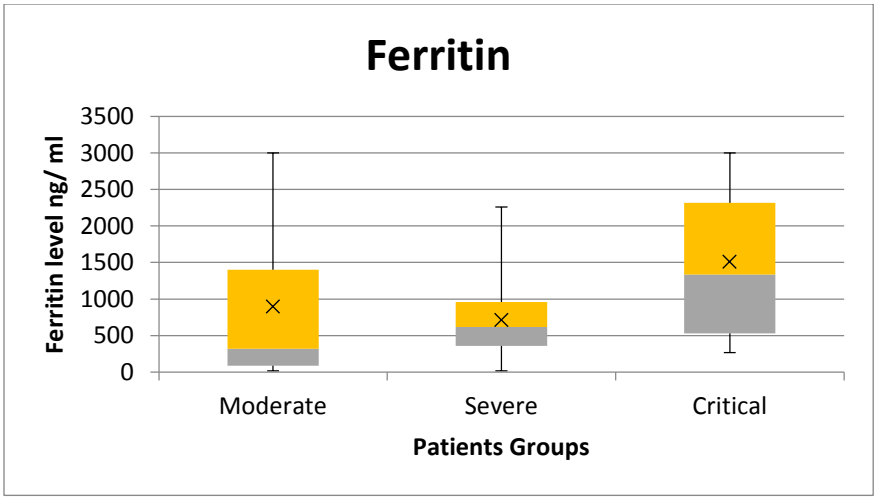

Figure (3-4): Boxplot of the Distribution of serum level of Ferritin ng/mL in COVID-19 patients based on their disease severity.

Table (3-4) shows that correlation between Ferritin and RDW-CV, WBC, NEU%, LYM%, and NLR in moderate, severe, and critical groups of COVID-19 patients. In severe group, there was a weakly significant positive-correlation between Ferritin and LYM% [rs=0.3, P value= 0.03]. Furthermore, a weakly significant negative-correlation was demonstrated between Ferritin and NLR [rs= -0.4, P value= 0.02]. In critical group, there was a moderately significant negative-correlation between Ferritin and RDW-CV [rs=-0.5, p value=0.02].

Table (3-4): Correlation coefficients by Spearman rank test between Ferritin and RDW-CV, WBC, NEU%, LYM%, and NLR in COVID-19 patients based on their disease severity.

	Ferritin in Moderate patients		**Ferritin in Severe patients**		**Ferritin in Critical patients**	
	rs	P value	rs	P value	rs	P value
RDW-CV	-0.4	0.29	-0.4	0.09	-0.5	0.02
WBC	0.1	0.9	-0.4	0.5	-0.1	0.7
NEU%	0.1	0.7	-0.4	0.07	0.1	0.8
LYM%	0.2	0.36	0.3	0.03	0.1	0.8
NLR	-0.4	0.5	-0.4	0.02	-0.4	0.8

There are two explanations that may explain the importance of ferritin: According to Shoenfeld et al., the clinical course of severe COVID-19 patients mimics that of macrophage activating syndrome, which is characterized by elevated ferritin levels and the presence of a cytokine storm. The H-chain of ferritin activating macrophages is responsible for the increased secretion of inflammatory cytokines in patients with COVID-19 [(230)]. Another possible explanation could be that ferritin elevation might be reflect how iron metabolism supports the immune system response to infecting microorganisms, including viral infections. Improved cellular metabolism and optimal iron levels among host cells are required for viral replication. Therefore, limiting the bioavailability of iron

is key to disturbing the replication of the virus. Despite the underlying aetiology, serum ferritin is mostly increased in patients with COVID-19 [231].

C) Serum amyloid A: Studies report that patients with severe acute respiratory syndrome have significantly increased level of SAA, suggesting that SAA could be used as a biomarker to monitor the progression of respiratory diseases [205]. Moderate COVID-19 patients were shown more variability of SAA than severe and critical groups.

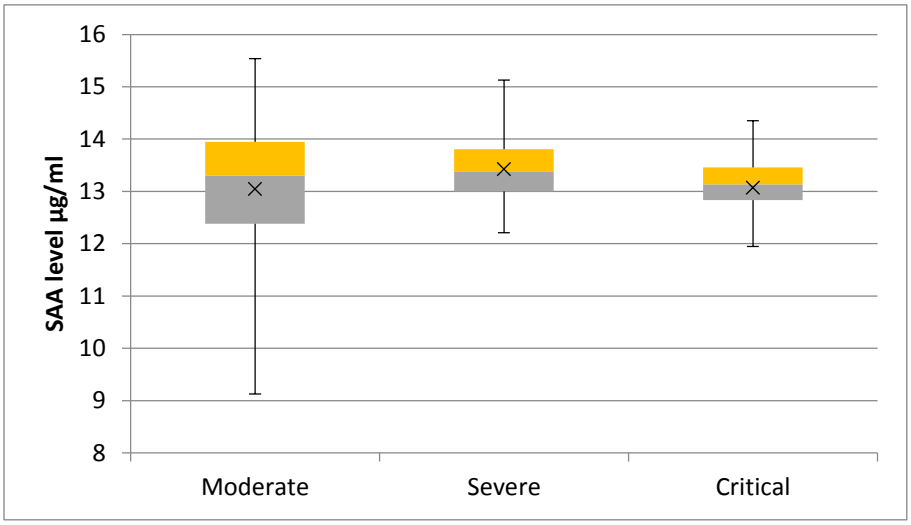

Figure (3-5): Boxplot of the Distribution of serum level of SAA in COVID-19 patients based on their disease severity.

Table (3-5) shows correlation between SAA and RDW-CV, WBC, NEU%, LYM%, and NLR in moderate, severe, and critical groups of COVID-19 patients. In severe group, there were a weakly significant negative-correlation between SAA and RDW-CV [rs=-0.4, P value =0.03], SAA and NEU% [rs= -0.4, P value= 0.005], and SAA and NLR [rs= -0.4, P value =0.01]. Furthermore, a weakly significant positive-correlation was demonstrated between SAA and LYM% [rs= 0.4, P value= 0.01].

Table (3-5): Correlation coefficients by spearman rank test between SAA and RDW-CV, WBC, NEU%, LYM%, and NLR in COVID-19 patients based on their disease severity.

	SAA in Moderate patients		SAA in Severe patients		SAA in Critical patients	
	Rs	P value	rs	P value	Rs	P value
RDW-CV	0.2	0.89	-0.4	0.03	0.1	0.22
WBC	0.3	0.23	-0.2	0.24	0.1	0.8
NEU%	0.2	0.57	-0.5	0.005	-0.4	0.13
LYM%	0.1	0.66	0.4	0.014	0.2	0.45
NLR	0.23	0.27	-0.4	0.01	-0.2	0.44

SAA is able to promote inflammatory response through activating chemokine and inducing chemotaxis even at a very low concentration [(232)]. Studies have suggested that patients infected with COVID-19 had a large amount of IL-1β, IFN-γ, IP-10, MCP-1 and other cytokines present in system, leading to the activation of Th1 cell. Compared with mild patients, severe patients may have more IL-1β, IL-6, MCP-1, MIP-1, TNF-α and other cytokines expressed, which boost liver cells to produce SAA [(100)]. Patients with respiratory virus infection usually have clinical symptoms after 36 to 48 h of infection, and SAA gradually increases and reaches to peak at 3–4 day post infection. During the phase of recovery, it is reported that SAA level continuously decreased and the decrease rate was faster than that of CRP [(203)].

Also, study examined the correlation between SAA and O_2 in moderate, severe, and critical groups of COVID-19 patients. Table (3-6) indicates that there was a significant correlation between SAA concentration and O_2 level in severe group.

Table (3-6): Correlation coefficients by Spearman rank test between SAA and saturation O_2 in COVID-19 patients based on their disease severity.

	Moderate		**Severe**		**Critical**	
	SAA level	O_2 level	SAA level	O_2 level	SAA level	O_2 level
Mean	13.06	92	13.42	76	13.04	38
P value	0.642486		0.006316		0.930383	

In COVID-19 patients, SAA and O_2 might represent an expression of lung damage and reflect the respiratory distress consequent to the abnormal inflammation status. In a small cohort of 27 patients, SAA correlated with CT scan findings and resulted significantly increased at the early stage of severe COVID-19 before changes to the critical [(112)].

Early identification and adequate treatment of COVID-19 patients at high risk for acute respiratory failure are paramount to avoid end-organ damage. As reported by Pan et al, chest CT scan has a pivotal role for the diagnosis and assessment of the severity of lung involvement in COVID-19 pneumonia [(233)]. Nowadays CT scan protocols are used to estimate the pulmonary damage [(234)], and CT scan findings can be useful to predict adverse outcome[(235)], but unfortunately CT scan scan is not available in all the Emergency Departments. Based on our results, we believe that serum SAA and O_2 level could be useful for the early identification of patients at high risk for acute respiratory failure, even in patients who do not complain dyspnea or affected by slight respiratory failure. These patients could benefit from a prompt hospitalization, a closer observation and correct treatments.

3.2.3. Examination the inflammatory indicators of COVID-19 by SAA/CRP ratio

Ratio of Predictive value for the inflammatory indicators (SAA-to-CRP) had shown statistical difference between the patient groups as shown in table (3-7). The SAA to CRP ratio was significantly higher in severe and critical groups than those of moderate patients.

Table (3-7): Correlation of inflammatory indicators SAA/CRP ratio between different stage of COVID-19 patients.

SAA/ CRP ratio				
	Moderate	*Severe*	*Moderate*	*Critical*
Mean	0.79	2.31	0.79	2.94
P value	0.03		0.01	

Viral infections can trigger inflammatory cytokine storms, which can result in worsening conditions or a poor prognosis in patients with COVID-19. In some severe cases, a cytokine storm, characterized by elevated levels of IL-1, IL-6, IL-12, and IFN-γ, was found [(236)]. A study of 99 COVID-19 patients in Wuhan Jinyintan Hospital showed that 52% of patients had elevated IL-6 levels[(177)]. In this study, levels of IL-6 were statistically different between patients with and without ARDS. However, levels of CRP and SAA, which are common inflammatory indicators, may be more conducive to universal screening. Increases in the levels of CRP, which is secreted by the liver, occur as a direct response to injury or infection. CRP activates the immune system, including the complement and mononuclear phagocytic system, resulting in clearance of viruses. During acute inflammation and infection, CRP levels can be correlated with disease severity [(237)], a finding also confirmed in this study. During the acute phase of disease, large amounts of

cytokines (IL-1, IL-6, and TNF-α) stimulate the synthesis and release of SAA by liver cells. During viral infection, SAA levels are more sensitive than CRP [238]. Lannergard *et al,* [239] proposed that SAA is more sensitive than CRP for mild inflammatory lesions and can be used for viral infections, and in noninvasive and early invasive bacterial infections.

3.3. Receiver operating characteristics (ROC) curves analysis of diagnostic markers of COVID-19 severity

In each patients group, ROC curves were performed for levels of CRP, SAA, Ferritin, and D-dimer, in addition to the SAA/CRP ratio. The AUP and cut-off values were calculated according to their specificity and sensitivity as predictive factors. In moderate group, CRP had the highest AUP, which was 0.7 [95% Cl= 0.509-0.795; Sensitivity% = 0.958; Specificity% = 0.896; Cut-off points = 3.02] .

Table (3-8): AUC, optimal threshold, Sensitivity, and specificity of CRP obtained by the ROC curves for prediction of moderate COVID-19 patients.

Moderate - COVID-19	AUP	Sensitivity %	Specificity %	Cut-off points	95% CI
CRP	0.7	0.958	0.896	3.02	0.509-0.795

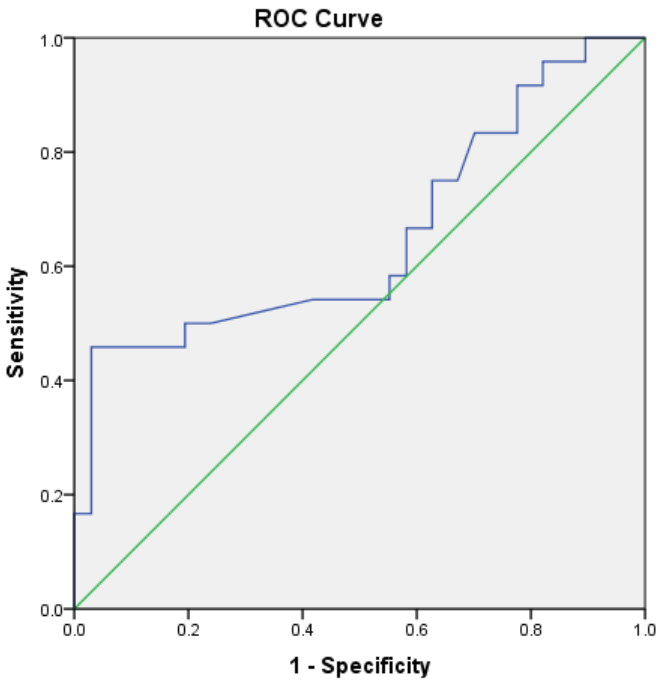

Figure (3-6): Receiver operating characteristics (ROC) curve analysis of CRP in the Moderate COVID-19 patients.

In severe group, SAA and SAA/CRP ratio had the highest AUP, which were 0.6 [95% Cl= 0.456-0.693; Sensitivity%= 0.978; Specificity%= 0.87; Cut-off points= 5.23] and 0.6 [95% Cl= 0.449-0.686; Sensitivity%= 0.978; Specificity%= 0.913; Cut-off points = 0.252] respectively.

Table (3-9): AUC, optimal threshold, Sensitivity, and specificity of SAA and SAA/CRP ratio obtained by the ROC curves for prediction of severe COVID-19 patients.

Severe- COVID-19	AUP	Sensitivity %	Specificity %	Cut-off points	95% CI
SAA	0.6	0.978	0.87	5.23	0.456- 0.693
SAA/CRP ratio	0.6	0.97	0.91	0.0252	0.449- 0.686

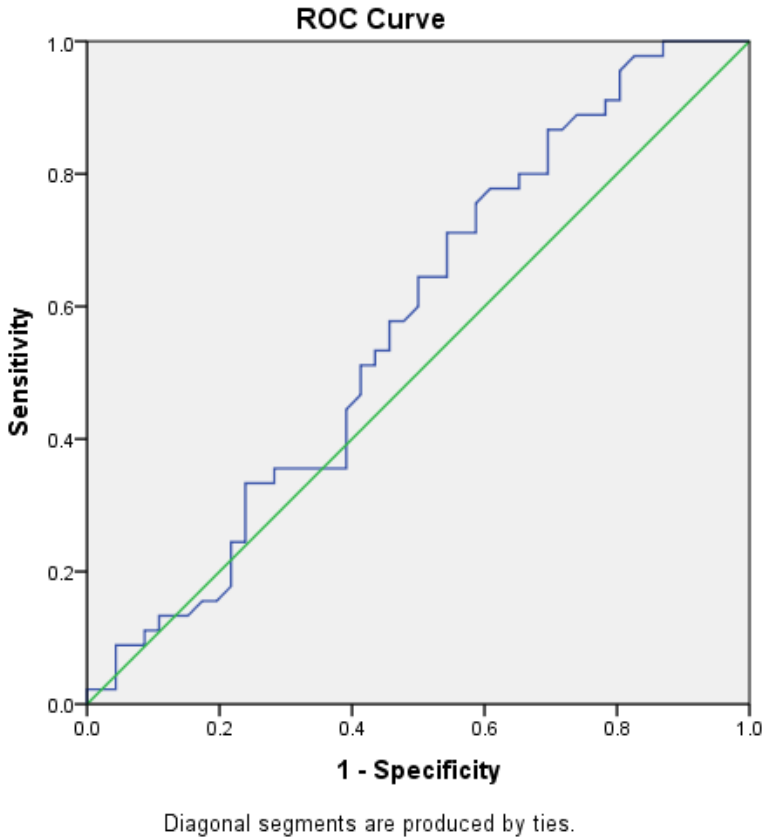

Figure (3-7): Receiver operating characteristics (ROC) curve analysis of SAA in the severe COVID-19 patients.

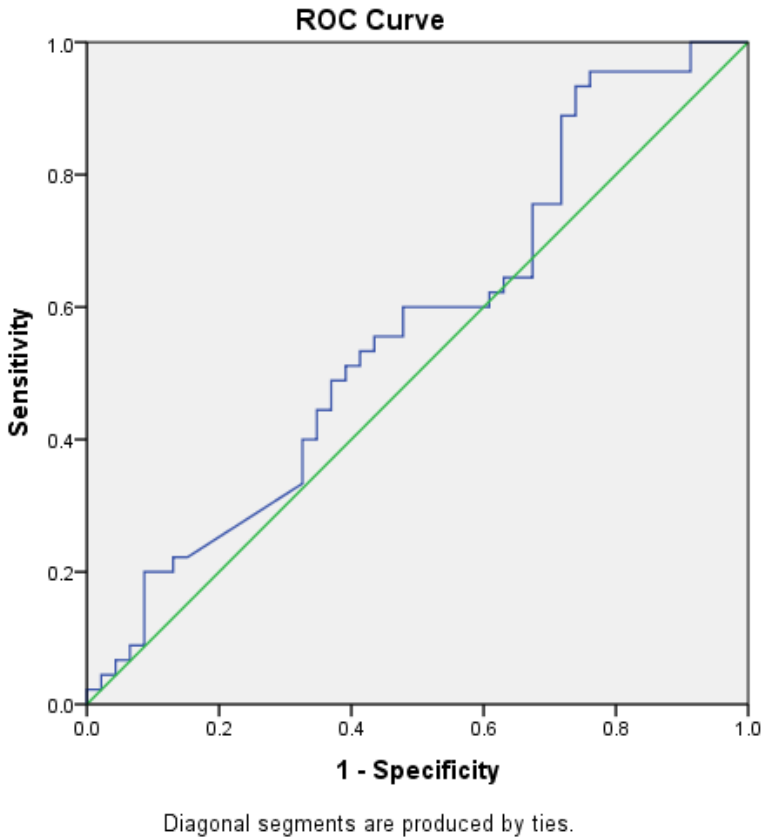

Figure (3-8): Receiver operating characteristics (ROC) curve analysis of SAA/CRP ratio in the severe COVID-19 patients.

In critical group, Ferritin , SAA/CRP ratio, and D-dimer had the highest AUP, which were 0.7 [95% Cl (Confidence internal)= 0.456 – 0.693; Sensitivity%= 0.978; Specificity% = 0.87; Cut-off points= 5.23], 0.7 [95% Cl (Confidence internal)= 0.524 – 0.768; Sensitivity% = 0.955; Specificity% = 0.754; Cut-off points = 0.661], and 0.6 [95%Cl (confidence internal) 0.482–0.779; Sensitivity%= 0.91; Specificity%= 0.986; Cut-off points =0.135] respectively.

Table (3-10): AUC, optimal threshold, Sensitivity, and specificity of Ferritin, d-dimer and SAA/CRP ratio obtained by the ROC curves for prediction of critical COVID-19 patients.

Critical- COVID-19	AUP	Sensitivity %	Specificity %	Cut-off points	95% CI
Ferritin	0.7	0.80	0.7	355.5	0.511-0.8
D-dimer	0.6	0.82	0.75	471	0.482-0.779
SAA/CRP ratio	0.7	0.955	0.754	0.0661	0.524-0.768

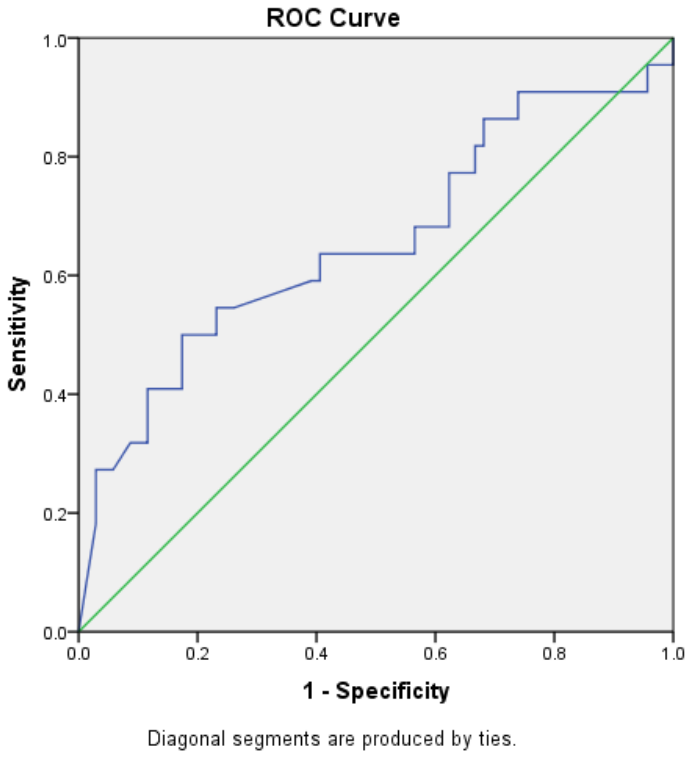

Figure (3-9): Receiver operating characteristics (ROC) curve analysis of Ferritin in the critical COVID-19 patients.

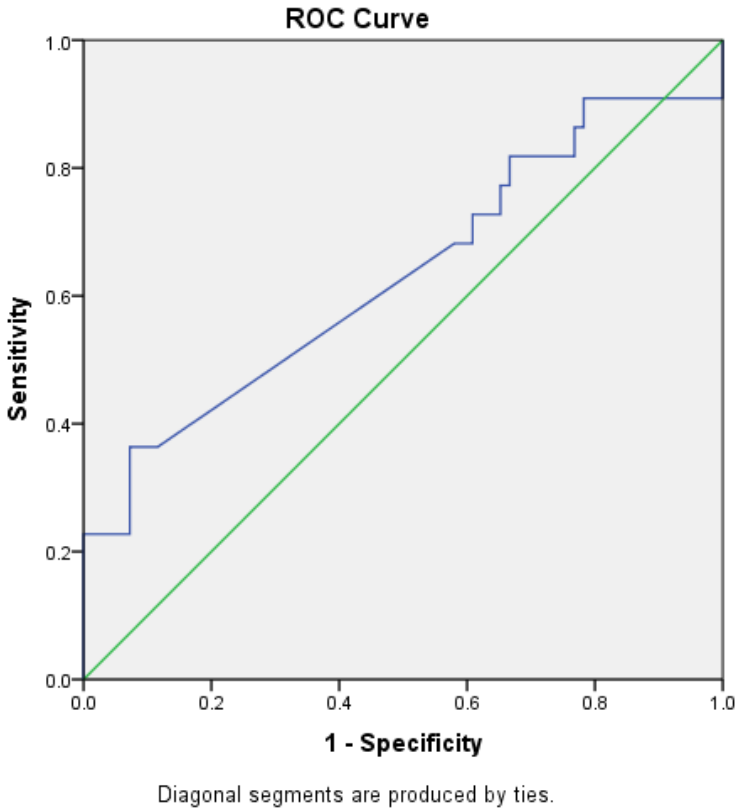

Figure (3-10): Receiver operating characteristics (ROC) curve analysis of d-dimer in the critical COVID-19 patients.

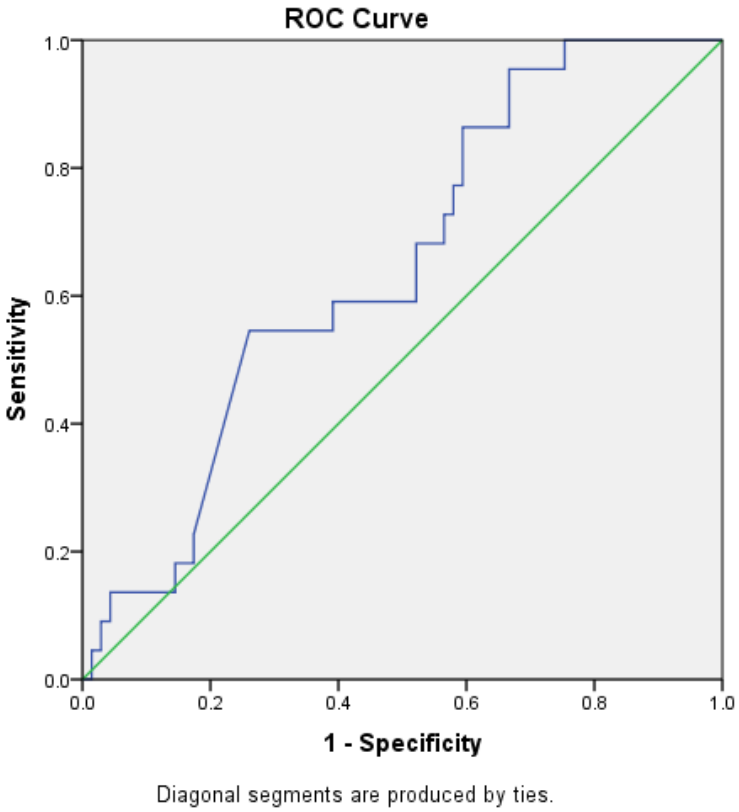

Figure (3-11): Receiver operating characteristics (ROC) curve analysis of SAA/ CRP ratio in the critical COVID-19 patients.

Levels of both CRP and SAA were statistically significant among COVID-19 patients groups. The combination of these levels were assessed. In both COVID-19 patient groups (severe and critical) the SAA/CRP ratio was indicated the high prognosis value of the combined inflammatory indicators. Receiver operating characteristics curves indicated that the diagnostic performance of the SAA/ CRP ratio in both COVID-19 groups exhibited much better predictive value than other tests. Infection causes changes in inflammatory markers and immune cells which were significantly different based on disease severity.

However, levels of CRP and SAA, which are common inflammatory indicators, may be more conducive to universal screening. Increases in the levels of CRP, which is secreted by the liver, occur as a direct response to injury or infection. During acute inflammation and infection, CRP levels can be correlated with disease severity [(237)], a finding also confirmed in this study. During the acute phase of the disease, large amounts of cytokines (IL-1, IL-6, and TNF-α) stimulate the synthesis and release of SAA by liver cells. During viral infection, SAA levels are more sensitive than CRP[(238)] .

Since severe cases of COVID-19 may be associated with a mix of viral and bacterial infections, using a combination of CRP and SAA levels may better predict the severity of disease.

FINAL CONCLUSION AND FUTURE WORK

Final conclusion and future work

A) Final conclusion:

An accurate prediction of disease severity during the early stages of the disease can effectively help in the essential interventions during these early disease stages. This study focused on the prognostic value of inflammatory markers such as SAA, CRP and other general tests in predicting COVID-19 severity, the study concluded that:

1) Levels of hematological parameters (RDW-CV, WBC, NEU%, LYM% and NLR) were varied based on severity of disease.

2) In critical COVID-19 patients, CRP had shown a significant positive-correlation with NEU% and NLR and a significant negative-correlation with LYM%.

3) Serum ferritin level was significantly higher in critical cases.

4) In severe COVID-19 patients, SAA level had shown a significant negative correlation with RDW-CV, NEU%, and NLR, while showing a positive correlation with LYM%.

5) Study indicated a significant correlation between SAA level and O2 levels in severe group.

6) Ratio of Predictive value for the inflammatory indicators (SAA/CRP) had shown to be statistical difference between the patient groups. The SAA/CRP ratio was significantly higher in severe and critical groups than those of moderate patients.

7) Levels of both CRP and SAA were statistically significant among COVID-19 patients groups. The combination of these levels were assessed. In both COVID-19 patient groups (severe and critical), the SAA/CRP ratio

demonstrated a high prognosis value of the combined inflammatory indicators. Receiver operating characteristics curves indicated that the diagnostic performance of the SAA/CRP ratio in both COVID-19 groups exhibited much better predictive value than other tests.

B) Future work:

1) The precise mechanism by which SAA plays a role in the pathogenesis of COVID-19 needs further investigation in the future.
2) Further research is warranted to better characterize and justify the routine clinical use of serial SAA assessments in COVID-19.
3) Correlation between SAA and monitoring the recovery process has important clinical values.
4) There is a potential needing for the methodological factors that contributed between-studies heterogeneity that include the use of different SAA detection methods based on immuno-based assays, different antibodies against various SAA components, and different calibrators in individual studies.

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Appendix

Questionnaire

Patient name	Sex	Age

Adress

Smoking : ☐ Autoimmune disease : ☐

Hypertensive : ☐ Diabetes mellitus : ☐

Disease state : Mild ☐ Moderate ☐

Severe ☐ Critical ☐

Temp.	SOB	Dry cough	O2 sat.
Other symptoms			

The main investigations :
CRP
Ferritin
Other investigations :

الملخص

نبذة: واجهت مصادر الصحة العالمية تحديات كبيرة من جائحة فيروس كورونا المستجد منذ شهر كانون الأول في عام ٢٠١٩. بتاريخ ٢٠ تموز/٢٠٢١ أقرت منظمة الصحة العالمية وجود حوالي ١٩٠١٦٩٨٣٣ حالة مؤكدة من المصابين بفيروس كورونا المستجد، و ٤٠٨٦٠٠٠ حالة وفاة مؤكدة بسبب الفيروس. لذا من المهم معرفة أفضل المؤشرات الحيوية التي قد تتعلق بشدة المرض لمنع حالات الوفاة قدر الإمكان. المؤشرات الحيوية التي درست في المصابين بفيروس كورونا المستجد كانت مختلفة و لها دور مهم في تشخيص المرض و العلاج و التنبؤ بالحصيلة السريرية للمرضى. يوجد حاليًا تقارير قليلة عن علاقة الأميلويد أ (SAA) مع شدة الإصابة بفيروس كورونا المستجد.

يعتبر أميلويد أ (SAA) أحد مكونات البلازما و السلف لمادة الأميلويد. وكذلك هو أحد بروتينات الطور الحاد (Acute phase reactants) و ينتج بشكل رئيسي في الكبد بالإستجابة إلى السايتوكينات المنشطة للإلتهابات (Proinflammatory cytokines) التي تفرز من الخلايا الوحيدة (Monocytes) النشطة. لذا كان الهدف من هذه الدراسة هو إختبار إحتمالية توظيف مستويات بروتين أميلويد أ (SAA) أمام شدة الإصابة بفيروس كورونا المستجد لسبب كونه قادر على تعزيز الاستجابة الإلتهابية من خلال تنشيط أجسام الكيماويات (Chemokines) و تحفيز عملية الإنجذاب الكيميائي (Chemotaxis) حتى بوجود تركيز واطئ جدًا منه.

طرق العمل و المواد: نوع الدراسة كان من الدراسات المستعرضة (Cross-sectional study). تم جمع بيانات طبية لـ ٩١ راقد مصاب بفيروس كورونا المستجد في ردهة الحياة في مدينة الإمام الحسين الطبية في كربلاء، و قسمت الحالات إلى ثلاث مجاميع إعتمادًا على شدة الإصابة (معتدلة، و حادة، وحرجة). لقد قيست مستويات المؤشرات الحيوية كالتالي:

١. إستخدام تقنية المقايسة الإمتصاصية المناعية للإنزيم المرتبط (ELISA) لقياس أميلويد أ (SAA) في مصل الدم.
٢. قياس بروتين سي التفاعلي (CRP) بالقياس الضوئي للمعقدات المناعية (Turbidimetry method) في مصل الدم.
٣. قياس الفرتين (Ferritin) في مصل الدم و دي دايمر (D-dimer) في البلازما بطريقة التضوء الكيميائي المناعي (CLIA).
٤. قياس عدد كريات الدم (CBC) في جهاز (XP-300™ Automated hematology analyzer Sysmex).

تم تقدير الإرتباط بين المؤشرات الكيماوية الحيوية و شدة المرض. و كذلك قدرت كفاءة القيمة التنبؤية بوساطة تحديد المنحنيات المميزة لأداء المستقبل (ROC).

النتائج: مستويات المؤشرات الدموية (معامل الإختلاف لعرض توزيع خلايا الدم الحمراء (RDW-CV)، و عدد كريات الدم البيضاء (WBC)، و نسبة خلايا العدلات (NEU%)، و نسبة الخلايا اللمفاوية (LYM%)، و النسبة بين خلايا العدلات إلى الخلايا اللمفاوية (NLR)) كانت متغايرة إعتمادًا على شدة الإصابة. بروتين سي التفاعلي أظهر ارتباط مهم مع (نسبة خلايا العدلات (NEU%)، و نسبة الخلايا اللمفاوية (LYM%)، و النسبة بين خلايا العدلات إلى الخلايا اللمفاوية (NLR)) في مجموعة مرضى الحالة الحرجة. أيضًا مستويات الفرتين في مصل الدم كانت مرتفعة بشكل ملحوظ إحصائيًا في الحالات الحرجة. مستويات الأميلويد أ (SAA) أظهرت إرتباطًا عكسيًا ذا معنى احصائي مع كل من (معامل الإختلاف لعرض توزيع خلايا الدم الحمراء (RDW-CV)، و نسبة خلايا العدلات (NEU%)، و النسبة بين خلايا العدلات إلى الخلايا اللمفاوية (NLR)). بينما كانت في إرتباط طردي مع نسبة الخلايا اللمفاوية (LYM%) في الحالات الحادة.

نسبة القيمة التنبؤية للمؤشرات الإلتهابية (بروتين أميلويد أ إلى بروتين سي التفاعلي (SAA/CRP)) أظهرت فرقًا إحصائيًا بين مجاميع المرضى. حيث كانت مرتفعة بين المجاميع الحادة و الحرجة بالمقارنة مع حالات المرضى المعتدلة. المنحنيات المميزة لأداء المستقبل أشارت إلى أن الأداء التشخيصي لنسبة بروتين أميلويد أ إلى بروتين سي التفاعلي (SAA/CRP) في كلا المجموعتين الحادة و الحرجة أظهرت قيمة تنبؤية أفضل بكثير من باقي الفحوصات.

الخلاصة: مستويات كل من بروتين سي التفاعلي (CRP) و بروتين أميلويد أ (SAA) كانت مرتفعة بشكل ملحوظ إحصائيا بين مجاميع مرضى فيروس كورونا المستجد. كذلك لقد قيس الإتحاد بين هذين المؤشرين (نسبة بروتين أميلويد أ إلى بروتين سي التفاعلي (SAA/CRP)) و كانت نتيجتها تبرهن قيمة تعقب عالية بين المؤشرات الالتهابية المتحدة في كلا مجاميع مرضى فيروس كورونا المستجد (الحادة و الحرجة).

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بروتين أميلويد أ في مصل الدم كمؤشر لشدة مرض كوفيد-١٩ مقارنة مع بروتينات الطور الحاد الأخرى

رسالة ماجستير

إلى مجلس كلية الطب/ فرع الكيمياء و الكيمياء الحياتية/ جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في الكيمياء السريرية

من قبل

سهيلة ريحان

بكالوريوس تحليلات مرضية – كلية العلوم الطبية التطبيقية – جامعة كربلاء – 2018

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2021 م **1443 هـ**