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Assessment of Vessels Endothelial Dysfunction by Protease-Activated Receptors (PARs) Signaling in COVID-19 Patients

A Thesis

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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

اقْرَأْ وَرَبُّكَ الْأَكْرَمُ ﴿ ٣ ﴾ الَّذِي عَلَّمَ بِالْقَلَمِ ﴿ ٤ ﴾ عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ ﴿ ٥ ﴾

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Supervisors Certification

We certify that this M.Sc. thesis entitled:-

"Assessment of Vessels Endothelial Dysfunction by Protease-Activated Receptors (PARs) Signaling in COVID-19 Patients"

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Dedication

To the one who gave me understanding and knowledge
To my Lord and Creator
To my beloved Prophet Muhammad
To the soul of grandfather Syed Jassim
To the soul of my professor, Dr. Shamma
To my beloved father and mother
To my kind wife and dear daughter
To my brother and sisters
To my friends
To The Iraqi people
To my great Arab nation
To my beautiful world
To All patients

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Summary

Background: One of the major factors in the development of thromboembolism in COVID-19 has become recognized to be endothelial dysfunction. Acute venous thromboembolism is another significant complication that significantly contributes to the substantial morbidity and mortality in patients with COVID-19. Despite of the fact that hypoxic respiratory failure caused by viral pneumonia and acute respiratory distress syndrome remains the leading cause of death in patients with COVID-19. The level of protease-activated receptors (PARs) would be correlated with the common coagulation and inflammation markers of COVID-19 because protease-activated receptor type 1 (PAR1) is the primary thrombin receptor and may reflect an important connection between coagulation and inflammation in the pathophysiology of COVID-19.

Methodology: A cross-sectional study for a total of 93 patient samples, including critical, severe, and post-COVID19 patient samples at Al-Shehid Hassan Al-Hatmy Hospital in Al-Najaf Governorate, Iraq. This study was done during 3 months. Serum biomarkers levels were measured for the following parameter: PAR-1, thrombin and prothrombin levels were measured using the ELISA technique.; D-Dimer assay was 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 the receiver operating characteristic (ROC) curve.

Results: Levels of hematological parameters were varied based on severity of the disease. Serum PAR-1 level was quietly increased in post COVID-19 and critical cases. Levels of thrombin were shown a massive significant increase in severe and critical cases ($p < 0.05$). In critical cases, a weakly correlation was confirmed between D-dimer and PAR-1. Also, a significant positive correlation was confirmed between Platelet-Lymphocyte Ratio (PLR) and Neutrophil-Lymphocyte Ratio (NLR) [$r = 0.55$, $P < 0.001$]

Results of the receiver operating curve (ROC) curve , area under the curve (AUC) analysis for the PAR-1 and Thrombin levels as possible diagnostic parameters. Both parameters were shown a good diagnostic performance for the prediction critical

cases of COVID-19 Patients compared to severe and post cases group. PAR-1 levels: (sensitivity = 96%, specificity = 92%) at a level = 596, while thrombin levels: (sensitivity = 0.96%, specificity = 0.76%) at a level = 39.5.

Conclusion: Levels of PAR-1 were statistically significant among groups of COVID-19 patients. Among the three groups of COVID-19 patients (severe, critical and post cases), D-dimer and PAR-1 levels were demonstrated a high prognosis value through the confirming results that obtained by the ROC curves for the prediction of coagulopathy in post cases of COVID-19.

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List of Abbreviations
2019-nCoV: 2019-new Coronavirus
ACE2: Angiotensin-Converting Enzyme 2
ADP: Adenosine Diphosphate
ALI: Acute Lung Injury
Ang II: Angiotensin II
ARDS: Acute Respiratory Distress Syndrome
AUC: Area Under Curve
CBC: Complete Blood Count
CD147: Clusters of Differentiation 147
COVID-19: 2019 Coronavirus Disease
CRP: C-reactive protein
CT scans: Computerized axilla Tomography scans
DIC : disseminated intravascular coagulation
DVT: deep vein thrombosis
EDTA: Ethylene Diamine Tetra Acetic acid
ELFA: Enzyme-Linked Fluorescent Assay
ELISA: Enzyme-Linked Immunosorbent Assay
FbDP: fibrin degradation products
GP: glycoprotein
GPCRs: G protein-coupled receptors
Hb: Hemoglobin
HRP : Horseradish Peroxidase
ICAM-1: Intercellular Adhesion Molecule 1
IFN-ϒ: interferon gama
IL-6: interleukin 6

IL-8: interleukin 8
MCP-1: Monocyte Chemoattractant Protein-1
MERS-CoV: Middle Eastern Respiratory Syndrome Coronavirus
MPV: Mean Platelet Volume
NLR: Neutrophil-Lymphocyte Ratio
OD: optical density
PAR-1: Protease Activated Receptor-1
PARs: Protease Activated Receptors
PE: Pulmonary Embolism
PLR: Platelet-Lymphocyte Ratio
RBC: Red Blood Cells
RBD: receptor-binding domain
ROC: Receiver Operating Characteristic
ROS: Reactive Oxygen Species
RT-PCR: real time –polymer chian reaction
S protein: spike protein
SARS-CoV: Severe acute respiratory syndrome coronavirus
SARS-CoV-2: severe acute respiratory syndrome coronavirus 2
SOB: shortness of breath
SPR: solid phase receptacle
SPSS: Statistical Package for the Social Sciences
STRs: sealed reagent strips
TF: tissue factor
TMPRSS-2: transmembrane protease serine 2
TNF-α: tumor necrotic factor- alpha
TXA2: Thromboxane A2

VASP: vasodilator-stimulated phosphoprotein
VCAM-1: vascular cell adhesion molecule-1
VEGF: vascular endothelial growth factor
Vwf: and von Willebrand factor
WBC: White blood cells
WHO: World Health Organization

Chapter One

Introduction

1. Introduction

1.1. The Pandemic of Coronavirus Disease 2019 (COVID-19)

In December 2019, a pneumonia outbreak of unknown etiology was discovered in Wuhan, Hubei Province, China, with these cases being patients who visited a local fish and wild animal market the previous month (Lu H, Stratton CW, & Tang YW, 2020). Using samples from the same pneumonia patients, Chinese investigators found a new strain of betacoronavirus, also referred to as the 2019-new coronavirus (2019-nCoV) (Zhu N, Zhang D, Wang W, & *et al,* 2020). The 2019 Coronavirus Disease (COVID-19) is caused by the 2019-nCoV identified as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Shreya R. S., Kedar S. P., & Lokesh K. B., 2021). It's a highly infectious and contagious illness. This condition has affected 2,761 people in China, resulting in 80 deaths, and has infected 33 people in ten other countries as of January 26, 2020 (Zhou P, Yang XL, Wang XG, & *et al.*, 2020). The World Health Organization (WHO) declared COVID-19 a global pandemic on March 11, 2020, because the disease has a global impact, affecting more than 200 countries, and it has gotten out of control, with well over one million cases and causing over 60, 000 deaths worldwide by the beginning of April 2020 (Mahmoud G. , Kaiming W., Anissa V., & *et al*., 2020) (Mitra Abbasifard & Hossein Khorramdelazad, 2020).

Coronaviruses are a huge family of enveloped single-stranded RNA viruses that are found in a variety of animal species, along with humans (Shreya R. S., Kedar S. P., & Lokesh K. B., 2021) (Wan Y, Shang J, Graham R, & *et al.*, 2020). Their envelope consists of the spike –S-, envelope –E-, and membrane –M- proteins, as well as the nucleocapsid –N- inside the virion, which completely covers the nucleic material as RNA, as shown in figure (1-1). Severe acute respiratory syndrome coronavirus (SARS-CoV), Middle Eastern respiratory syndrome coronavirus (MERS-CoV), and now SARS-CoV-2 are three zoonotic strains of the beta-coronavirus genus that have been proven to cause severe respiratory illnesses like severe pneumonia, acute lung injury in humans (ALI), and acute respiratory distress syndrome (ARDS) (James D. M., Hannah S., & Karlheinz P., 2020) (Shreya R. S., Kedar S. P., &

Lokesh K. B., 2021).

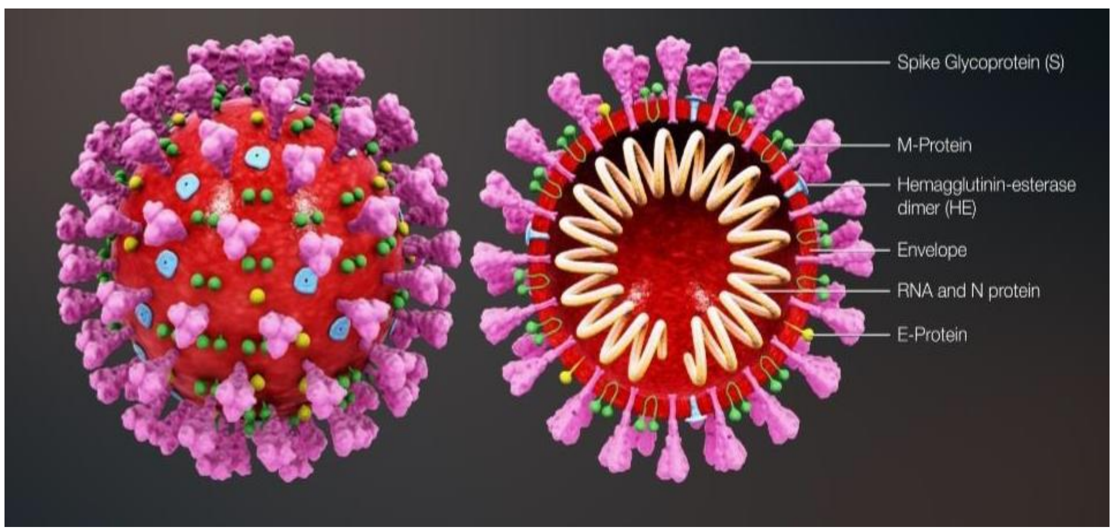


Figure 1-1: It shows the structure of SARS-CoV-2. It represents the SARS-CoV-2 structure and the important proteins that it contains (Fenna & Ian, 2020).

1.2. Infection Process

Coronaviruses enter host cells by binding to a host receptor, angiotensin-converting enzyme 2 (ACE2), but then fuse viral and host membranes through the use of an envelope-anchored spike protein (S protein), which is defined by the SARS-CoV spike's receptor-binding domain (RBD) (Wan Y, Shang J, Graham R, & *et al.,* 2020). On the other hand, more recent studies have indicated that clusters of differentiation 147 (CD147) and CD209 can act as receptors for SARS-CoV-2 (Mitra Abbasifard & Hossein Khorramdelazad, 2020) (Wang, Chen, Zhou, & *et al.,* 2020). An S-protein is a glycoprotein with S1 and S2 subunits. The S1 subunit is in charge of binding to the receptor on the host cell, while the S2 subunit aids in viral and host cell fusion (James D. M., Hannah S., & Karlheinz P., 2020) (Walls A.C., Park Y.J., Tortorici M.A, & *et al*., 2020). The presence of an a polybasic furin cleavage site at the junction of S1 and S2 is a novel structural feature of SARS-CoV-2 compared to SARS-CoV, in addition to different amino acid residues within the RBD of the S protein. The infectivity and host range of SARS-CoV-2 are thought to be influenced by the cleavage of the S protein by cellular proteases such as furin and transmembrane protease serine 2 (TMPRSS-2) (James D. M., Hannah S., & Karlheinz P., 2020) (Walls A.C., Park Y.J., Tortorici M.A, & *et al*., 2020).

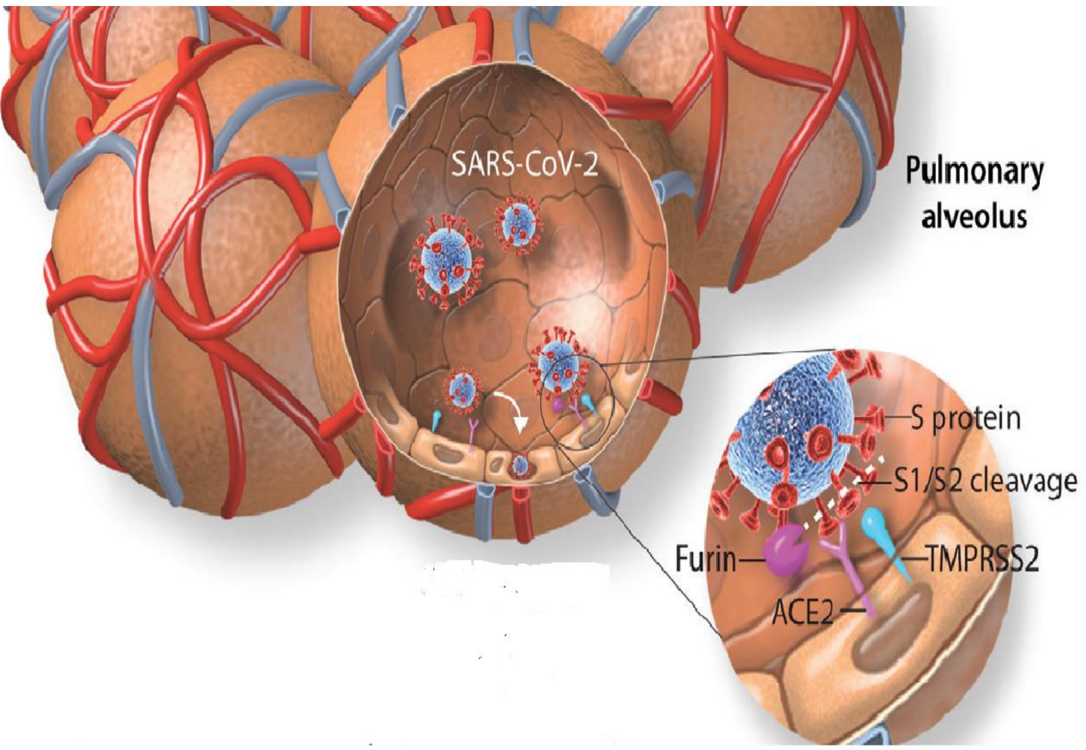


Figure 1-2: An overview of SARS-CoV-2 entry into host lung epithelial cells via the spike (S) glycoprotein bound to angiotensin-converting enzyme 2 (ACE-2) by furin and TMPRSS-2 (transmembrane protease serine 2) (James D. M., Hannah S., & Karlheinz P., 2020).

It's worth noting that ACE2 is strongly expressed on the surface of alveolar type II epithelial cells in the human lung, inferring that SARS-CoV-2 could find a suitable host there (Markus Hoffmann, Hannah K W, Simon Schroeder, & *et al.*, 2020). On the other hand, more types of cells, like immune cells such as macrophages and monocytes, can also express ACE2, as shown in figure (1-3), but to a lesser extent than alveolar type II endothelial cells, and as a result, they are susceptible to infection (Zaid Abassi , Yara Knaney, Tony K., & Samuel N. H., 2020). At the time of SARS-CoV-2 infection, dysregulated inflammatory responses, immunocyte infiltration, and uncontrolled release of inflammatory cytokines are considered vital factors in the development of severe pneumonia, ARDS, or multi-organ failure (Mitra Abbasifard & Hossein Khorramdelazad, 2020) (Huang C. , Wang Y. , Li X., & *et al.*, 2020).

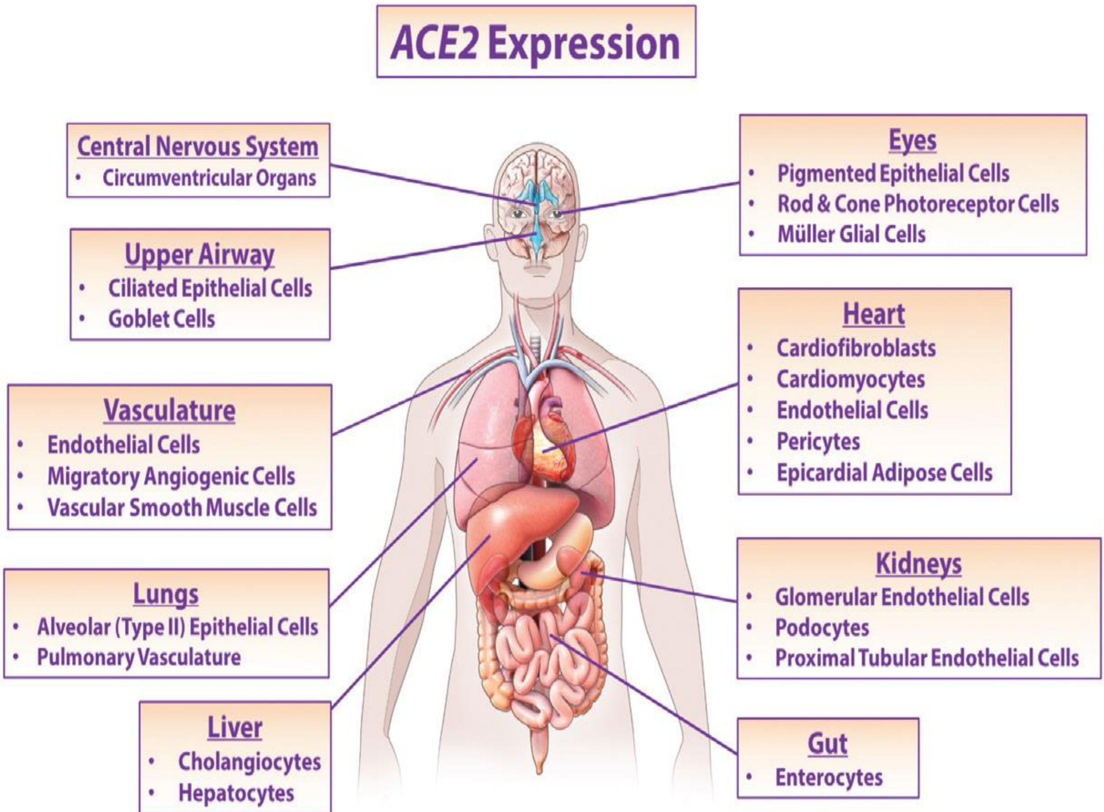


Figure 1-3: It shows the expression of ACE2 in the vascular system, heart, kidneys, liver, intestines, central nervous system, and lung cells (Mahmoud G. , Kaiming W., Anissa V., & *et al.*, 2020).

1.3. Clinical Features of COVID-19

COVID-19 causes a wide range of symptoms, ranging from complete asymptomatic infection to mild flu-like symptoms to life-threatening ARDS (Kieron South, Laura McCulloch, Barry W McColl, & *et al.*, 2020). Estimates suggest that up to 86 percent of cases in China are asymptomatic or mildly symptomatic, and thus go untreated (Kieron South, Laura McCulloch, Barry W McColl, & *et al.*, 2020) (Li R, Pei S, Chen B, & *et al*, 2020). COVID-19 cases are organized and listed in the table (1-1).

Table1-1: COVID-19 cases according to World Health Organization (WHO, 27 May 2020)

Cases	Clinical Features
Mild Cases	Symptomatic patients: COVID-19 without evidence of hypoxia or pneumonia. Fever, cough, fatigue, anorexia, dyspnea, and myalgia are common symptoms.
Moderate Cases	Fever, cough, dyspnea, and rapid breathing but no signs of severe pneumonia.
Severe Cases	Fever, cough, dyspnea, and rapid breathing plus one of the following symptoms: 1. a rate of respiration greater than 30 breaths per minute 2. Exacerbation of respiratory distress. 3. Room air with a SpO2 of 90 percent.
Critical Cases	The appearance of ARDS, sepsis, or septic shock, as well as other complications such as acute pulmonary embolism, acute coronary syndrome, acute stroke, and delirium.

Fever, cough, myalgia, anosmia, and fatigue are the most common symptoms at the beginning of an illness (Kieron South, Laura McCulloch, Barry W McColl, & *et al.*, 2020). Bilateral ground–glass opacity, interlobular septal thickening, and pleural thickening are all familiar chest radiological findings (Shi H, Han X, Jiang N, & *et al.*, 2020) (Kieron South, Laura McCulloch, Barry W McColl, & *et al.*, 2020). Pleural effusion, lymphadenopathy, and round cystic changes were also seen in patients who developed ARDS (Kieron South, Laura McCulloch, Barry W McColl, & *et al*., 2020). Patients with ARDS also have severe hypoxemia, and the leading causes of death are respiratory failure, heart failure, fulminant myocarditis, and multi-organ failure (Kieron South, Laura McCulloch, Barry W McColl, & *et al.*, 2020).

A condition may occur in COVID-19 patients, which is known as the cytokine storm, when the inflammatory response is abnormal, resulting in damage to the heart, kidney, and liver tissues, as well as respiratory or multi-organ failure (Shreya R. S., Kedar S. P., & Lokesh K. B., 2021). This uncontrolled 'cytokine storm' was discovered to play a key role in symptom exacerbation and disease progression, making it a significant contributor to COVID-19-related mortality (Cao x, 2020) (Shreya R. S., Kedar S. P., & Lokesh K. B., 2021). Due to the presence of a limited inflammatory condition, individuals over 60 years of age with co-morbid conditions such as cardiovascular disease, diabetes, and chronic respiratory and inflammatory diseases are at a higher risk of developing COVID-19 or progressing to a severe disease state (Baviskar T, Raut D, & Bhatt LK, 2021).

1.4. Vessels Endothelial Cells

Endothelial cells are usually composed of an inner layer of blood vessels, and based on the tissues and organs, they have various structures and functions (Yuefei Jin, Wangquan Ji, Haiyan Yang, & et al., 2020). Pericytes normally shield them, which support the vessel structure (Sturtzel, 2017). Attempting to control vascular permeability and regulating vascular tone are multiple commonly accepted roles and responsibilities of endothelial cells in body physiology, as depicted in figures (1-4).

1.5. Vessels Endothelial Dysfunction (Vasculitis) in COVID-19

Endothelial dysfunction is caused by endothelial hyperactivation (Kieron South, Laura McCulloch, Barry W McColl, & *et al.*, 2020). Endothelial activation occurs by the activation of protease activated receptor-1 (PAR-1) on endothelial cells (Yuefei Jin, Wangquan Ji, Haiyan Yang, & *et al.*, 2020).

1.5.1. Endothelial Hyperactivation

Thromboxane A2 (TXA2), reactive oxygen species (ROS), and angiotensin II (Ang II), which act as proinflammatory, vasoconstrictive, vasodilatative, and/or procoagulatory factors (Yuefei Jin, Wangquan Ji, Haiyan Yang, & *et al.*, 2020). All these can be synthesized and released when thrombin activates PAR-1 on endothelial cells. Additionally, endothelial cells play a role in the activation, adhesion, and migration of leukocytes as well as the balance of fibrin (Kruger-Genge A, Blocki A.,

Franke R. P. , & Jung F, 2019) (Ekholm M & Kahan T , 2021).

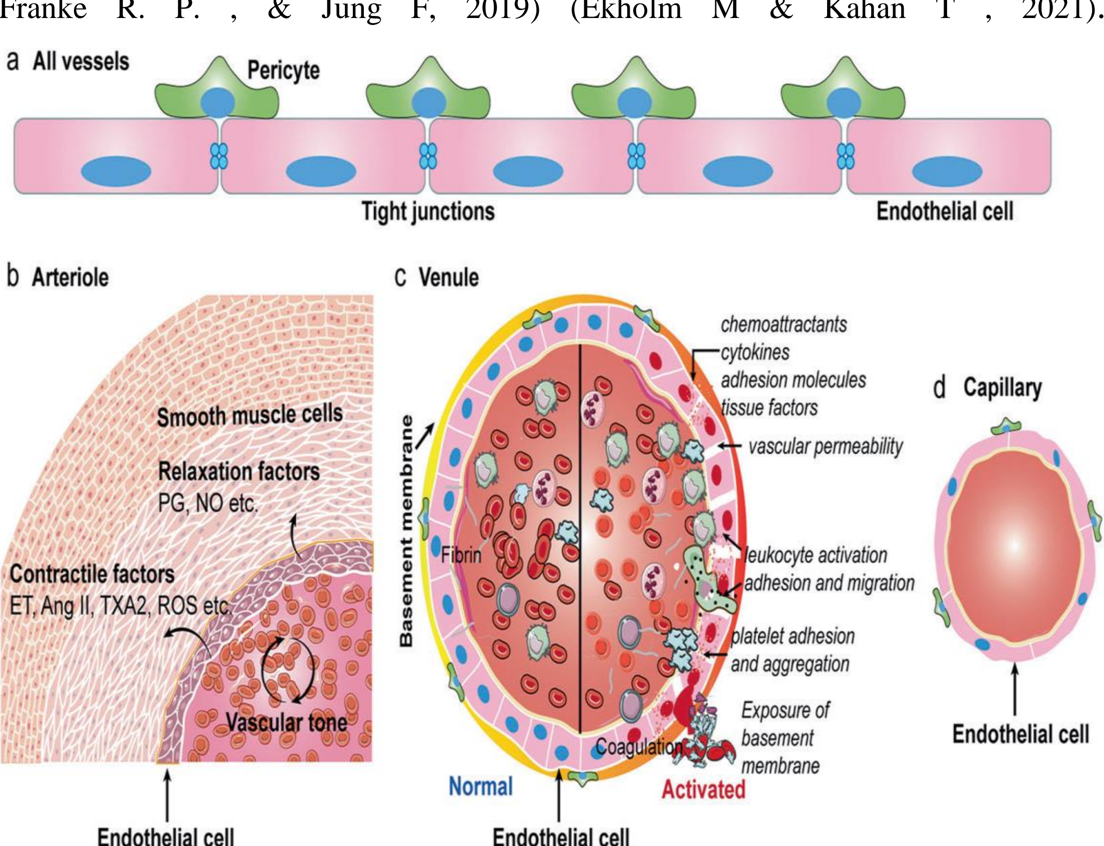


Figure 1-4: An overview of normal endothelial cells, which serve as permeability barriers in the inner layer of blood vessels like arteries, veins, and capillaries (a), b, and c). Endothelial cells that are activated release cytokines, assist in the adhesion and movement of immune cells, and facilitate coagulation (Yuefei Jin, Wangquan Ji, Haiyan Yang, & *et al.*, 2020).

In addition to mobilizing P-selectin and von Willebrand factor (Vwf), activation of PAR-1 also motivates endothelial activation, endothelial cell rolling, and subsequently firm adhesion of platelets and leukocytes (Ekholm M & Kahan T , 2021). Inflammatory cytokines like interleukin 6 (IL-6), interleukin 8 (IL-8), and monocyte chemoattractant protein-1 (MCP-1), adhesion molecules like intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), vascular adhesion protein-1 (VAP-1), and vascular endothelial growth factor (VEGF), and chemokines are extensively produced when endothelial cells are directly or indirectly activated (Teuwen L. A., Geldhof V., Pasut A., & Carmeliet P, 2020) (Yuefei Jin, Wangquan Ji, Haiyan Yang, & *et al.*, 2020). This may cause a cytokine storm and localized inflammation of inflammatory cells. As a result, pulmonary hemorrhage and alveolar edema are known to result from endothelial hyperactivation

during viral infection, which also causes the loss of tight junctions, increased vessel permeability, and endothelial dysfunction (Kieron South, Laura McCulloch, Barry W McColl, & *et al.*, 2020).

1.5.2. Endothelial Dysfunction in COVID-19

Endothelial cells' expression of PAR-1 is more strongly during inflammation and endothelial dysfunction (Ekholm M & Kahan T , 2021). Also, viral infection can cause an unbalanced endothelial dysfunctional response, which can stimulate the coagulation pathway (Sturtzel, 2017). Among the above-mentioned causes, pulmonary endothelial dysfunction and damage have been linked to ARDS (Teuwen L. A., Geldhof V., Pasut A., & Carmeliet P, 2020). Accordingly, ARDS, pulmonary edema, cytokine storm, sepsis, and diffuse coagulopathy are the leading causes of death in patients with critical COVID-19 (Jin Y., Yang H., Ji W., & et al., 2020). The early detection of individuals at high risk of developing serious complications will be made easier with an understanding of the mechanisms of endothelial activation and dysfunction during COVID-19 infection (Yuefei Jin, Wangquan Ji, Haiyan Yang, & *et al.*, 2020).

Patients who died from COVID-19 had dysfunctional epithelial cells that were swollen with foamy decay and a few spots of segmental fibrin thrombus in the glomerular capillary loops (Varga Z, Flammer A.J., Steiger P., & *et al.*, 2020). The thrombogenic basement membrane is exposed and the coagulation cascade is activated as a result of endothelial activation and dysfunction, disruption of vascular integrity, and epithelial cell apoptosis (Sturtzel, 2017). Patients with COVID-19 frequently experience a stroke, thrombosis in the extracorporeal circuits, arterial thrombosis, deep vein thrombosis (DVT), and pulmonary embolism (PE) (Merrill J. T., Erkan D. , Winakur J., & James J. , 2020) (Teuwen L. A., Geldhof V., Pasut A., & Carmeliet P, 2020). It has been suggested that organ failure and ARDS are related to microthrombus formation (Ranucci M., Ballotta A., Dedda U. D., & *et al.*, 2020). Figure 1–5 provides an overview of endothelial activation and dysfunction in COVID-19.

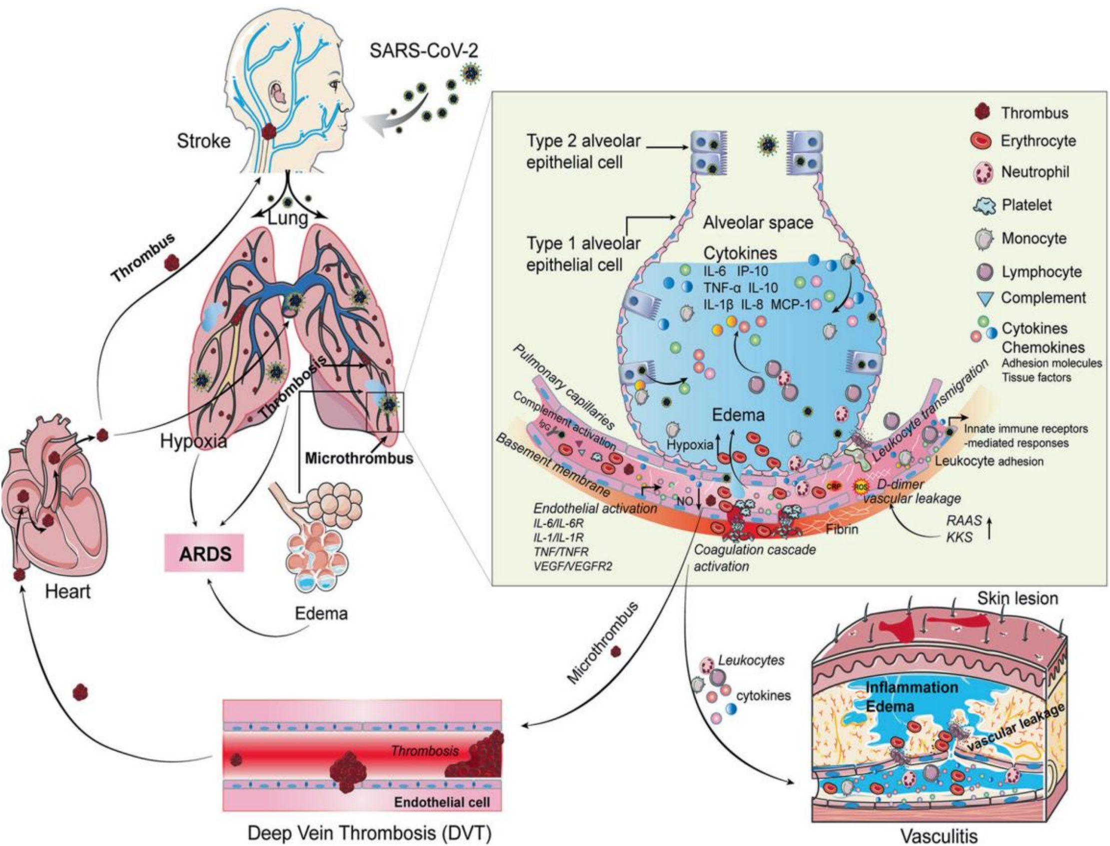


Figure 1-5 : An Overview of endothelial activation and dysfunction in COVID-19. SARS-CoV-2 infection is followed by an overabundance of cytokines released by immune and epithelial cells. (Yuefei Jin, Wangquan Ji, Haiyan Yang, & et al., 2020).

1.6. Endothelial Dysfunction-Mediated Coagulopathy in COVID-19

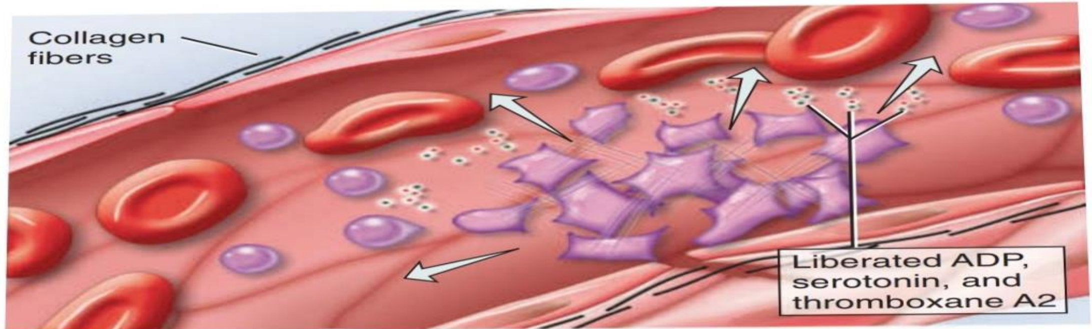
The thrombotic formation response includes platelet activation and a coagulation cascade (Parker WAE & Storey RF , 2018).

1.6.1. Platelet Activation

Several stimulants initiate platelet activation, such as collagen via glycoprotein (GP) VI receptors and thrombin via protease-activated receptors (Gąsecka, Borovac, Guerreiro, & *et al,* 2021). Platelets change shape from discoid to stellate upon activation due to calcium mobilization and dephosphorylation of vasodilator-stimulated phosphoprotein (VASP). Platelets aggregate as a result of this conformational change (Gąsecka, Borovac, Guerreiro, & *et al,* 2021).

Furthermore, platelets release extracellular vesicles or their outer membrane, which contain a variety of substances such as TXA2 (pro-aggregatory and vasoconstrictor factor), adenosine diphosphate ADP (stimulating and amplifying platelet activation), serotonin (aggregatory factor), P-selectin (proinflammatory and procoagulant factor), and phosphatidylserine (procoagulant) (Gasecka A, Böing AN, Filipiak KJ, & Nieuwland R, 2017), as shown in figure (1-6).

In reality, platelet activation is nevertheless activated by thrombin via PAR1 and PAR4, but platelets can also catalyze thrombin generation via membrane scramblase enzyme activity (Gąsecka, Borovac, Guerreiro, & *et al*, 2021).



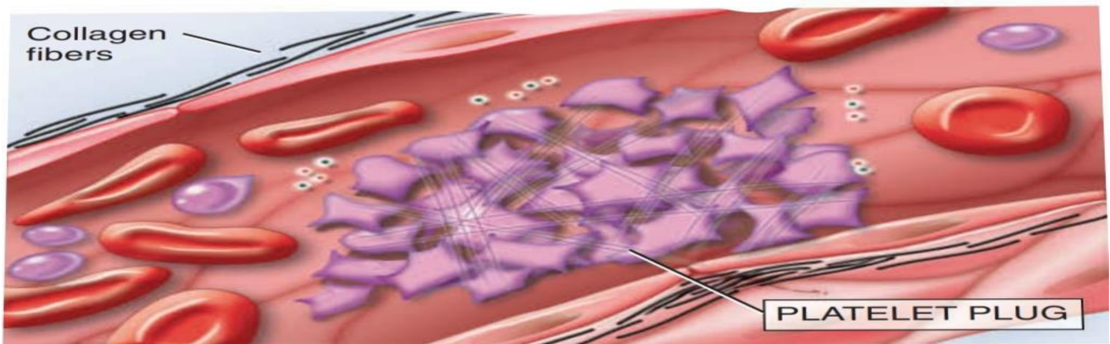


Figure 1-6: An Overview of platelet aggregation and platelet plug formation in blood vessels following platelet activation and platelet releasing processes (Tortora G J & Derrickson B, 2016).

1.6.2. The Coagulation Cascade

The coagulation cascade is categorized into three pathways:

1- The extrinsic pathway, also known as the tissue factor (TF) pathway.

2- The intrinsic pathway, also known as the contact pathway. Both lead to the activation of factor X (Tortora G J & Derrickson B, 2016).

3- The common pathway

In which actives factor X act with factor V and Ca^{+2} to convert prothrombin into thrombin by prothrombinase (Tortora G J & Derrickson B, 2016), as shown in figure (1-7).

Thrombin converts soluble fibrinogen to insoluble fibrin, which forms interweaving strands that are stabilized further by factor XIII (Ekholm M & Kahan T , 2021). The coagulation cascade includes many coagulation (clotting) factors, which are listed in the table (1-2).

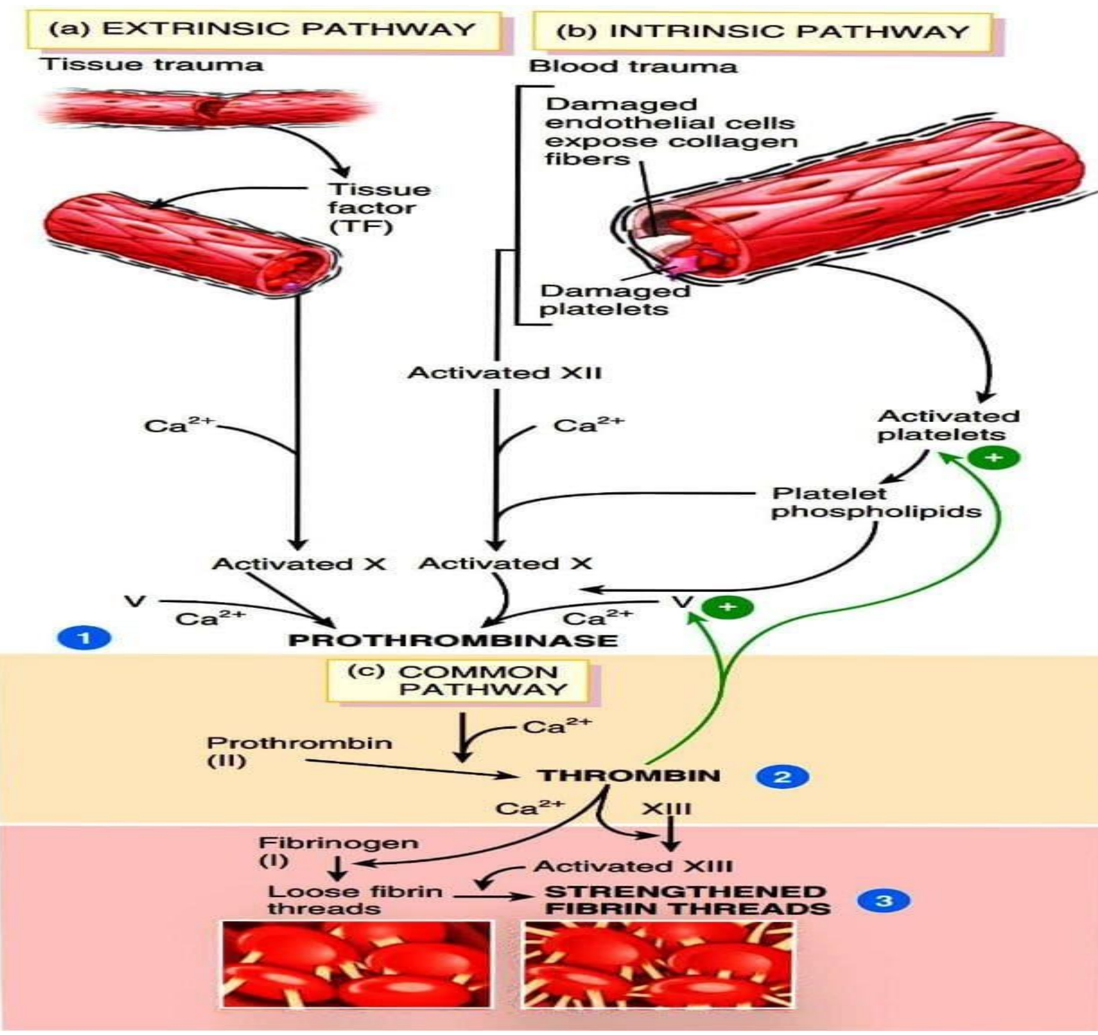


Figure 1-7: Overview of the clotting cascade (Tortora G J & Derrickson B, 2016).

Table1-2: The coagulation (clotting) factors (Anne Waugh & Allison Grant, 2018)

Number of factor	Name of factor
I	Fibrinogen
II	Prothrombin
III	Tissue factor (Thromboplastin)
IV	Calcium (Ca^{+2})
V	Labile factor(Proaccelerin)(Ac-globulin)
VII	Stable factor (Proconvertin)
VIII	Antihaemophilic globulin (AHG)(Antihaemophilic factor A)
IX	Christmas factor (plasma Thromboplastin component)(PTC)(Antihaemophilic factor B)
X	Stuart factor (thrombokinase)
XII	Hageman factor
XIII	Fibrin stabilizing factor
Vitamin K is	essential for synthesis of factors II, VII, IX, and X

1.6.3. Coagulopathy Complications in COVID-19

COVID-19 is in line with the coagulopathy model, which indicates that inflammation and coagulation are closely linked in a bidirectional manner (Jackson SP, Darbousset R, & Schoenwaelder SM, 2019). Inflammation can activate the coagulation system through proinflammatory cytokines, whereas the coagulation system can influence inflammatory activity through thrombin, which is the most important player in activating PARs, which up-regulate inflammatory molecules such as cytokines, chemokines, growth factors, and adhesion molecules (Ekholm M & Kahan T , 2021). Thrombocytopenia has been observed in some COVID-19 patients as a result of increased "platelet consumption" as a result of the complex thrombosis in serious COVID-19 cases (Thachil J, 2020).

Coagulopathy can also be caused by angiotensin signaling, which promotes the release of tissue factor (TF), the first step in the coagulation cascade (Krishna Sriram & Paul A. Insel, 2021) (Grover S. P & Mackman N., 2018). Endothelial cells, alveolar epithelial cells, fibroblasts, and innate immune cells, among others, have TF in response to injury and inflammation (Joly BS, Siguret V, & Veyradier A., 2020). Accordingly, angiotensin II can stimulate the production and expression of TF in a variety of cell types. Patients with the metabolic syndrome, such as hypertension, diabetes, and obesity, have increased TF synthesis, particularly from inflammatory and vascular cells. As a result, COVID-19 patients with metabolic syndrome may have an increased risk of coagulopathy (Krishna Sriram & Paul A. Insel, 2021).

Recent research demonstrates the formation of disseminated intravascular coagulation (DIC) in SARS-CoV-2 infection patients (Tang N, Li D, Wang X, & *et al,* 2020) (Guan W, Ni Z, Hu Y, & *et al,* 2020). DIC is a condition in which blood clots form all over the body's small vessels (AlSubaie AM, 2020). It's possible that the lungs are the source of this coagulopathy, which then spreads to other vital organs and causes multi-organ failure and ARDS as shown in figure (1-8).

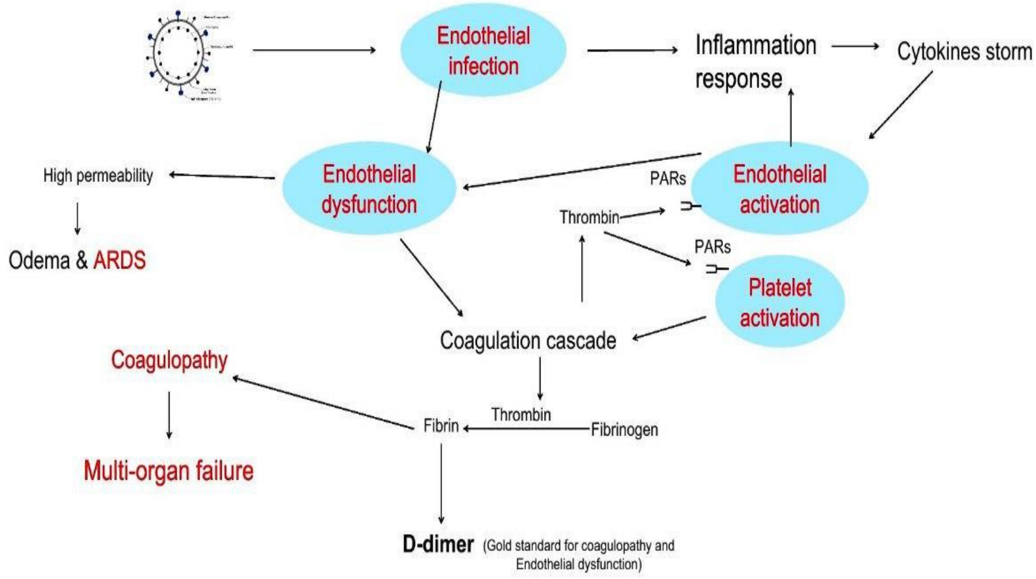


Figure 1-8: Overview of endothelial dysfunction-mediated coagulopathy in COVID-19. This coagulopathy causes multi-organ failure and ARDS.

As a result, there were two proofs for the formation of clots in the lungs. First, D-dimer and platelets, as well as other markers like ferritin and C-reactive protein

(CRP), are included in the acute phase response. Second, postmortem examinations have revealed the presence of microthrombi in COVID-19 patients (Gąsecka, Borovac, Guerreiro, & *et al*, 2021).

1.7 Diagnosis of Endothelial Dysfunction and Coagulopathy in COVID-19 by D.Dimer

Once the thrombin breaks down fibrinogen into monomers, the monomers can conform and are able to form fibrin in response to activated factor XIII, calcium, and platelets. Immediately after that, plasmin dissolves the cross-linked fibrin to produce fibrin degradation products like D-dimers (E. Giannitsis, J. Mair, C. Christersson , & *et al.*, 2017). D-dimer measurements can be used as diagnostic tests to diagnose thromboembolic conditions or DIC. This D-dimer, which is produced during fibrinolysis but also serves as a marker for thrombin activity and fibrin retention, is a good indicator of both blood clotting and fibrinolysis (E. Giannitsis, J. Mair, C. Christersson , & *et al.*, 2017).

It has a half-life of about eight hours and becomes detectable in the blood about two hours after the formation of an index thrombus (E. Giannitsis, J. Mair, C. Christersson , & *et al.*, 2017). Because severe cases of COVID-19 are associated with increased fibrinolytic activity, an elevated level of this parameter suggests the possibility of thrombosis and even pulmonary embolism (Ulanowska M. & Olas B., 2021). In China, patients were found to have D-dimers above 0.5 mg/L in 46.4% of cases; if the illness was not severe, only 43% of patients had elevated D-dimers, and about 60% of patients had serious illness (Guan W, Ni Z, Hu Y, & *et al,* 2020). Evaluate D-dimer in COVID-19 patients in accordance with the International Society of Thrombosis and Haemostasis (ISTH) guidance (J .Thachil, N Tang, S Gando, & *et al.*, 2020).

1.8 Assessment Endothelial Dysfunction in COVID-19 by PAR-1 signaling

A family of G protein-coupled receptors (GPCRs) are called proteinase-activated receptors (PARs), which stimulate cells' activity in response to serine proteases in the body (M. Cunningham, K. McIntosh, T. Bushell, & *et al.*, 2016). The PAR family has four mammalian members: PAR1, PAR2, PAR3, and PAR4 (D. M.

Heuberger & R. A. Schuepbach , 2019). According to the description of the PAR activation process, trypsin-related proteases preferentially target PAR2 and, to a lesser extent, PAR4, while thrombin is the predominant serin protease for PAR1 and PAR3 (M. Cunningham, K. McIntosh, T. Bushell, & *et al.*, 2016).

Because thrombin has the capacity to recognize specific amino acid residues, it frequently targets substrates, primarily PAR-1 (Gallwitz M, Enoksson M, Thorpe M, & Hellman L , 2012). Both the activation of platelets by PAR-1 signals and the activation of endothelial cells by PAR-1 signals are affected by thrombin. Endothelial activation may result in endothelial dysfunction and disruption, and platelet activation may result in thrombotic response or coagulopathy (Yuefei Jin, Wangquan Ji, Haiyan Yang, & *et al.*, 2020).

Endothelial dysfunction, coagulopathy, and tissue damage are linked to increased levels of thrombin signaling of PAR1 on the endothelium and platelets (Rezaie A.R., 2014) (Krishna Sriram & Paul A. Insel, 2021). According to this mechanism, COVID-19 will exhibit greater tissue damage and coagulopathy (Ackermann M, Verleden SE, Kuehnel M, & *et al*, 2020). Endothelial barrier function, vasoreactivity, intimal hyperplasia, inflammation, and coagulation are all significantly impacted by PAR1 (K. Erturk, D. Tastekin, E. Bilgin, & *et al.*, 2016).

1.9. Role of Complete Blood Count in The diagnosis of COVID-19

1.9.1. Platelets Count

Platelet count is a significant variable in COVID-19 infection because thrombocytopenia correlates with disease severity and suggests the presence of a coagulopathy (Palladino, 2021). Higher mortality and critical cases are associated with low platelet counts (Lippi G, Plebani M, & Henry BM , 2020).

Megakaryocyte fracture and increased platelet consumption or a low platelet count can result from viral interactions with megakaryocytes, damaged lung tissues, and pulmonary endothelial dysfunction (Palladino M., 2021). The majority of patients with thrombocytopenia have high D-dimer concentrations and altered coagulation parameters, which supports the theory that these factors cause

intravascular coagulation (Zaid Y., Puhm F., Allaeys I., & *et al.*, 2020). Lastly, it was discovered that critically ill COVID-19 patients had a significantly higher mean platelet volume (MPV) than critically ill non-COVID-19 patients when their platelet counts were matched (Palladino M., 2021).

1.9.2. Lymphocytes Count

Lymphopenia, or a low lymphocyte count, is the most widely recognized hematopoietic abnormality in COVID-19 infection patients, and it is typically regarded as an insufficient immune response to viral infection (Qin C, Zhou L, Hu Z, & *et al.*, 2020). Lymphopenia may also result from the diffusion of circulating lymphocytes into inflamed lung tissues (Palladino M., 2021).

When compared to patients with mild or moderate COVID-19 disease, lymphocyte cell counts in patients with severe or critical disease showed a statistically significant decline (Huang W, Berube J, McNamara M, & et al., 2020). Clinicians may use low lymphocyte counts in risk stratification to identify patients who will develop severe and fatal COVID-19 (Palladino M., 2021).

1.9.3. Neutrophils Count

A hyperinflammatory state and cytokine storm are associated with neutrophilia or an elevated lymphocyte count, with the exception of patients who have bacterial infections or superinfections (Palladino M., 2021). Due to the fact that the number of circulating neutrophils gradually rises as COVID-19 progresses, it has been recognized as a marker of severe respiratory disease and a poor outcome (Zhang L, Huang B, Xia H, & *et al.*, 2020). Correspondingly, as the COVID-19 disease progressed, the severe groups' leukocyte and neutrophil counts increased (Soraya G.V. & Ulhaq Z.S., 2020).

1.9.4. Neutrophil-Lymphocyte Ratio(NLR)

Patients with COVID-19 infection have elevated levels of neutrophil-lymphocyte ratio (NLR), which is the absolute neutrophil count/absolute lymphocyte count. According to reports, NLR and IgG may be more accurate predictors of COVID-19 severity than neutrophil count alone (Zhang B, Zhou X, Zhu C, & *et al.,* 2020).

1.9.5. Platelet-Lymphocyte Ratio(PLR)

Platelet-lymphocyte ratio(PLR), which is defined as absolute platelet count divided by absolute lymphocyte count, has been shown to be helpful in the diagnosis, monitoring, and survey of a variety of systemic inflammatory processes (Palladino M., 2021). The severity of COVID-19 disease is correlated with the PLR level (Chan AS & Routa A, 2020). Increased ferritin and PLR were discovered to be separate predictors of disease severity in COVID-19 patients (Bastug A, Bodur H, Erdogan S, & *et al.* , 2020).

1.10. Knowledge gap

This is an active area of research; more data are needed to clarify similarities and differences of the PARs and their modulators in Post and Critical cases of COVID-19. Such issues are especially relevant given the potential use of PAR-1 as a marker of promoting thrombosis and endothelial dysfunction or tissue injury through a proposed mechanism between inflammation and thrombosis that was reported previously. Moreover, assessment of the diagnostic value of Protease-activated receptors (PARs) level as an early thrombotic marker in COVID-19 patients correlated with the standard coagulation and inflammation markers of COVID-19 such as d-dimer.

1.11. Aims and objectives

The study aims were

1- Evaluation of the diagnostic value of Protease-activated receptors (PARs) level.

2-Protease-activated receptor type 1 (PAR1) may represent an essential link between coagulation and inflammation in the pathophysiology of COVID-19, therefore, the level of Protease-activated receptors would be correlated with the standard coagulation and inflammation markers of COVID-19 such as d-dimer, platelets count. etc.

3-Prothrombin & thrombin level would also be measured and correlated with the Protease-activated receptors

Chapter Two

Materials and Methods

2. Materials and Methods

2.1. Study Design

A cross-sectional study for a total of 93 patients, including 31 critical patients, 31 severe patients, and 31 post-COVID19 patients, was included in the current study. The sample size was calculated according to WHO's data sets relating to the prevalence of COVID-19 in Iraq. The project began in December 2021 and was completed in March 2022. Patients with COVID-19 were sampled at Al-Shehid Hassan Al-Hatmy Hospital. Additionally, they were examined medically by a qualified physician for COVID-19 symptoms and signs. Positive results from RT-PCR (real time –polymer chain reaction) tests at the Laboratory of Public Health in Najaf or lung CT scans (computerized axilla tomography scans) at Al-Hakim Hospital were used to accurately diagnose COVID-19 patients.

2.2. Instruments and Materials

The instruments and tools that were used in the study are listed in the table (2-1). While the materials that were used in the study are scheduled in table (2-2).

Table 2-1: The instruments used in the study

Instrument/Tool	Suppliers	Model
Centrifuge	HETTICH/ Germany	ROTOFIX 32 A
Deep freezer	COOLTECH/ China	Chittoor
Pipette(100-1000µl)	Dragon / USA	Pipettor toppette
Multichannel pipette (10-100 µl)	Dragon / USA	Pipettor toppette
Micropipette(10-100 µl)	Dragon / USA	Pipettor toppette
ELISA system (Wisher,Printer,Reader)	Bio tek /USA	Bio tek 800
Gilson Tips,1000µl (blue)	China	
Gilson Micro-tips, 100µl (yellow)	China	

Eppendorf Tubes	China	
XP-300™ automated hematology analyzer Sysmex	Sysmex / Germany & Japan	XP-300™
Vidas	Biomeroix /France	miniVidas
Sodium citrate tubes	China	Vacuumed Sodium citrate tube
EDTA tubes	China	Vacuumed EDTA tube
Gel tubes	China	Vacuumed gel tube
mixer	China	
Incubator	Memmert/UK	MM-02

Table 2-2: The materials used in the study

Materials	**Suppliers**
PAR-1 Kit	Sunlong Biotech/China
Thrombin Kit	Sunlong Biotech/China
Prothrombin Kit	Sunlong Biotech/China
D-dimer Kit	Biomeroix /France
CBC Kit	Sysmex / Germany & Japan

2.3. Inclusion and Exclusion criteria

2.3.1. Inclusion Criteria

All patients who have been classified into severe cases and critical cases according to specialized physician and had a positive RT-PCR test or a lung CT scan. They were reported to the hospital as confirmed COVID-19 cases. A complete clinical history, a clinical scan, and any necessary laboratory testing were performed.

Post-COVID-19 cases, had passed at least 21 days after the appearance of infection according to specialized physician**.** The post-infection period should not exceed three months.

2.3.2. Exclusion Criteria

Patients with COVID-19 who have Lung Cancer because PAR-1 level increases in this case (K. Erturk, D. Tastekin, E. Bilgin, & et al., 2016) .

2.4. Study Variables

2.4.1. Dependent Variables

The dependent variables of this study are D-dimer, PAR1, Thrombin level ,Prothrombin level , Red Blood Cells (RBC) count, Hemoglobin (Hb) , White Blood Cells (WBC) count, NLR, Platelets count, and PLR.

2.4.2. Independent Variables

The study's independent variables are age, gender, smoking status, body temperature, dry cough, oxygen saturation level, shortness of breath(SOB), and any ongoing chronic diseases (hypertension, diabetes, and autoimmune diseases).

2.5. Approval of the Ethical Committee

The research protocol was confirmed in accordance with the final approvals from the Scientific and Ethical Committee for Research in the AL-Najaf Health Directorate and the Medical Research Bioethical Committee in the College of Medicine, University of Kerbala (a copy of each letters in appendix). Additionally, samples were only taken with the consent of patients or patients' relatives.

2.6. Measurement and Data Collection

2.6.1. Data Collection

The questionnaire was specifically designed to gather data that would help in the selection of participants in accordance with the study's selection criteria. Age, gender, and the presence of any active chronic diseases were collected from the patients' self-reported social demographic data (hypertension, diabetes, cancer, and

autoimmune diseases). Each patient had their smoking status, body temperature, dry cough, and oxygen saturation measured.

2.6.2. Blood Collection and Storage

By using a disposable 5 mL syringe for venipuncture, blood samples totaling 5 mL were drawn from the patient. Each sample of blood was divided into three tubes:

1) The first tube, which was a gel tube containing 2 milliliters of blood, was left at room temperature for fifteen minutes. A gel tube was used to centrifuge the serum for 10 minutes at a speed of about 4000 rpm to separate it. An eppendroff tube was used to collect the serum sample, and it was then kept at -20°C to prevent repeated freezing and thawing cycles. Serum samples were used to measure the levels of PAR1, thrombin, and prothrombin.

2) The second tube contained 1.5 mL of blood and was filled with sodium citrate anticoagulant. By centrifuging a tube of plasma for 10 minutes at about 4000 rpm, the plasma was separated. Plasma was used to measure the levels of D-dimer.

3) The third tube, containing the anticoagulant ethylenediaminetetraacetic acid (EDTA), contained 1.5 mL of blood. In order to prevent clot formation, the samples were left on the mixruller at room temperature. The samples were employed to evaluate the CBC.

These blood collection tubes were disposed of, non-pyrogenic, and incapable of producing any endotoxins.

2.7. Methods

2.7.1. Determination of PAR1 using the ELISA Technique

To measure the concentrations of PAR1, an enzyme-linked immunosorbent assay system (ELISA) was carried out using the sandwich method.

Principle:- A specific human PAR1 antibody was used to precoat the plate. Standards or samples were combined with the specific antibody in the appropriate plate wells. Then, each plate well received a Horseradish Peroxidase (HRP)-conjugated antibody that was targeted for PAR1. Free parts were removed by washing. To each well, the TMB substrate solution was added. Only the wells containing PAR1 and HRP-

conjugated PAR1 antibodies would initially appear blue before changing to yellow after the stop solution was added. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm. Due to the fact that the OD value is proportional to the concentration of PAR1, the concentration of PAR1 in the samples can be calculated by contrasting their OD values with a standard curve.

Reagents:- The reagents were listed in the table (2-3).

Table 2-3: List of reagents in PAR1 kit

Materials	Quantity
1- Pre-coated Plate	**96 wells**
2-Standard of human PAR1 (Concentration is 1800 pg/ml)	**0.5 mL x 1 bottle**
3-Standard diluent	**1.5 mL x 1 bottle**
4-Sample diluent	**6 mL x 1 bottle**
5-wash solution	**20 mL (30X)×1bottle**
6-Stop Solution	**6 mL× 1 bottle**
7-Chromogen Solution A	**6 mL× 1 bottle**
8-Chromogen Solution B	**6 mL × 1 bottle**
9-HRP-Conjugate reagent	**6 mL × 1 bottle**
10-Closure plate membrane	**2 pcs.**
11-User manual	**1 pcs.**
12-Sealed bags	**1 pcs.**

Sample preparation: The serum samples were stored in eppendroff tubes at -20°C, from which one of these tubes was taken from each sample. These tubes were kept at room temperature before use.

Procedure

1. **Dilution of Standards**

The standard solution was diluted using small tubes as shown table (2-4) and figure (2-1), the 50µl from each tube was pipetted into each plate well.

Table 2-4: Standard solutions of PAR1 Kit

Standard Concentration	Standard tube No.	Standard Dilution
1200pg/mL	Standard No.1	300µL Original Standard + 150 µL Standard diluents
800pg/ Ml	Standard No.2	300 µL Standard No.1 + 150 µL Standard diluents
400pg/ mL	Standard No.3	150 µL Standard No.2+ 150 µL Standard diluents
200pg/ mL	Standard No.4	150 µL Standard No.3 + 150 µL Standard diluents
100pg/ mL	Standard No.5	150 µL Standard No.4 + 150 µL Standard diluents

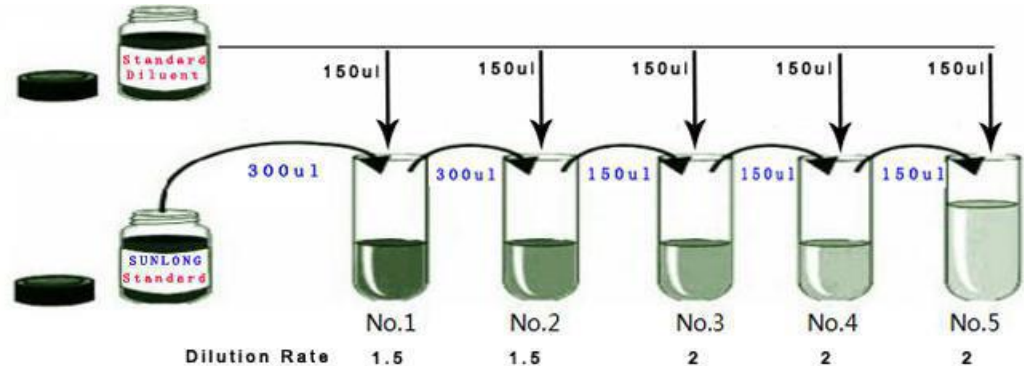


Figure 2-1: Dilution of standard solutions in human PAR1 kit

2- The 40 µL of sample dilution buffer and 10 µL of sample were added (dilution factor is 5) to each sample well. Samples should be loaded onto the bottom without touching the well, and mixed well with gentle shaking.

3-After being sealed with a closure plate membrane, it was incubated for 30 min at 37°C.

4-The wash solution was prepared by dilution of the concentrated washing buffer with distilled water (30 times).

5- After carefully peeling off the closure plate membrane, it was aspirated and refilled with the wash solution. The wash solution was discarded after resting for 30 seconds. This washing procedure was repeated five times.

6- The 50 µL from HRP-Conjugate reagent was added to each well.

7-Incubation was repeated as described in Step 3.

8-Washing was repeated as described in Step 5.

9- The 50 µL of Chromogen Solution A and 50 µL of Chromogen Solution B were added to each well, mixed with gentle shaking, and incubated at 37°C for 15 minutes to allow coloring, avoiding light in this process.

10- The 50 µl of stop solution was added to each well to terminate the reaction. The color in the well should change from blue to yellow.

11- Absorbance O.D. of the plate wells was read at 450nm using an ELIZA Reader.

Calculation of Results: Human PAR1 standard concentrations and their corresponding OD readings were plotted on the log scale (x-axis) and log scale (y-axis), respectively. The concentration of human PAR1 in samples was determined by comparing the OD of the samples to the standard curve shown in figure (2-2). The original concentration was calculated by multiplying the dilution factor.

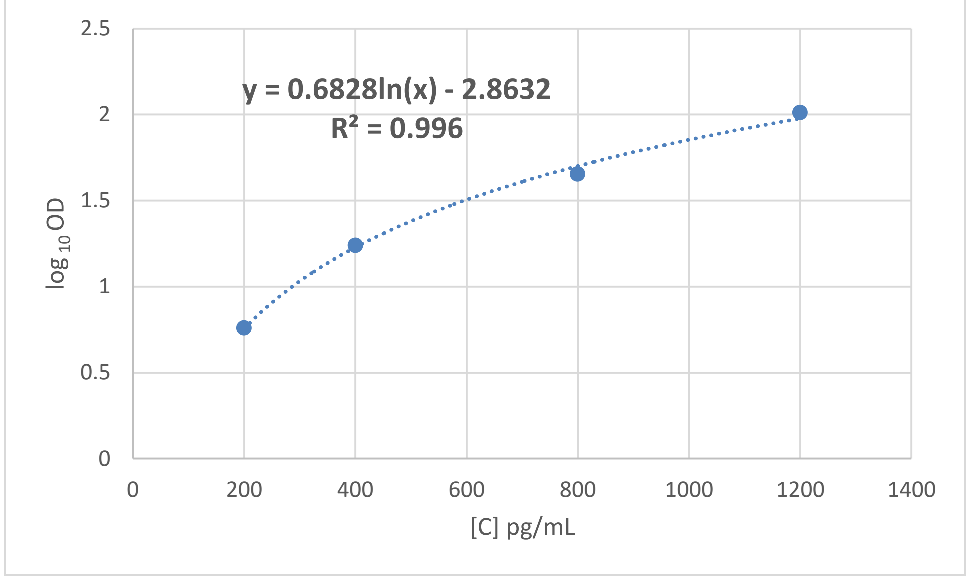


Figure 2-2 : The PAR1 standard curve.

2.7.2. Determination of Thrombin Level Using the ELISA Technique

To measure the concentrations of thrombin, an enzyme-linked immunosorbent assay system (ELISA) was carried out using the sandwich method.

Principle:- A specific human thrombin antibody was used to precoat the plate. Standards or samples were combined with the specific antibody in the appropriate

plate wells. Then, each plate well received a Horseradish Peroxidase (HRP)-conjugated antibody that was targeted for thrombin. Free parts were removed by washing. To each well, the TMB substrate solution was added. Only the wells containing thrombin and HRP-conjugated thrombin antibodies would initially appear blue before changing to yellow after adding the stop solution. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm. Due to the fact that the OD value is proportional to the concentration of thrombin, the concentration of thrombin in the samples can be calculated by contrasting their OD values with a standard curve.

Reagents:- The reagents were listed in the table (2-5).

Table 2-5: List of reagents in Thrombin kit

Materials	Quantity
1- Pre-coated Plate	96 wells
2-Standard of human thrombin (Concentration is 108 pg/ml)	0.5 mL x 1 bottle
3-Standard diluent	1.5mL x1 bottle
4-Sample diluent	6 mL x 1 bottle
5-wash solution	20 mL (30X)×1bottle
6-Stop Solution	6 mL ×1 bottle
7-Chromogen Solution A	6 mL ×1 bottle
8-Chromogen Solution B	6 mL ×1 bottle
9-HRP-Conjugate reagent	6 mL ×1 bottle
10-Closure plate membrane	2 pcs.
11-User manual	1 pcs.
12-Sealed bags	1 pcs.

Sample preparation : The serum samples were stored in eppendroff tubes at -20°C, from which one of these tubes was taken from each sample. These tubes were put at room temperature before use.

Procedure

1. Dilution of Standards

The standard solution was diluted by small tubes as shown table (2-6) and figure (2-3), then the 50ul from each tube was pipetted to each plate well.

Table 2-6: Standard solutions of Thrombin Kit

Standard Concentration	Standard tube No.	Standard Dilution
72pg/Ml	Standard No.1	300µL Original Standard + 150 µL Standard diluents
48pg/ mL	Standard No.2	300 µL Standard No.1 + 150 µL Standard diluents
24pg/ mL	Standard No.3	150 µL Standard No.2+ 150 µL Standard diluents
12pg/ mL	Standard No.4	150 µL Standard No.3 + 150 µL Standard diluents

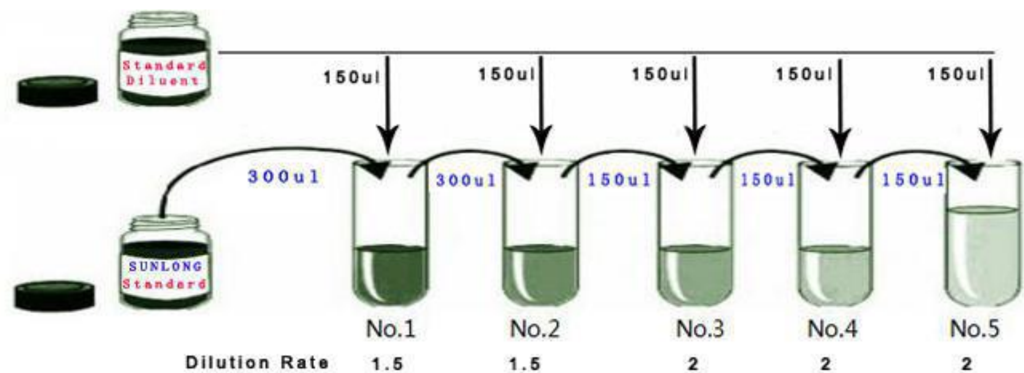


Figure 2-3: Dilution of standard solutions in human Thrombin kit

2- The 40 µL of sample dilution buffer and 10 µL of sample were added (dilution factor is 5) to each sample well. Samples should be loaded onto the bottom without touching the well, and mixed well with gentle shaking.

3-After being sealed with a closure plate membrane, it was incubated for 30 min at 37°C.

4-The wash solution was prepared by dilution of the concentrated washing buffer with distilled water (30 times).

5- After carefully peeling off the closure plate membrane, it was aspirated and refilled with the wash solution. The wash solution was discarded after resting for 30 seconds. This washing procedure was repeated five times.

6- The 50 µL from HRP-Conjugate reagent was added to each well.

7-Incubation was repeated as described in Step 3.

8-Washing was repeated as described in Step 5.

9- The 50 µL of Chromogen Solution A and 50 µL of Chromogen Solution B were added to each well, mixed with gentle shaking, and incubated at 37°C for 15 minutes for coloring, avoiding light in this process.

10-The 50 µl of stop solution was added to each well to terminate the reaction. The color in the well should change from blue to yellow.

11- Absorbance O.D. of the plate wells was read at 450nm using an ELIZA Reader.

Calculation of Results: Human thrombin standard concentrations and their corresponding OD readings were plotted on the log scale (x-axis) and log scale (y-axis), respectively. The concentration of human thrombin in samples was determined by comparing the OD of the samples to the standard curve shown in figure (2-4). The original concentration was calculated by multiplying the dilution factor.

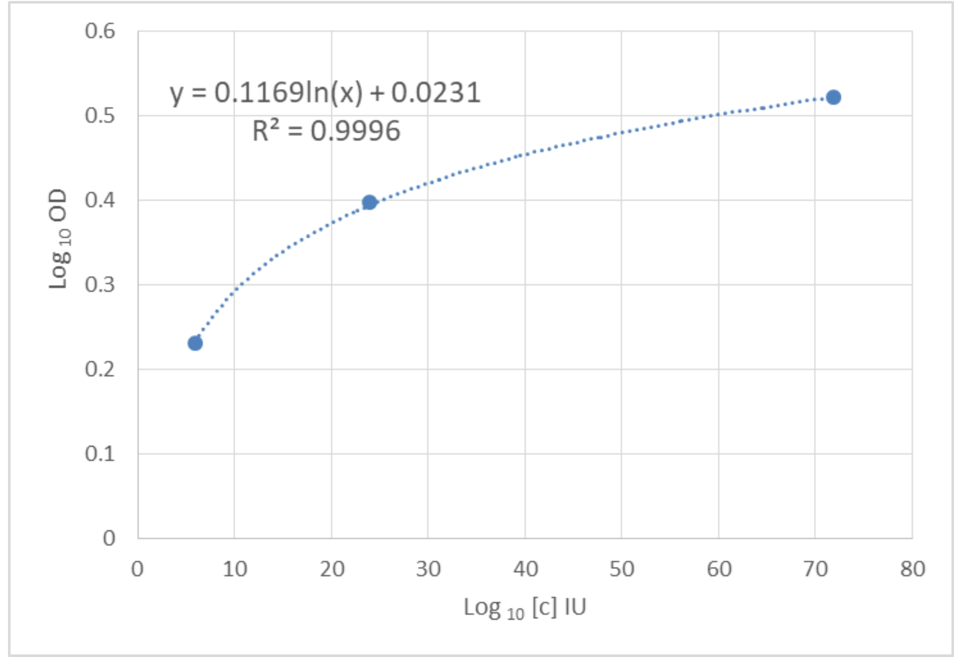


Figure 2-4 : The Thrombin standard curve.

2.7.3. Determination of Prothrombin Level Using ELISA Technique

To measure the concentrations of prothrombin, an enzyme-linked immunosorbent assay system (ELISA) was carried out using the sandwich method.

Principle:- A specific human prothrombin antibody was used to precoat the plate. Standards or samples were combined with the specific antibody in the appropriate plate wells. Then, each plate well received a Horseradish Peroxidase (HRP)-conjugated antibody that was targeted for prothrombin. Free parts were removed by washing. To each well, the TMB substrate solution was added. Only the wells containing prothrombin and HRP-conjugated prothrombin antibodies would initially appear blue before changing to yellow after the stop solution was added. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm. Due to the fact that the OD value is proportional to the concentration of prothrombin, the concentration of prothrombin in the samples can be calculated by contrasting their OD values with a standard curve.

Reagents:- The reagents were listed in the table (2-7).

Table 2-7: List of reagents of Prothrombin kit

Materials	Quantity
1- Pre-coated Plate	96 wells
2-Standard of human prothrombin (Concentration is 225 pg/ml)	0.5mL x1 bottle
3-Standard diluent	1.5mL x1 bottle
4-Sample diluent	6mL x1 bottle
5-wash solution	20mL (30X)×1bottle
6-Stop Solution	6mL ×1 bottle
7-Chromogen Solution A	6mL ×1 bottle
8-Chromogen Solution B	6mL ×1 bottle
9-HRP-Conjugate reagent	6mL ×1 bottle
10-Closure plate membrane	2 pcs.
11-User manual	1 pcs.
12-Sealed bags	1 pcs.

Sample preparation : The serum samples were stored in eppendroff tubes at -20°C, from which one of these tubes was taken from each sample. These tubes were put at room temperature before use.

Procedure

1. Dilution of Standards

The standard solution was diluted by small tubes shown as table (2-8) and figure (2-5), then the 50ul from each tube was pipetted to plate well.

Table 2-8 Standard Solutions of Prothrombin Kit

Standard Concentration	Standard tube No.	Standard Dilution
150pg/mL	Standard No.1	300µL Original Standard + 150 µL Standard diluents
100pg/ mL	Standard No.2	300 µL Standard No.1 + 150 µL Standard diluents
50pg/ mL	Standard No.3	150 µL Standard No.2+ 150 µL Standard diluents
25pg/ mL	Standard No.4	150 µL Standard No.3 + 150 µL Standard diluents
12.5pg/ mL	Standard No.5	150 µL Standard No.4 + 150 µL Standard diluents

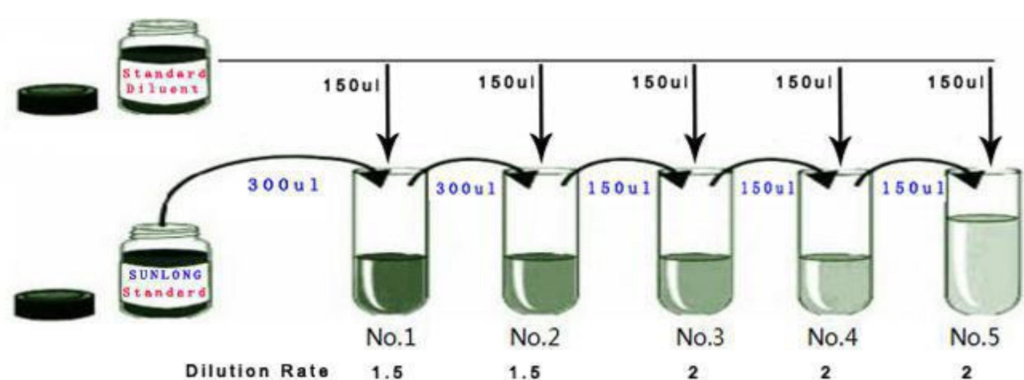


Figure 2-5: Dilution of standard solutions in human Prothrombin Kit

2- In sample wells, the 40 µL of sample dilution buffer and 10 µL of sample were added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well, and mixed well with gentle shaking.

3-After being sealed with a Closure plate membrane, it was incubated for 30 min at 37°C.

4-The wash solution was prepared by dilution of the concentrated washing buffer with distilled water (30 times).

5- After carefully peeling off the closure plate membrane, it was aspirated and refilled with the wash solution. The wash solution was discarded after resting for 30 seconds. This washing procedure was repeated five times.

6- The 50 μL from HRP-Conjugate reagent was added to each well.

7-Incubation was repeated as described in Step 3.

8-Washing was repeated as described in Step 5.

9- The 50 μL of Chromogen Solution A and 50 μL of Chromogen Solution B were added to each well, mixed with gentle shaking, and incubated at 37°C for 15 minutes for coloring, avoiding light in this process.

10-The 50 μl of stop solution was added to each well to terminate the reaction. The color in the well should change from blue to yellow.

11- Absorbance O.D. of plate wells was read at 450nm using an ELIZA Reader.

Calculation of Results: Human prothrombin standard concentrations and their corresponding OD readings were plotted on the log scale (x-axis) and log scale (y-axis), respectively. The concentration of human prothrombin in samples was determined by comparing the OD of the samples to the standard curve shown in figure (2-6). The original concentration was calculated by multiplying the dilution factor.

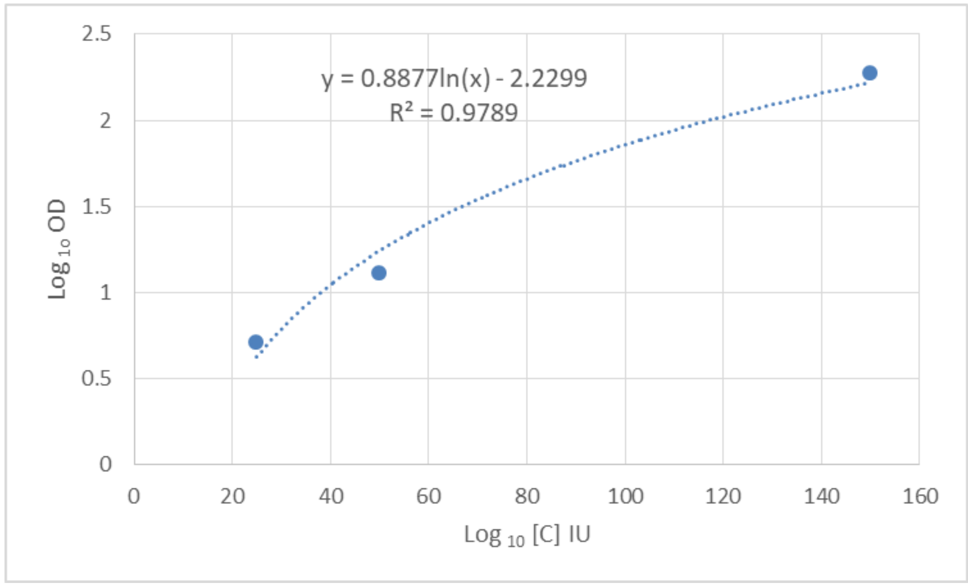


Figure 2-6 : The Prothrombin standard curve.

2.7.4. Measurement of D-dimer Using the VIDAS technique:

Principle: The VIDAS D-Dimer assay is an automated quantitative test for the determination of the fibrin degradation products (FbDP)in human plasma (in sodium citrate tube) using the ELFA (Enzyme-Linked Fluorescent Assay) technique. The assay combines a two-step enzyme immunoassay sandwich method with a final fluorescent detection . The assay reagents are ready-to-use and pre-dispensed in the sealed reagent strips (STRs). The solid phase receptacle (SPR), a pipette tip-like device, serves as the solid phase as well as the pipetting device for the assay. All of the assay steps are performed automatically by the instrument .

In first step, the sample is taken by the SPR . The antigen binds to the anti-FbDP immunoglobulins coated on the SPR. A detection step is then performed . The substrate (4-methyl-umbelliferyl phosphate) is cycled in

and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-methyl-umbelliferone), the fluorescence of which is measured at 450 nm .The intensity of fluorescence is proportional to the concentration of antigen present in the sample.

At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory. The results are then printed.

Reagents:- A reagents used are listed in tables (2-9) and (2-10).

Table 2-9: List of reagents in D-Dimer kit

Components	Contents
Solid Phase Receptacle (SPR)	Coating solution includes Anti-FbDP monoclonal mouse antibodies
Strip	Ready to use./ Description of the D-Dimer Strip contents in table (2-5)
Calibrators and Controls (ng/dL)	S1: 3700-4400 S2: N/A C1: 5200-6040 C2: :250-380

Table 2-10: Description of the D-Dimer Strip contents

Well	Reagent
1	Sample
2,3,4	Empty
5	Conjugate: Mouse monoclonal anti- D-Dimer antibodies conjugated to alkaline phosphatase with 1 g/L sodium azide (600 µL).
6,7	Wash buffer: Sodium phosphate (0.01 mol/L , pH 7.4) with 1 g/L sodium azide (600 µL)
8	Wash buffer: diethanolamine* (1.1 mol/L , or 11.5%, pH 9.8) with 1 g/L sodium azide (600 µL)
9	Empty
10	10 Reading Cuvette with Substrate: 4-methylumbelliferyl phosphate (0.6 mmol/L) with diethanolamine** (0.62 mol/l or 6.6%, pH 9.2) + 1 g/L sodium azide (300 µl)

Calculation of Results : The results were automatically calculated by the instrument in relation to the calibration curve stored in memory. The results were then printed.

2.7.5. Determination of Complete Blood Count (CBC):

The complete blood count (CBC) were done by XP-300™ Automated hematology analyzer Sysmex .

Principle :

The direct current (DC) detection method for WBC, RBC and PLT count measurement. This method detects the size of blood cellular contents by changes in DC resistance on the density of cells interior .

Procedure:- The 50uL of whole blood in EDTA tube was dispensed automatically by CBC device .

2.8. Statistical Analysis

Information from the questionnaire and all test results from study groups samples were entered a data sheet. The data analysis for this work was generated using the Statistical Package for the Social Sciences software, version 28.0 (IBM, SPSS, Chicago, Illinois, USA) and the Real Statistics Resource Pack software for Mac (Release 7.2) of the resource pack for Excel 2016. Copyright (2013 – 2020).

Descriptive statistics were performed on the data of each group. Values were illustrated by n (%) for categorical, Scale variables were presented by mean ± standard deviation for normal data while non-normal data, continuous variables were presented by interquartile range (IQR) and median. The distribution of the data was checked using Shapiro-Wilk test and box–blot as numerical means of assessing normality.

For abnormal distribution, the univariate analysis was performed using an independent Kruskal Wallis Test for continuous variables. Biomarkers were compared using Sparman rank test to evaluate the relationship within the case study. Results of all hypothesis tests with p-values <0.05 (two-side) were considered to be statistically significant.

The optimal threshold with high specificity and sensitivity for critical cases was detected using receiver operating characteristic (ROC) analysis. It was found out that all the values of P were two-sided, and a $P < 0.05$ was considered to be statistically significant.

Chapter Three
Results and Discussion

3. Results and Discussion

As the coagulation cascade is dysregulated in COVID-19, coagulopathy is a major challenge with a high incidence of thromboembolic complications. It has been reported as a complex and could differ in important ways from the common mechanisms of thrombosis reported in critically patients (Bonaventura A., Vecchié A., Dagna L. , & et al., 2021). This study was design to identify coagulopathy and endothelial dysfunction in COVID-19 patients based on the role of PAR1 plus other parameters that associated with clinical COVID-19 severity.

3.1. Demographic and Clinical Characteristics

The demographic characteristics and laboratory parameters of studied groups of COVID-19 patients were listed in Table (3-1). The age range of participants was within (95-28) years old, while gender distribution was 63% male and 37% female. It has been reported that male being more infected than female, this could be due to a number of factors, including males having higher levels of ACE 2; coronavirus receptors, sex-created immunological alterations caused by sex hormone, and the X chromosome (G.M. Bwire, 2020).

Almost all of the participants in this study were using different ventilator devices based on their clinical cases. In some cases, O_2 saturation in blood SPO reached 70%. As a coagulation marker, D-dimer was highly variable and above the reference range of (> 500 ng/mL), especially in critical cases. Overall, Coagulation parameters (PAR-1, thrombin levels) were increased markedly in critical and post COVID-19 groups.

Also, results indicated that most of the covid patients' were shown wide variability in the hematological parameters based on disease severity.

Table (3.1) the Descriptive of Demographic the study .

Parameters	Sever			Critical			Post		
	Mean ± SD	Median	(Max.-Min.)	Mean ± SD	Median	(Max.-Min.)	Mean ±SD	Median	(Max.-Min.)
Age	53.2 ±12.95	50	85-32)(	64.9±16.4	64.5	95-28)(	69.5±10.05	71	(85-50)
Gender (male/female)	(14/17)			(21/10)			15/16)(		
Temperature (^{o}c)	36.83 ± 0.66	37	(38.5-36)	36.83 ± 0.4	37	(37.1-36)	36.85±0.3	37	(37-36)
SPO_2 with ventilator	96.11 ± 2.47	97	(99-92)	89.77 ± 7.4	93	(98-72)	90.47±7.48	93	(99-70)
d-dimer (ng/dl)	988.7± 1152.8	703	5014-240)(	3901±2985	3666	(10000-588)	2033±1593	1717	(5605-355)
WBC (*10^3 /ml)	12.8± 8.46	10.2	(46.4-4.3)	19.04±15.59	12.7	(62.6-4.4)	13.13±5.77	12.2	(25-4.6)
lymphocyte (%)	12.33± 10.28	10.6	(35.9-0.6)	6.57±3.69	5.7	20-1.5)(	10.1±7.34	10.1	(31.3-2.3)
Neutrophil (%)	73.91± 18.31	79.8	95.3-27.3)(	90.18±4.87	90.7	(97.4-75.2)	86±7.49	86.2	(97-66)
lymphocyte count	1.36± 0.19	1.1	(1.5-0.1)	0.92±0.64	0.7	(3.3-0.3)	1.08±0.67	0.9	(2.1-0.2)
neutrophil count	11.86± 9.69	8.95	(36-1.5)	17.57±14.6	11.65	(55.3-3.8)	11.53±5.3	11.1	(22.3-3.9)
NLR	9.0± 8.37	6.23	(34.04-1.37)	19.44±13.4	15.9	64.9-3.76)(	14.65±11.8	8.53	(38.5-2.1)
RBC (*10^6/ml)	4.46 ± 0.82	4.5	(6.65-2.69)	4.39±0.94	4.56	(6.57-2.31)	4.21±0.74	4.25	(5.3-2.8)
Hb (g/ml)	12.05 ± 2.01	12.2	(17-7.6)	12.46±2.6	12.85	16-7)(	11.72±1.9	11.6	(15.3-8.2)
HCT (%)	37.35 ± 6.5	37.1	(53.9-22.7)	37.65±7.94	38.8	54.2-20.8)(	37.76±5.05	38.9	(48.3-27.6)
MCV	84.07 ± 5.28	83.9	(96.2-76.3)	85.8±7.4	86	(98-71)	90.57± 8.83	92	(115.2-71)
MCH	27.23 ± 2.63	27.6	(33.1-19.7)	28.53±3.4	28.55	(34.8-19.9)	28.15± 3.82	28	(37.2-20.9)
PLT (*10^3/ml)	277.9 ± 135	254	(643-46)	191.2±84.8	202.5	(341-48)	251.5± 112	238	(533-138)
MPV	9.75± 1.4	9.4	12.9-7.1)(	8.5±0.99	8.6	(11-6.9)	8.43± 0.66	8.6	(11.3-7.1)
PLR	651.69± 205.6	203	(880-65.7)	281.9±166	311.25	(696.7-28.4)	311 ± 187.4	231.7	(730-60.3)
PAR1 (ng/ml)	657.4 ± 56.4	643	(807-572)	809±484.8	683	(885-594)	727.6± 66.7	704	(978-654)
Thrombin (ng/ml)	67.3± 72.58	38	(267-16)	110.8±53.9	94.5	(287-37)	108.5± 26.9	105	(175-75)
Prothrombin (ng/ml)	112.8 ± 13.2	112	(143-87)	115.5±10.5	111	(128-95)	106.3± 14.3	111	(126-71)

Furthermore, Figure (3-1) demonstrated the characteristics and risk factors which were collected through the self-reported technique, Most patients were having moderate to severe shortness of breath, the last generation of COVID, which was included in the study, was characterized by severe dry mouth and dry cough. Regarding the risk factors, more than one-third of the participants were reported to suffer from diabetes and Hypertensive.

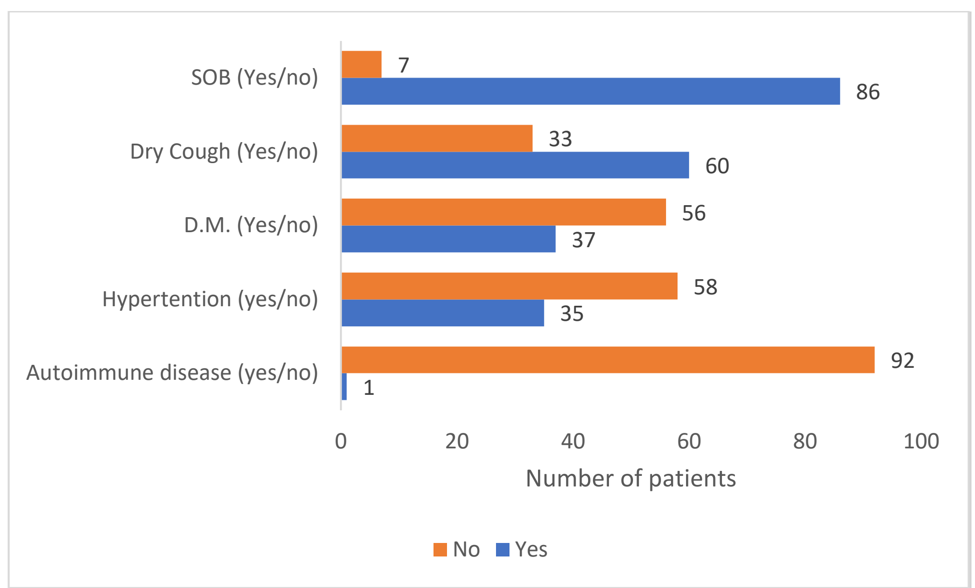


Figure 3-1: the Percentage of selected underlying medical conditions under the COVID-19 associated to study groups.

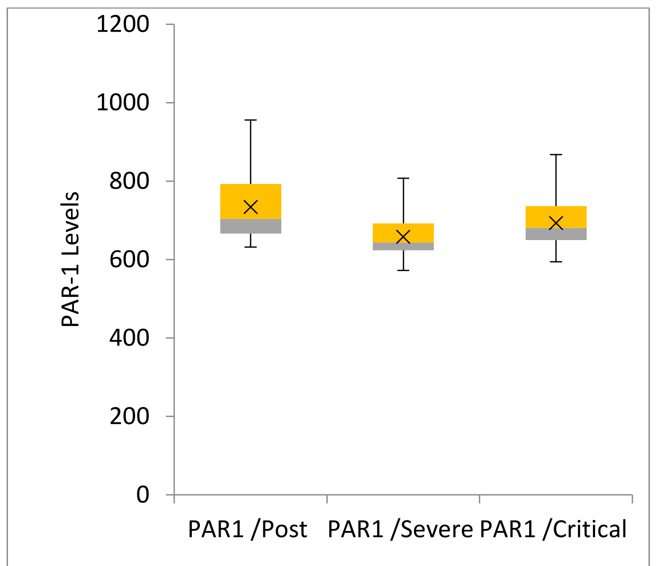
3.2. Distribution of Coagulation Markers

The distribution of serum levels of D-dimer and PAR-1 were examined, and presented in Figure (3-2). Serum D-dimer level was increased noticeably in critical COVID-19 and post-COVID19 cases. The range levels of serum D-dimer levels was 10000-588 ng/mL in critical group, while it was 5605-355ng/mL in the post group and 5014-240ng/mL in the severe group. According to nonparametric Kruskal-Wallis and the Mood's median tests, which were used as a robust method against outliers, the D-dimer test was highly significantly differed among the studied groups [p = 0.001], data were illustrated in Table (3-2).

Previous research reported the clinical outcome of COVID-19 patients can be significantly predicted with mortality by coagulation biomarkers like D-dimer > 1000 ng/dL

[p = 0.008] (T. M. Stanne, *et al.*, 2021). This can be explained because D-dimer level can be a good indicator of both blood clotting and fibrinolysis (E. Giannitsis, J. Mair, C. Christersson , & *et al.,* 2017).

Also, serum PAR-1 level was quietly increased in post COVID-19 and critical cases. The range of serum PAR-1 levels was 885-594pg/mL in critical group, while it was 978-654ng/mL in post group and 807-572pg/mL in severe group. The PAR-1 test was significantly predictable with studied groups [p = 0.033] consistent with Kruskal-Wallis and the Mood's median tests, as shown in Table (3-2). Results were in agreement with previous reported data of (T. M. Stanne, *et al.,* 2021) who were indicated that patients with worsening conditions of covid had elevated plasma levels of PAR1. That might be related to the endothelial barrier function, vasoreactivity, intimal hyperplasia, inflammation, and coagulation which are all significantly squeezed by PAR1 (K. Erturk, *et al.*, 2016).



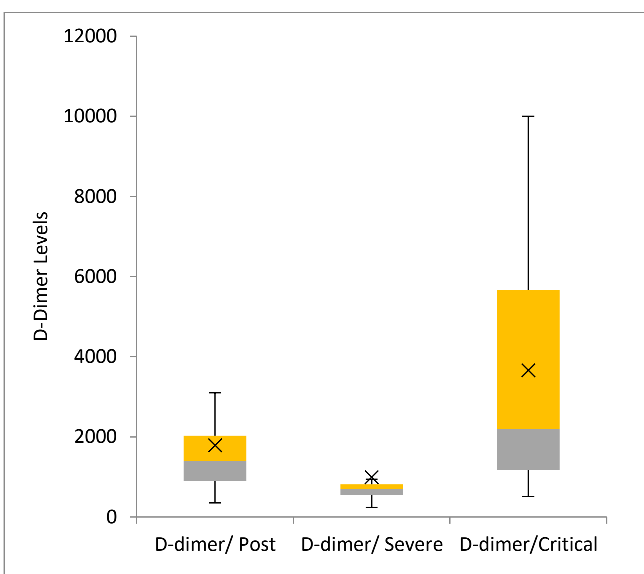


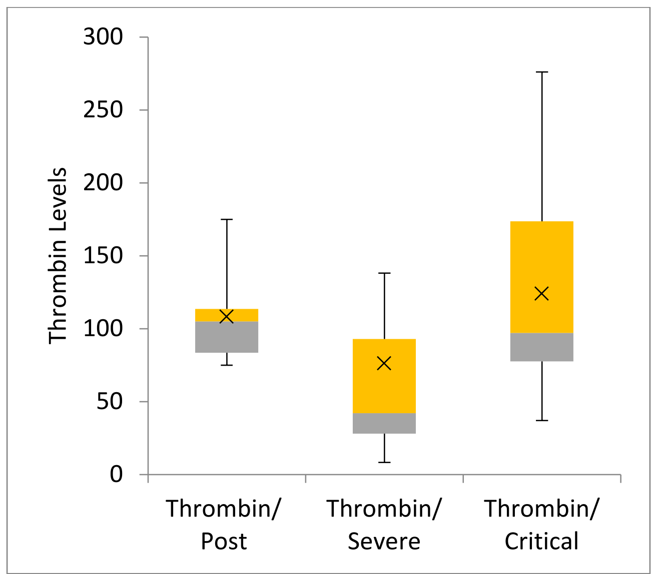
Figure 3-2 : the Boxplot of the distribution of serum level of PAR-1 Pg/mL & D-dimer ng/mL in COVID-19 patients based on their disease severity.

Table 3-2 : Kruskal-Wallis and Mood's median test for the differences between the median levels of D-dimer and PAR1 with COVID-19 groups.

Median Test		Study groups			Test Statistics	
		Post COVID-19	Critical COVID-19	Severe COVID-19	Chi-Square	P value
D-dimer	> Median	15	22	8	12.239	0.001
	≤ Median	16	9	23		
PAR1	> Median	18	18	10	6.801	0.033
	≤ Median	13	13	21		

Also, the distribution of serum level of Thrombin and Prothrombin in the studied groups were presented in Figure (3-3). The serum level of thrombin was shown a massive increase in severe and critical cases. The variety of serum thrombin levels was 287-37pg/mL in critical group, while it was 175-75 pg/mL in post group and 267-16pg/mL in severe group. The thrombin test was significantly expectable with studied groups [p = 0.022] consistent with Kruskal-Wallis and the Mood's median tests , as presented in Table (3-3). In a previous study, it was compared to the reference population, the patients' thrombin generation was significantly greater in venous diseases and at their height peak (A. J. ten Cate-Hoek , A.W J. H. Dielis , & H. M. H. Spronk, 2008).

The range of serum prothrombin levels was 128-95ng/mL in critical group, while it was 126-71 ng/mL in post group and 143-87pg/mL in severe group. The prothrombin test was not significantly predictable with studied groups [p = 0.899] consistent with Kruskal-Wallis and the Mood's median tests, as shown in Table (3-3). However, previous research had reported that patients with venous diseases had significantly higher levels of factor II (prothrombin) than healthy individuals (P. C. S. van Paridon, M P Noeva, R van Oerle, & *et al*., 2022). Because there was no healthy group in our study, this result differs from the findings of that study.



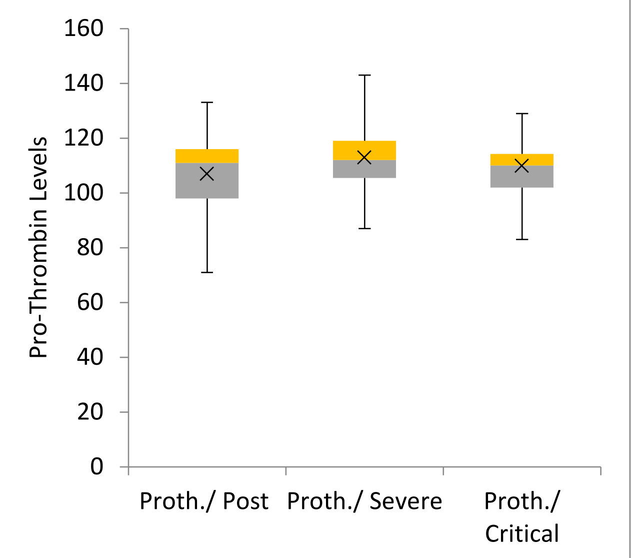


Figure 3-3: the Boxplot of the Distribution of serum level of Thrombin & Prothrombin in COVID-19 patients based on their disease severity.

Table 3-3: Kruskal-Wallis and Mood's median test for The differences between the median levels of Thrombin and Prothrombin with COVID-19 groups

Median Test		Study groups			Test Statistics	
		Post COVID	Critical COVID	Severe COVID	Chi-Square	P value
Thrombin	> Median	20	17	8	7.670	0.022
	≤ Median	11	14	23		
Prothrombin	> Median	16	14	17	0.212	0.899
	≤ Median	15	17	14		

3.3. Distribution of Hematological Parameters & Inflammation Markers

Increasing evidence shows that hematological patterns are closely associated with disease progression of patients infected with viruses. A decrease in peripheral T cell

subsets is a unique characteristic in patients with severe acute respiratory syndrome (SARS) (Guan W. J. & *et al,* 2020) . It has been shown that SARS-CoV-2 disrupts normal immune responses, leading to an impaired immune system and uncontrolled inflammatory responses in severe and critical patients with COVID-19. These patients exhibit lymphopenia, lymphocyte activation and dysfunction, granulocyte and monocyte abnormalities, high cytokine levels (Li Yang & *et al*, 2020).

The distribution of hematological parameters and inflammation markers was presented in Figures (3-4),(3-5), and (3-6). The WBC count was increased noticeably in critical and severe COVID-19 cases compared to post case . The range levels were (62.6-4.4 $*10^3$/mL); (46.4-4.3$*10^3$/mL) and (25-4.6$*10^3$/mL) respectively.

The lymphocytes count were reduced markedly in critical COVID-19 cases. That might be because COVID-19 infection causes lymphopenia in patients, which is present in progressive cases and is correlated to the disease outcome (C. Qin, *et al.,* 2020). The range levels were (3.1-0.1$*10^3$/mL) in critical group compared to (1.5-0.1$*10^3$/mL) and (2.1-0.2$*10^3$/mL) respectively in severe and post cases.

The platelets count were also reduced distinctly in critical cases. The range levels were (341-48$*10^3$/mL) in critical group compared to (643-46$*10^3$/mL) and (533-138$*10^3$/mL) respectively in severe and post cases.

Thus, the risk of COVID-19 progression was more than five times higher in people who had thrombocytopenia (J .Thachil, N Tang, S Gando, & *et al.*, 2020). Since it proposed that platelets count is consistent prognosticator for COVID-19 improvements(J .Thachil, N Tang, S Gando, & et al., 2020).

The PLR was increased in critical and severe cases than post, and the range levels were (880-65.7); (696.67-28.4) and (730-60.3) respectively, while The NLR was increased only in critical and post cases, the range levels were (64.9-3.76); (38.5-2.1) and (34.04-1.37) respectively. The progression of COVID-19 was correlated with NLR and PLR levels (Palladino, 2021). NLR and PLR have been regarded as independent variables linked to COVID-19 progression, but the underlying mechanisms are still unclear (Ai-Ping Yang, *et al.,* 2020).

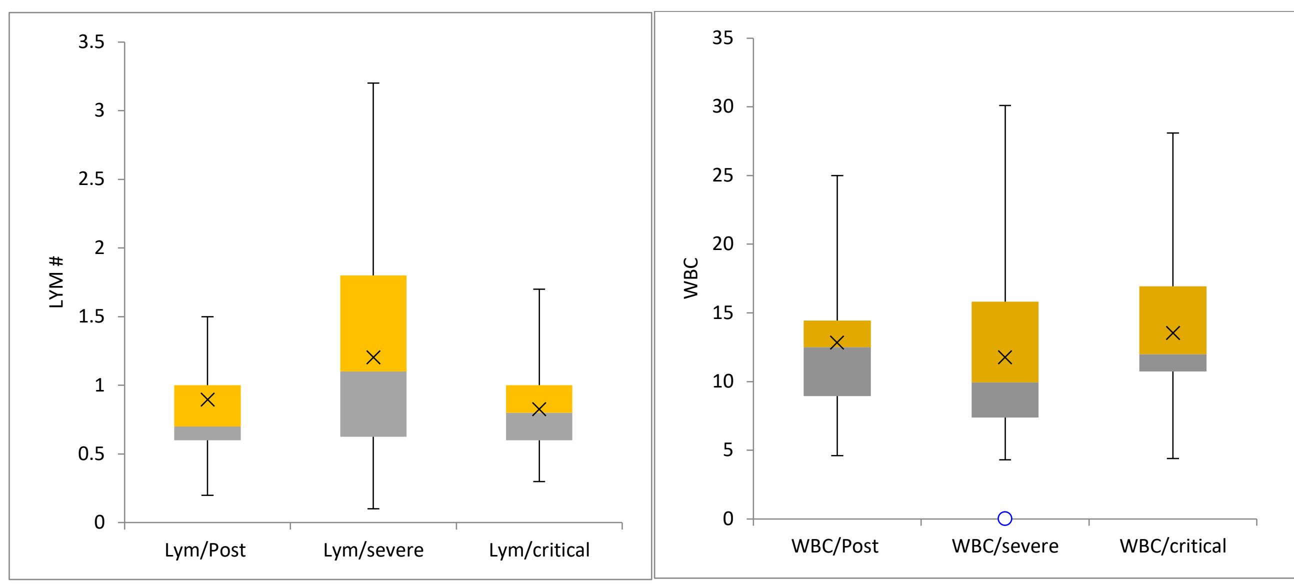


Figure 3.4: The Boxplot of the distribution of lymphocyte count and WBCs count in COVID-19 patients based on their disease severity.

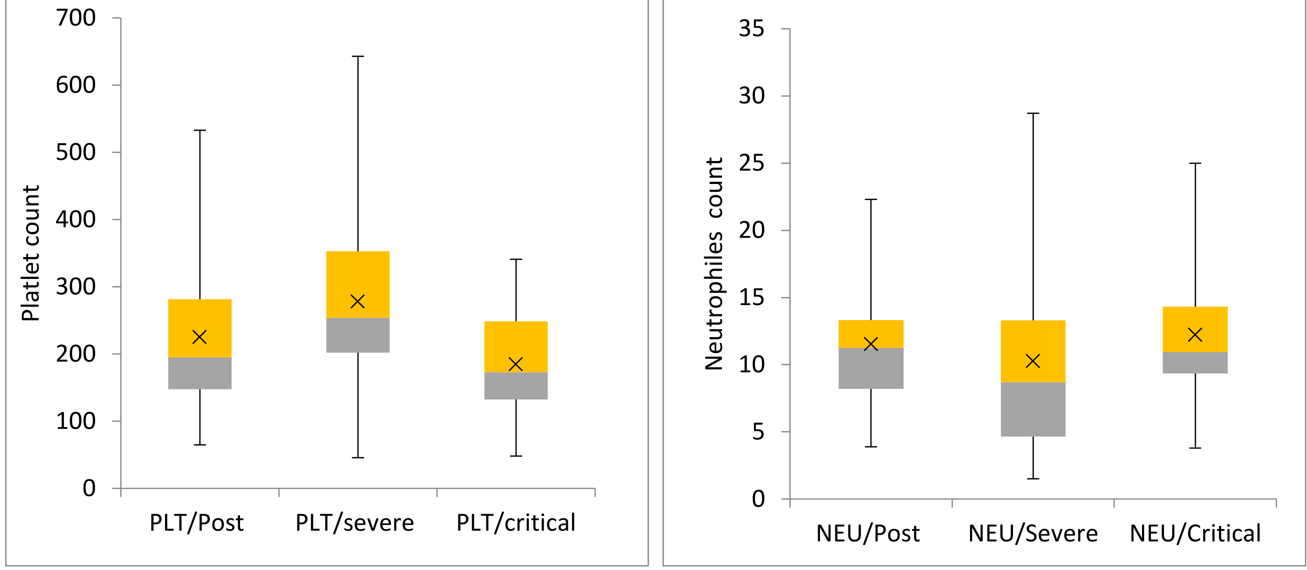
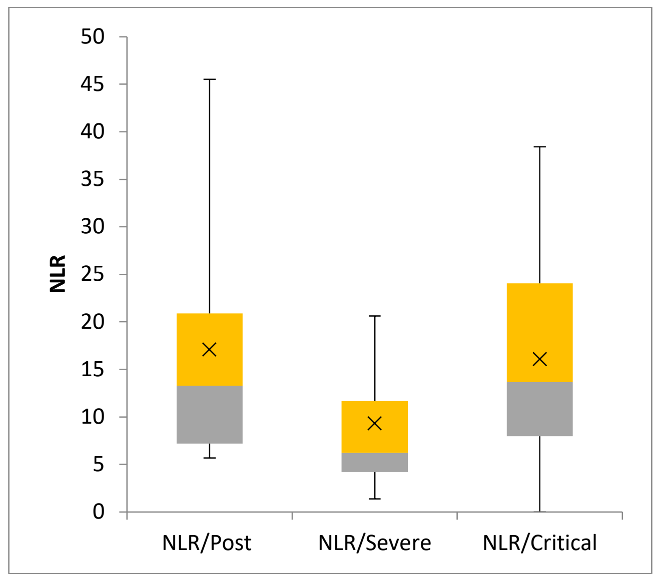


Figure 3.5: the Boxplot of the distribution of hematological parameters & inflammation markers in COVID-19 patients based on their disease severity.



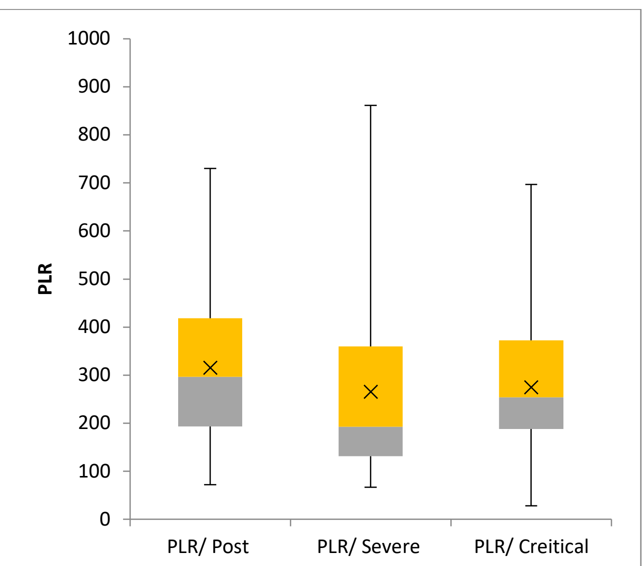


Figure 3.6: the Boxplot of the distribution of NLR & PLR in COVID-19 patients based on their disease severity.

A decrease in the lymphocytes was observed in early infected 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 (HENRY, 2020). The decreased lymphocytes count was observed in the severe COVID-19 patients individuals which could be attributed to increased inflammation and suppression of the immune system caused by COVID-19 infection (WARIS, Abdul & et al. , 2021). Various studies have supported lymphocytopenia as a reliable and effective biomarker for the severity of COVID-19 disease (Yonggang & *et al*., 2020).

Furthermore, Neutrophils count was shown a wide variability in early infected of COVID-19. It has been previously reported that the Neutrophils count increased with the severity of the COVID-19 disease (Mardani R, Ahmadi Vasmehjani A, Zali F, & *et al.*, 2020). With regard to leukocytosis (The increase in the WBC levels), both groups were significantly associated with leukocytosis.

Previous investigations have also reported neutrophil to lymphocytes ratio as important prognostic factor for disease progression (Yang AP, Liu JP, Tao WG, & Li HM. , 2020). These differences in the research parameters were related to morphology of neutrophils, monocytes, and lymphocytes between COVID-19– patients. The presence of

different parameter values between COVID-19–patients groups could be attributed to the severity of the infection (Olga Pozdnyakova MD & *et al.,* 2020).

As COVID-19 causes a systemic inflammatory response, neutrophils are activated by virus-induced inflammatory markers IL-6, IL-8, gama interferon (IFN-ϒ) and alpha tumor necrotic factor (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 (WARIS, Abdul & *et al.* , 2021) .

Liao et al, (Liao D., Zhou F., Luo L., & *et al*, 2020) also found the wide range variability of 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 (Yang AP, Liu JP, Tao WG, & Li HM. , 2020).

Neutrophilia was more prominent in moderate and critical than in severe patients. Qin et al, (C. Qin, 2020) reported significantly higher neutrophil count in severe patients (LI, 2020). The presence of neutrophilia could be related to the cytokine storm that characterizes COVID-19 disease (Zhang B, Zhou X, Qiu Y, & *et al.* , 2020).

3.4 Correlation between Coagulation Markers among COVID-19 Severity

Considering the important role of the measured biomarkers in the severity of COVID-19 patients, the multivariable linear regression model was used to analyze the response relationship between parameters. In critical cases, a weakly correlation was confirmed between D-dimer and PAR-1 [rs= 0.32, P value =0.05]. The relationship between the parameters and d-dimer was presented in Table (3.4).

Previous research has shown that D-dimer and PAR-1 are highly significant in the life-threatening cases (T. M. Stanne, et al., 2021). When elevated levels of the proteins PAR and D-dimer were investigated for their associations with disease progression in multivariate ordinal logistic regressions (T. M. Stanne, Annie Pedersen, Magnus Gisslén, & Christina Jern, 2021).

Table 3-4: Correlation coefficients by Spearman test between D-dimer and PAR-1, Thrombin and Prothrombin based on COVID-19.

	D-dimer Sever group		D-dimer Critical group		D-dimer Post group	
	P value	rs	P value	rs	P value	Rs
PAR-1	0.31	0.22	0.05	0.32	0.35	0.15
Thrombin	0.65	-0.28	0.51	0.13	0.25	-0.26
Prothrombin	0.82	-0.1	0.59	0.1	0.45	0.17

On the other hand, a simple linear regression of PAR-1 and NLR among COVID patient groups was also performed. Figure (3-7) pointed weak significant negative correlation of PAR-1 toward NLR and thrombin [r = -0.3, 0.4; P <0.05].

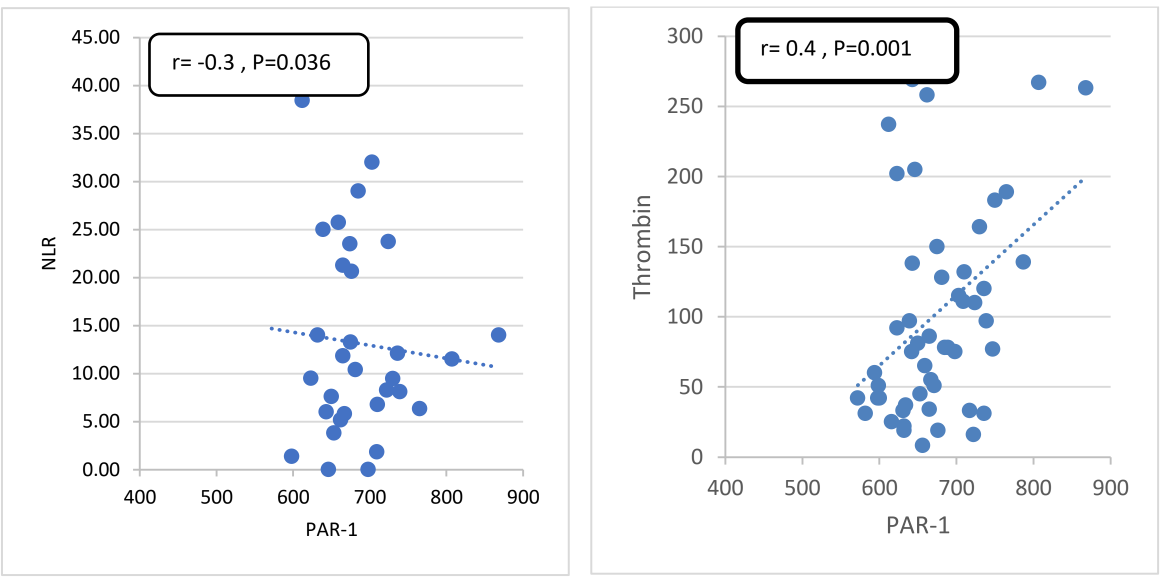


Figure 3.7: Simple linear regression of PAR-1 vs NLR & Thrombin levels

PAR-1 on the surface of platelets are how thrombin activates them (J B Larsen, et al., 2021). Endothelial dysfunction, coagulopathy, and tissue damage are correlated to higher levels of thrombin activation of PAR1 on the endothelium and platelets (Jin Yuefei, Ji Wangquan, Yang Haiyan, & *et al,* 2020).

A significant positive correlation was confirmed between PLR and NLR [r = 0.55, P <0.001] as presented in figure (3.8). This ensures that the severity of COVID-19 disease is correlated with NLR and PLR levels (Palladino, 2021). Thus, NLR and PLR values were higher in patients with serious disease in comparison to non-serious disease (Chan AS, *et al.*, 2020). Also, a significant positive correlation was confirmed between NLR and D-dimer [r = 0.5, P =0.001] as shown in figure (3.7).

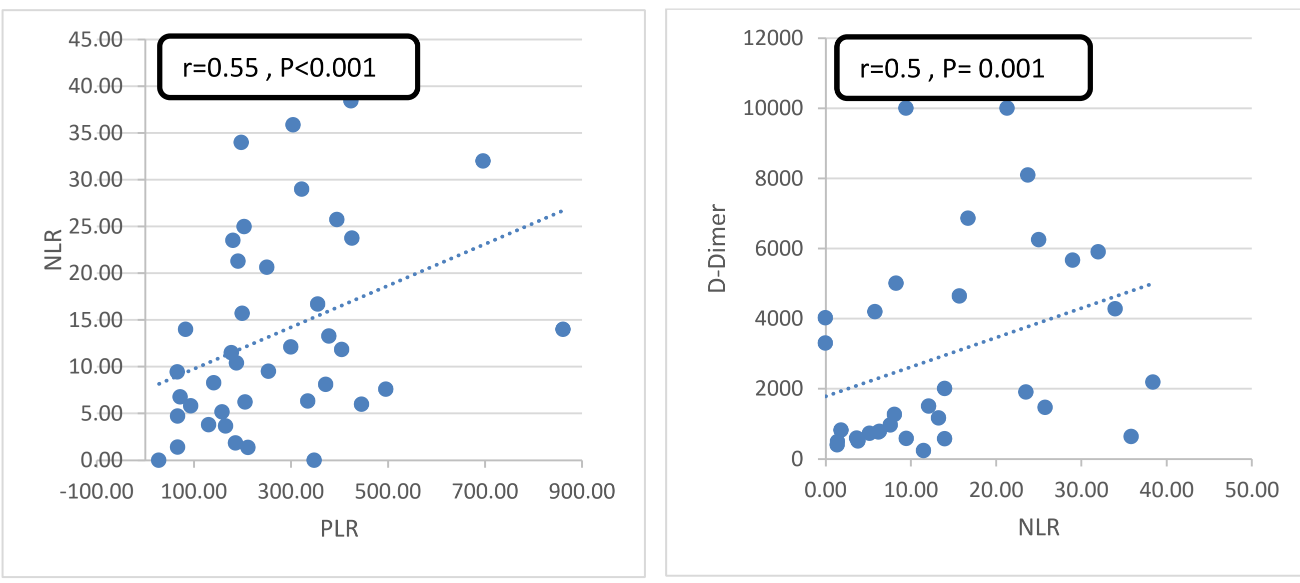


Figure 3.8: Simple linear regression of biochemical markers levels among COVID -19 patients groups: PLR *vs* NLR & NLR *vs* D-dimer.

3.5 Receiver Operating Characteristic (ROC) curve of serum PAR-1 and Thrombin levels for diagnosis of critical cases of COVID-19.

Results of the receiver operating curve (ROC) curve and AUC analysis for the PAR-1 and Thrombin levels as possible diagnostic parameters. Both markers were shown a good diagnostic performance for prediction critical cases of covid-19 Patients compared to severe and post cases group, data are presented in table (3.5) and figures (3.9); (3.10); and (3.11).

For PAR-1 levels: (sensitivity = 96%, specificity = 92%) at a level = 596, while thrombin levels: (sensitivity = 0.96%, specificity = 0.76%) at a level = 39.5. interestingly, the diagnostic points of thrombin levels in predicting critical cases of COVID-19 were

indicated a significant threshold, p-values of the AUC for thrombin were 0.028 and statistically significant.

Levels of NLR were also statistically significant differed, NLR ratio was indicated a good prognosis value of the combined inflammatory indicators. Receiver operating characteristics curves indicated that the diagnostic performance of the NLR ratio in COVID-19 groups exhibited as a predictive value. Infection causes changes in inflammatory markers and immune cells which were significantly different based on disease severity.

Table 3.5: AUC, optimal threshold, Sensitivity, and specificity of PAR-1and thrombin level obtained by the ROC curves for prediction of critical cases of COVID-19.

Test Variable	AUC	Sensitivity %	Specificity %	Cut-off points	P vale	CI (95%)
PAR-1	0.6	0.96	0.92	596	0.402	0.426 - 0.686
Thrombin	0.7	0.968	0.761	39.5	0.028	0.526- 0.772
NLR	0.7	0.95	0.74	3.5	0.012	0.565-0.819

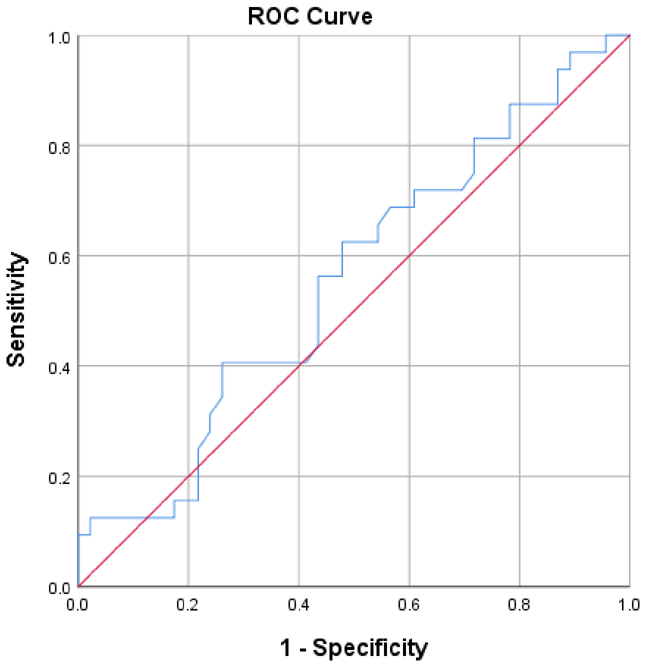


Figure 3.9: ROC curves for PAR-1 levels in COVID-19 patients to analyze the optimal diagnostic points for predicting critical cases.

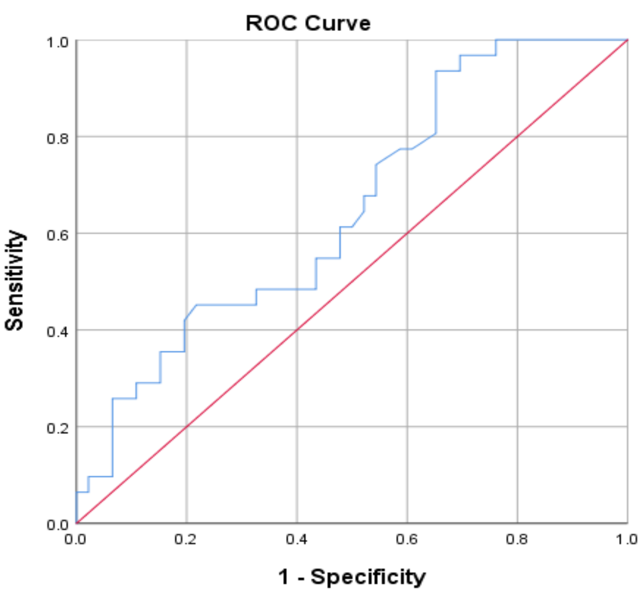


Figure (3.10) ROC curves for Thrombin levels in COVID-19 patients to analyze the optimal diagnostic points for predicting critical cases.

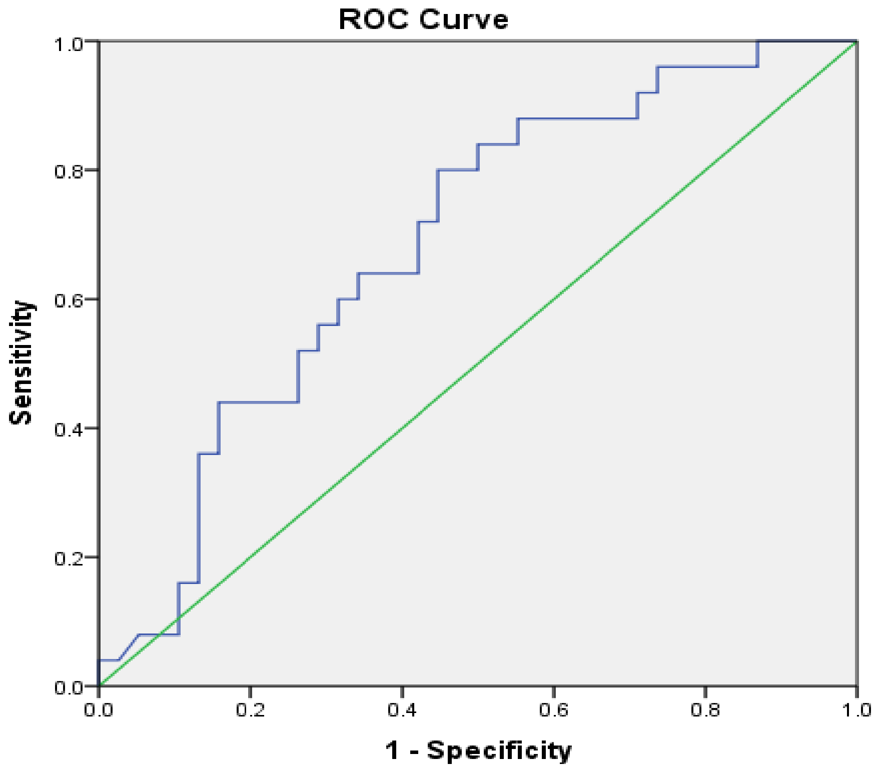


Figure 3.11: ROC curves for NLR levels in COVID-19 patients to analyze the optimal diagnostic points for predicting critical cases.

3.6 Receiver Operating Characteristic (ROC) Curve of Serum PAR-1 and Thrombin Levels for Diagnosis of Post Cases of COVID- 19

Furthermore, the analysis of the optimal diagnostic points for prediction coagulopathy in post cases of Covid-19 was performed.

Results were indicated that d-dimer and PAR-1 were demonstrated the most interesting significant prediction about covid complication in post cases. Diagnostic thresholds of noninvasive models for such cases were presented in figures (3.12); (3.13) and (3.14), and table (3.6), Results of this study confirmed the association of serum d-dimer and PAR-1 levels to the coagulopathy process after covid infection . To the best of our knowledge, this is an detailed study about the analysis of the optimal diagnostic points for

predicting advanced complication in post cases. No Previous study was pointed any data about the role of PAR-1 in post cases of COVID-19.

Table 3.6 : AUC, optimal threshold, sensitivity, and specificity of D-dimer, PAR-1and thrombin level obtained by the ROC curves for prediction of coagulopathy in post cases of COVID-19.

Test Variable	AUC	Sensitivity %	Specificity %	Cut-off points	P vale	CI (95%)
PAR-1	0.7	0.957	0.764	633	0.017	0.547-0.796
D-dimer	0.75	0.913	0.837	592	0.001	0.62-0.864
Thrombin	0.6	0.957	0.556	75.5	0.14	0.484-0.727

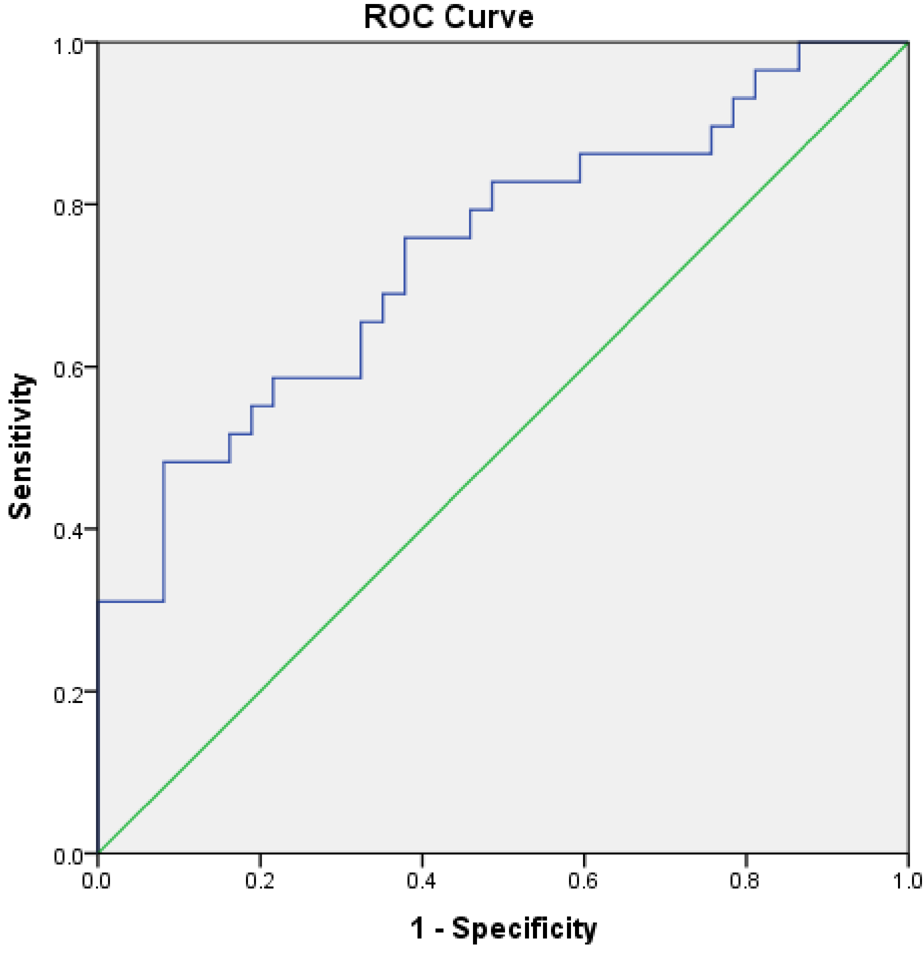


Figure 3.12 : ROC curves for D dimer levels in COVID-19 patients to analyze the optimal diagnostic points for prediction of coagulopathy in post cases.

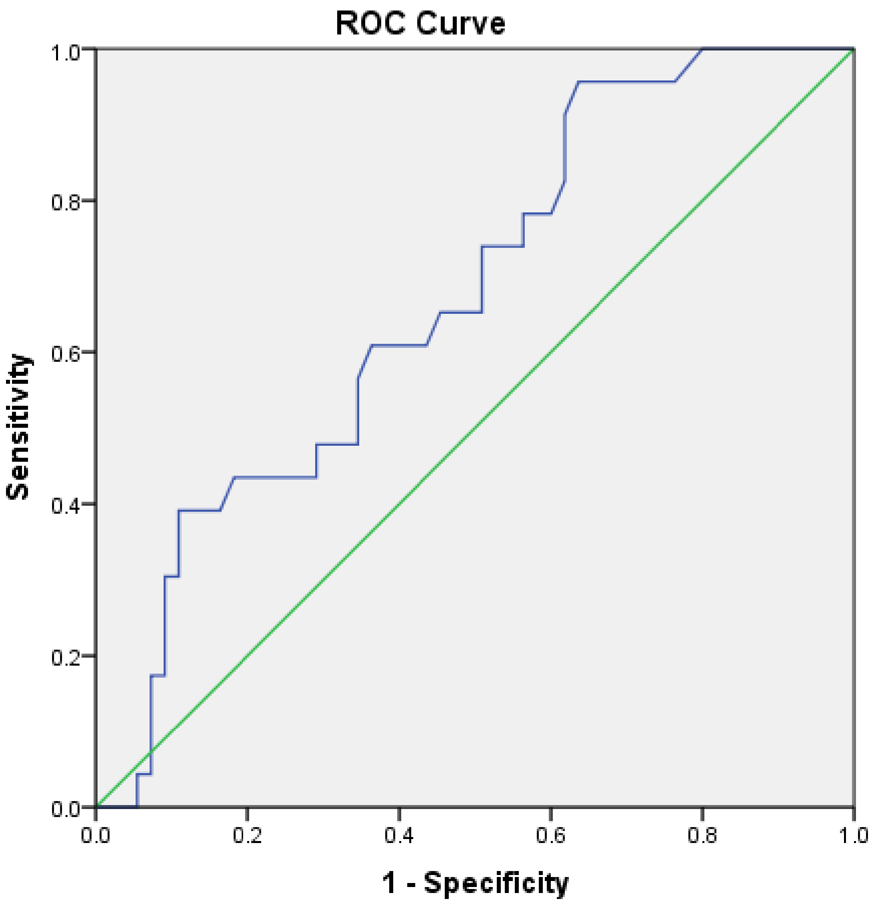


Figure 3.13: ROC curves for PAR-1 levels in COVID-19 patients to analyze the optimal diagnostic points for prediction of coagulopathy in post cases.

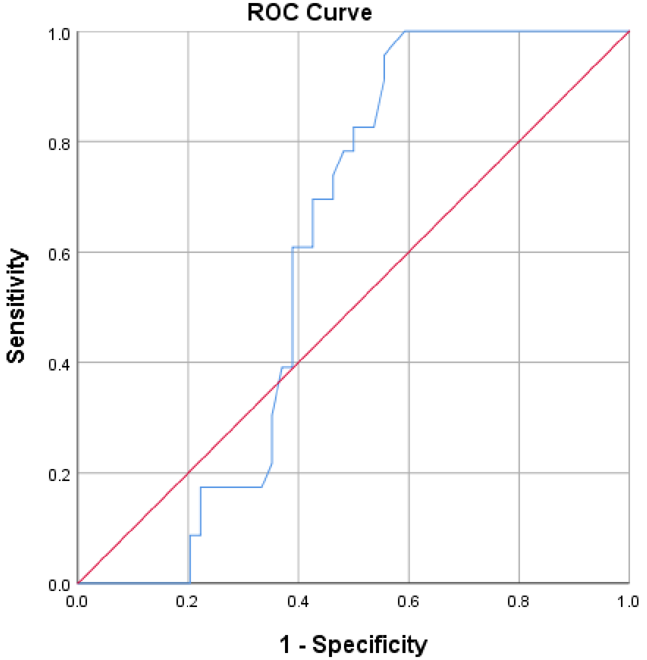


Figure 3.14: ROC curves for Thrombin levels in COVID-19 patients to analyze the optimal diagnostic points for prediction of coagulopathy in post cases.

The results of this study confirmed the association of serum PAR-1 levels to coagulopathy process in post Covid cases. To the best of our knowledge, this is an detailed study about the analysis of the optimal diagnostic points for predicting coagulopathy complication in Covid cases. Previously reported Endothelial dysfunction, coagulopathy, and tissue damage are linked to increased levels of thrombin signaling of PAR1 on the endothelium and platelets (Rezaie A.R., 2014) (Krishna Sriram & Paul A. Insel, 2021). According to this mechanism, COVID-19 will exhibit greater tissue damage and coagulopathy (Ackermann M, Verleden SE, Kuehnel M, & *et al,* 2020). Endothelial barrier function, vasoreactivity, intimal hyperplasia, inflammation, and coagulation are all significantly impacted by PAR1 (K. Erturk, D. Tastekin, E. Bilgin, & *et al.*, 2016). Inflammation process in covid 19 cases can activate the coagulation system through proinflammatory cytokines, whereas the coagulation system can influence inflammatory activity through thrombin, which is the most important player in activating PARs, which up-regulate inflammatory molecules such as cytokines, chemokines, growth factors, and adhesion molecules (Ekholm M & Kahan T , 2021).

Conclusion and future work

Conclusion and Future Work

Conclusion

The direct COVId-19 infection of endothelial cells may cause endothelial dysfunction, which contributes to the coagulopathy . This study were concluded:

- Serum PAR-1 level was slightly increased in post COVID-19 and critical cases.
- In critical cases, a weakly correlation was confirmed between D-dimer and PAR-1. Also A significant positive corrlelation was confirmed between PLR and NLR
- The PAR-1 and Thrombin levels as possible diagnostic parameters were shown a good diagnostic performance for prediction critical cases of COVID-19 Patients compared to severe and post cases groups.
- NLR ratio was indicated a good prognosis value of the combined inflammatory indicators.
- D-dimer and PAR-1 were demonstrated the most interesting significant prediction about COVID-19 complication in post cases.

Future Work

1) More research is needed in the future to determine the precise mechanism through which PAR-1 contributes to the endothelial dysfunction of COVID-19.
2) Additional studies are required to characterize and support the regular clinical usage of adhesion molecules such VAP-1, ICAM-1, and VEGF evaluations in Endothelial dysfunction.
3) Also, It would be a good idea to characterize and support the clinical application of proinflammatory and procoagulant factors, such as P-selectin and E-selectin evaluations in Endothelial dysfunction and coagulopathy.
4) Furthermore, there is needed to characterize the clinical application of pro-aggregatory and vasoconstrictor factor evaluations, such as TXA2, in endothelial dysfunction.
5) The clinical usage of procoagulant factors like phosphatidylserine evaluations in coagulopathy and endothelial dysfunction might be worth doing for the future.

References

References

Ackermann M, Verleden SE, Kuehnel M, & et al. (2020). Pulmonary vascular endothelialitis,thrombosis, and angiogenesis in COVID-19. *N Engl J Med*, 383: 120–128 .

AlSubaie AM. (2020). Coagulopathies in novel coronavirus (SARS-CoV-2) pandemic: emerging evidence for hematologists. *Saudi J Biol Sci.*, 28(1):956–961.

Anne Waugh , & Allison Grant. (2018). The blood. In A. Waugh, & A. Grant, *Ross & Wilson Anatomy and physilology in health and illness 13th edition* (70-71).

A. J. ten Cate-Hoek , A.W J. H. Dielis , & H. M. H. Spronk. (2008). Thrombin generation in patients after acute deep-vein thrombosis. *Thromb Haemost*, 100(02): 240-245.

Ai-Ping Yang, Jian-ping Liu, Wen-qiang Tao, & Hui-ming Li. (2020). The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients,. *International Immunopharmacology*, 84,106504.

Bastug A, Bodur H, Erdogan S, & et al. (2020). Clinical and laboratory features of COVID-19: Predictors of severe prognosis. *Int Immunopharmacol.*,88:106950.

Baviskar T, Raut D, & Bhatt LK. (2021). Deciphering Vaccines for COVID-19:where do we stand today? *Immunopharmacol.Immunotoxicol*, 43(1):8–21.

Bonaventura A., Vecchié A., Dagna L. , & et al. (2021). Endothelial dysfunction and immunothrombosis as key pathogenic mechanisms in COVID-19. *Nat Rev Immunol*, 21, 319–329.

Cao x. (2020). COVID-19: immunopathology and its implications for therapy. *Nat Rev Immunol.*, ;20(5):269–270.27.

C. Qin, L. Zhou, Z. Hu, & et al. (2020). Dysregulation of Immune Response in Patients With Coronavirus 2019 (COVID-19) in Wuhan, China. *Clinical Infectious Diseases*, 71/15, P 762–768.

Chan AS, & Routa A. (2020). Use of Neutrophil-to-Lymphocyte and Platelet-to-Lymphocyte Ratios in COVID-19. . *J Clin Med Res*, 12:448-53.

D. M. Heuberger , & R. A. Schuepbach . (2019). Protease-activated receptors (PARs): mechanisms of action and potential therapeutic modulators in PAR-driven inflammatory diseases. *Thrombosis J* , 17, 4.

E. Giannitsis, J. Mair, C. Christersson , & et al. (2017). “How to use D-dimer in acute cardiovascular care,. *European Heart Journal Acute Cardiovascular Care*, 6(1) 69–80.

Ekholm M , & Kahan T . (2021). The Impact of the Renin-Angiotensin Aldosterone System on Inflammation,Coagulation, and Atherothrombotic Complications, and to Aggravated COVID-19. *Front. Pharmacol.* , 12:640185.

Fenna, & Ian. (2020). *SARS-COV-2 (2019-nCoV) Proteins.* Crech Republic Biovendor.

G.M. Bwire. (2020). Coronavirus: Why Men are More Vulnerable to Covid-19 Than Women?. *SN Comprehensive Clinical Medicine volume 2*, 874–876 .

References

Gallwitz M, Enoksson M, Thorpe M, & Hellman L . (2012). The Extended Cleavage Specificity of Human Thrombin. *PLOS ONE*, 7(2): e31756.

Gasecka A, Böing AN, Filipiak KJ, & Nieuwland R. (2017). Platelet extracellular vesicles as biomarkers for arterial thrombosis. *Platelets*, ;28(3):228–34.

Gąsecka, A., Borovac, J., Guerreiro, R. A., & et al. (2021). Thrombotic Complications in Patients with COVID-19:Pathophysiological Mechanisms, Diagnosis, and Treatment. *Cardiovascular Drugs and Therapy* , 35:215–229.

Grover S. P, & Mackman N. (2018). Tissue Factor. . *Arterioscler Thromb. Vasc.Biol.* , pp. 38 (4), 709–725.

Guan W, Ni Z, Hu Y, & et al. (2020). Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med.*, 382(18):1708–1720.

HENRY, B. M. (2020). COVID-19, ECMO, and lymphopenia: a word of caution. . *The Lancet Respiratory Medicine*, 8.4: e24.

Huang C. , Wang Y. , Li X., & et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China,. *The Lancet*, 497-506.

Huang W, Berube J, McNamara M, & et al. (2020). Lymphocyte Subset Counts in COVID-19 Patients:A Meta-Analysis. *Cytometry A.*, 97:772-6.

J .Thachil, N Tang, S Gando, & et al. (2020). ISTH interim guidance on recognition and management of coagulopathy in COVID-19. *Journal of Thrombosis and Haemostasis*, 18.5: 1023-1026.

Jackson SP, Darbousset R, & Schoenwaelder SM. (2019). Thromboinflammation:challenges of therapeutically targeting coagulation and other host defense mechanisms. . *Blood* , 133: 906–918.

James D. M., Hannah S., & Karlheinz P. (2020, 07 31). The Emerging Threat of (Micro)Thrombosis in COVID-19 and Its Therapeutic Implications. *Circulation Research*, 127:571–587.

Jin Y., Yang H., Ji W., & et al. (2020). Virology, Epidemiology, Pathogenesis, and Control of COVID-19. *Viruses*, 12, 372.

Joly BS, Siguret V, & Veyradier A. (2020). Understanding pathophysiology of hemostasis disorders in critically ill patients with COVID-19. . *Intens Care Med* , 46: 1603–1606 .

J B Larsen, & A-M Hvas. (2021). Thrombin: A Pivotal Player in Hemostasis and Beyond. *Seminars in Thrombosis and Hemostasis*, 47(07): 759-774.

K. Erturk, D. Tastekin, E. Bilgin, & et al. (2016). Clinical significance of serum protease activated receptor1 levels in patients with lung cancer. *European Review for Medical and Pharmacological Sciences*, 20: 243-249.

Kieron South, Laura McCulloch, Barry W McColl, & et al. (2020). Preceding infection and risk of stroke: An old concept revived by the COVID-19 pandemic. *International Journal of Stroke*, Vol. 15(7) 722–732.

References

Krishna Sriram, & Paul A. Insel. (2021). Inflammation and thrombosis in COVID-19 pathophysiology: Proteinase-activated and purinergic receptors as drivers and candidate therapeutic targets. *Physiol Rev*, 101: 545–567, 2021.

Kruger-Genge A, Blocki A., Franke R. P. , & Jung F. (2019). Vascular endothelial cell biology: an update. *Int. J. Mol. Sci.*, 20, 4411.

Li R, Pei S, Chen B, & et al. (2020). Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV-2). *Science*, 368: 489–493.

Lippi G, Plebani M, & Henry BM . (2020). Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections:a meta-analysis. . *Clinica Chimica Acta*, 506:145–8.https://doi.org/10.1016/j.cca.2020.03.022.

LI Qiubai, & et al. . (2020). Hematological features of persons with COVID-19. *Leukemia*, 34.8: 2163-2172.

Li Yang , & et al. (2020). COVID-19: immunopathogenesis and Immunotherapeutics. *Signal Transduction and Targeted Therapy*, 5:128.

Liao D., Zhou F., Luo L., & et al. (2020). Haematological characteristics and risk factors in the classification and prognosis evaluation of COVID-19: a retrospective cohort study. *Lancet Haematol.*, ;S2352-3026(20)30217-9.

Lu H, Stratton CW, & Tang YW. (2020). Outbreak of pneumonia of unknown etiology in Wuhan, China: the mystery and the miracle. *J Med Virol*, 92(4):401–402.

M. Cunningham, K. McIntosh, T. Bushell, & et al. (2016). Proteinase-activated receptors (PARs) as targets for antiplatelet therapy. *Biochem Soc Trans*, 44 (2): 606–612.

Mahmoud G. , Kaiming W., Anissa V., & et al. (2020, 05 8). Angiotensin-Converting Enzyme 2: SARS-CoV-2 Receptor and Regulator of the Renin-Angiotensin System. *Circulation Research*, 126:1456–1474.

Markus Hoffmann, Hannah K W, Simon Schroeder, & et al. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*, 181 (2) 271–280.e8.

Mardani R, Ahmadi Vasmehjani A, Zali F, & et al. (2020). Laboratory parameters in detection of COVID-19 patients with positive RT-PCR: a diagnostic accuracy study. *Arch Acad Emerg Med.*, 8(1):e43.

Merrill J. T., Erkan D. , Winakur J., & James J. . (2020). Emerging evidence of a COVID-19 thrombotic syndrome has treatment implications. *Nat. Rev. Rheumatol*, 16,581–589 .

Mitra Abbasifard, & Hossein Khorramdelazad. (2020). The bio-mission of interleukin-6 in the pathogenesis of COVID-19: A brief look at potential therapeutic tactics. *Life Sciences*, 257.

Olga Pozdnyakova MD, & et al. (2020). Clinical Significance of CBC and WBC Morphology in the Diagnosis and Clinical Course of COVID-19 Infection. *Am J Clin Pathol*, :1-12.

Palladino M. (2021). Complete blood count alterations in COVID-19 patients: A narrative review. *Biochemia Medica (Zagreb)*, 31(3):030501.

References

Parker WAE, & Storey RF . (2018). 'Thrombotic Response' in ESC. In P. WAE, & S. RF, *Textbook of Cardiovascular Medicine 3rd edition.*

P. C. S. van Paridon, M P Noeva, R van Oerle, & et al. (2022). Relationships between coagulation factors and thrombin generation in a general population with arterial and venous disease background. *Thrombosis J*, 20, 32 .

Ranucci M., Ballotta A., Dedda U. D., & et al. (2020). The procoagulant pattern of patients with COVID-19 acute respiratory distress syndrome. *J. Thromb. Haemost.*, 18, 1747–1751 .

Rezaie A.R. (2014). Protease-activated receptor signalling by coagulation proteases in endothelial cells. *Thromb Haemost*, 112: 876–882.

Shi H, Han X, Jiang N, & et al. (2020). Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan,China: a descriptive study. . *Lancet Infect Dis* , 20:425–434.

Shreya R. S., Kedar S. P., & Lokesh K. B. (2021). Cytokine storm associated coagulation complications in COVID-19 patients: Pathogenesis and Management. *Expert Review of Anti-infective Therapy*,

Soraya G.V., & Ulhaq Z.S. (2020). Crucial laboratory parameters in COVID-19 diagnosis and prognosis: An updated metaanalysis. *Med Clin (Engl Ed).*, 155:143-51.

Sturtzel, C. (2017). Endothelial cells. In *The Immunology of Cardiovascular Homeostasis and Pathology* (71-91).

Tang N, Li D, Wang X, & et al. (2020). Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. . *J Thromb Haemost.* , 18(4):844–847.

Teuwen L. A., Geldhof V., Pasut A., & Carmeliet P. (2020). COVID-19: the vasculature unleashed. *Nat. Rev. Immunol.*, 20, 389–391.

T. M. Stanne, Annie Pedersen, Magnus Gisslén, & Christina Jern. (2021). Low admission protein C levels are a risk factor for disease worsening and mortality in hospitalized patients with COVID-19. *J.Thrombosis Research*,

Thachil J. (2020). What do monitoring platelet counts in COVID-19 teach us? *J Thromb Haemost* , 18: 2071–2072. doi:10.1111/jth.14879.

Tortora G J, & Derrickson B. (2016). Hemostasis. In *Principles of anaiomy & physiology 15th edition* (676-678).

Ulanowska M., & Olas B. (2021). Modulation of Hemostasis in COVID-19; Blood Platelets May Be Important Pieces in the COVID-19 Puzzle. *Pathogens*, 10, 370.

Varga Z, Flammer A.J., Steiger P., & et al. (2020). Endothelial cell infection and endotheliitis in COVID-19. *Lancet*, 395, 1417–1418.

Varga, Z., Flammer, A., Steiger, P., Haberecker, M., & et al. (2020). Endothelial cell infection and endotheliitis in COVID-19. . *Lancet*, 395, 1417–1418 .

Walls A.C., Park Y.J., Tortorici M.A, & et al. (2020). Structure,function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell*, 181:281–292.e6.

References

Wan Y, Shang J, Graham R, & et al. (2020, 05 17). Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. *J Virol*, 94:e00127-20.

Wang, K., Chen, W., Zhou, Y.-S., & et al. (2020). SARS-CoV-2 invades host cells via a novel route: CD147-spike protein. *bioRxiv*.

WHO, W. H. (27 May 2020). *Clinical management of COVID-19: interim guidance.*

WARIS, Abdul, & et al. . (2021). Evaluation of hematological parameters as an indicator of disease severity in Covid-19 patients: Pakistan's experience. *Journal of Clinical Laboratory Analysis.*, e23809.

Yang AP, Liu JP, Tao WG, & Li HM. . (2020). The diagnostic and predictive role of NLR,d-NLR and PLR in COVID-19 patients. *Int Immunopharmacol.*, 84:106504.

Yonggang, Z., & et al. (2020). Pathogenic T-cells and inflammatory monocytes incite inflammatory storms in severe COVID-19 patients. *National Science Review*, 7.6: 998-1002.

Yuefei Jin, Wangquan Ji, Haiyan Yang, & et al. (2020). Endothelial activation and dysfunction in COVID-19: from basic mechanisms to potential therapeutic approaches . *Signal Transduction and Targeted Therapy*, 5:293 . https://doi.org/10.1038/s41392-020-00454-7.

Zaid Abassi , Yara Knaney, Tony K., & Samuel N. H. (2020). The Lung Macrophage in SARS-CoV-2 Infection: A Friend or a Foe? *Front. Immunol*, 11:1312.

Zaid Y., Puhm F., Allaeys I., & et al. (2020). Platelets Can Associate With SARS-CoV-2 RNA and Are Hyperactivated in COVID-19. *Circ Res.* ., 127:1404–18.

Zhang B, Zhou X, Zhu C, & et al. (2020). Immune Phenotyping Based on the Neutrophil-to-Lymphocyte Ratio and IgG Level Predicts Disease Severity and Outcome for Patients With COVID-19. *Front Mol Biosci.*, 7:157.

Zhang B, Zhou X, Qiu Y, & et al. . (2020). Clinical characteristics of 82 death cases with COVID-19. *PLoS One.*, 15(7):e0235458.

Zhang L, Huang B, Xia H, & et al. (2020). Retrospective analysis of clinical features in 134 coronavirus disease 2019 cases. *Epidemiol Infect*, 2020;148:e199. https://doi.org/10.1017/S0950268820002010.

Zhou P, Yang XL, Wang XG, & et al. (2020, 02 03). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Natuer*, 579(7798):270–3. https://doi.org/10.

Zhu N, Zhang D, Wang W, & et al. (2020). A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*, pp. 382 (8):727–733.

Questionnaire

Patient name	Sex	Age	Marital state	Occupation
....				

Adress

Smoking ☐ **Autoimmune disea** ☐

Hypertensive : ☐ **Diabetes mellitus :** ☐

Disease state : **Mild** ☐ **Moderate** ☐

Severe ☐ **Critical** ☐

Patients data: Vital signs

Body temperature		
<37 ^{0}C	38 ^{0}C -38 ^{0}C	>38 ^{0}C

Oxygen saturation rate			
< 90%	90% -92.9 $^{\%}$	93% -95.9%	>96 %

Blood pressure			
< 120; <80 mmHg	120-140; 80-89 mmHg	141-159; -90-99 mmHg	>159; >99mmHg

Main investigation results	
PCR	
Other	

Appendices

Report symptoms at presentation n %	
Duration of symptoms prior to the test	
Fever	
Chills	
Body ache	
Cough	
Sore throat	
Breathing difficulty	
Loss of taste	
Loss of smell	
Chest pain	
Running nose	
Diarrhea	
Tiredness	
Vomating	
Blurred vision	

Risk factors	
Close contact with confirmed covid 19 patients	
Travel history	

University of Kerbala
College of Medicine
Medical Research Bioethical Committee

FINAL APPROVAL LETTER

Muntadhar Ali, MSc student
University of Kerbala, College of Medicine, Dept of Biochemistry
Date. December, 22nd, 2021
Dear Muntadhar
Title of Project:
Assessment of vessels endothial dysfunction by protease active receptors (PARs) signal in COVID-19 patients.

Reference Number: 22

Thank you for your recent correspondence. This is to certify that your responses have satisfactorily addressed the research bioethical guidelines. You may now proceed with your research.
Please consider the following requirements of approval:
1. Approval will be valid for one year. By the end of this period, if the project has been completed, abandoned, discontinued or not commenced for any reason, you are required to announce the Committee.
2. However, at the end of the one-year period if the project is still current you should instead submit an application
for renewal of the approval.This allows the Committee to fully re-review research in an environment where legislation, guidelines and requirements are continually changing.
3. Please remember the Committee must be notified of any alteration to the project.
4. You must notify the Committee immediately in the event of any adverse effects on participants or of any unforeseen events that might affect continued ethical acceptability of the project.
5. At all times you are responsible for the ethical conduct of your research in accordance with the standard bioethical guidelines.
6. The Committee should be notified if you will be applying for or have applied for internal or external funding for the above project.

Yours sincerely

--Signed--
Assist. Professor Ali Mansoor Al-Ameri

11-

Chair, Medical Research Bioethical Committee
Date: December, 22nd, 2021

Appendices

Republic of Iraq
Al-Najaf Al-Ashraf Governorate
Najaf Health Directorate
Training and Human Development Center

No.
Date:

مركز التدريب و التنمية البشرية
العدد: ٥٨٢٠١
التاريخ: ١٢/١٢/٢٠٢١

إلى/ جامعة كربلاء/كلية الطب

م / تسهيل مهمة

تحية طيبة ...

إشارة إلى كتابكم ذي العدد ٣٣٢١ في ٥/١٢/٢٠٢١ بخصوص تسهيل مهمة الباحث طالب **الماجستير** **(منتظر علي جاسم)** للحصول على الموافقة الاخلاقية لإجراء البحث الموسوم:

Assessment of vessel endothelial dysfunction by protease actived receptor in COVID-19

حصلت موافقة اللجنة العلمية للبحوث في مركز دائرتنا على إجراء البحث في **(مستشفى الشهيد حسن هلوس الحاتمي)** في دائرتنا مع التأكيد على الالتزام الكامل بتعليمات السلامة الحيوية والضوابط الاخلاقية والحصول على موافقة المشاركين قبل الشروع بالبحث والحفاظ على خصوصيتهم وعدم افشاء البيانات او استخدام العينات لغير اغراض البحث العلمي ... على أن لا تتحمل دائرتنا أية تبعات مادية ..

للتفضل بالاطلاع ...... مع الاحترام.

الدكتور
رضوان كامل الكندي
المدير العام
١٢/١٢/٢٠٢١

نسخة منه إلى/
مكتب المدير العام / للعلم مع الاحترام .
مركز التدريب و التنمية البشرية / مع الأوليات .
مستشفى الشهيد حسن هلوس الحاتمي/ /...... تسهيل مهمة الباحثين مع التقدير.

تسلسل الاستمارة ٣٨٤

الملخص

نبذة: يظهر الخلل البطاني كواحد من المساهمين الرئيسيين في التسبب في أحداث الانسداد الخثري في كوفيد-19. على الرغم من أن السبب الرئيسي للوفيات في المرضى الذين يعانون من كوفيد-19 هو فشل الجهاز التنفسي الناجم عن الالتهاب الرئوي الفيروسي ومتلازمة الضائقة التنفسية الحادة ، فقد أظهرت الأدلة المتراكمة أن خطر الإصابة بالجلطات الدموية مرتفع بشكل كبير في المرضى الذين يعانون من كوفيد-19 الحاد وأن حدث الانسداد الخثري هو من المضاعفات الرئيسية الأخرى التي تسهم في ارتفاع معدلات الاعتلال والوفيات لدى المرضى الذين يعانون بعد كوفيد. نظرًا لأن نوع المستقبل المنشط بالإنزيم الحال للبروتين 1 (PAR1) هو مستقبل الثرومبين الرئيسي وقد يمثل رابطًا أساسيًا بين التخثر والالتهاب في الفيزيولوجيا المرضية لكوفيد-19 ، وبالتالي ، فإن مستوى مستقبلات المنشطة بالإنزيم الحال للبروتين سيكون مرتبطًا بالتخثر القياسي وعلامات التهاب بكوفيد-19.

طريقة العمل والمواد: دراسة مقطعية لما مجموعه (93) عينة من المرضى ، بما في ذلك عينات المرضى الحرجة والحادة وعينات ما بعد اصابة بكوفيد-19 في مستشفى الشهيد حسن الحاتمي لأمراض الأنتقالية في محافظة النجف ، العراق. تم قياس مستوى المؤشرات الحيوية في الدم للمعلمة التالية: تم قياس مستويات المستقبل المنشط بالانزيم الحال لبروتين-1 (PAR-1) والثرومبين والبروثرومبين باستخدام تقنية اليزا (ELISA) ؛و تم إجراء مقايسة ديدايمر D-Dimer بواسطة تقنية التفلور الكيمائي المناعي. تم إجراء تعداد الدم الكامل بواسطة محلل الدم الآلي (Sysmex ™ XP-300). تم تقييم العلاقة بين العلامات البيوكيميائية وشدة المرض. تم تقييم كفاءة قيمة التنبؤ باستخدام منحنى خاصية تشغيل المستقبل (ROC).

النتائج: تفاوتت مستويات المؤشرات الدموية على أساس شدة المرض. ازداد مستوى PAR-1 في المصل بصورة طفيفة في حالات ما بعد اصابة بكوفيد-19 والحالات الحرجة. أظهر مستوى الثرومبين في الدم زيادة كبيرة في الحالات الشديدة والحرجة ($p < 0.05$) كانت مستويات PAR-1 في المصل مرتبطة بشكل إيجابي بـ d-dimer تم تأكيد وجود ارتباط ضعيف بين D-dimer و PAR-1. في الحالات الحرجة . أيضًا ، تم تأكيد ارتباط إيجابي معنوي بين PLR و [NLR r=0.55، P <0.001]

أما نتائج منحنى تشغيل المستقبل (ROC) وتحليل منحنيات لمستويات PAR-1 و ثرومبين كمعلمات تشخيصية محتملة. أظهر كلاهما أداءً تشخيصيًا جيدًا للتنبؤ بالحالات الحرجة لمرضى كوفيد-19 مقارنة بمجموعة الحالات الشديدة والحالات مابعد اصابة بكوفيد .وكانت مستويات PAR-1: (الحساسية = 96٪ ، النوعية = 92٪) عند مستوى = 596 بيكوغرام لكل مليلتر ، بينما مستويات الثرومبين: (الحساسية = 0.96٪ ، النوعية = 0.76٪) عند مستوى = 39.5بيكو غرام لكل مليلتلر.

الأستنتاج: كانت مستويات PAR-1 ذات دلالة إحصائية بين مجموعات مرضى كوفيد-19. أظهرت مستويات D-dimer و PAR-1 قيمة عالية للتنبؤ من خلال النتائج المؤكدة التي حصلت عليها منحنيات ROC للتنبؤ باعتلال التخثر في حالات ما بعد كوفيد -19 من بين المجموعات الثلاث لمرضى COVID-19 (الحالات الشديدة والحرجة)

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء

كلية الطب

فرع الكيمياء والكيمياء الحياتية

تقييم الخلل البطاني للأوعية الدموية بواسطة المستقبل المنشط لإنزيم الحال للبروتين -1 -PAR لدى مرضى كوفيد-19

رسالة ماجستير

الى مجلس كلية الطب / جامعة كربلاء كجزء من متطلبات لنيل درجة الماجستير في الكيمياء السريرية

من قبل

منتظر علي جاسم السمباوي

(بكالوريوس تقنيات تحليلات مرضية /كلية التقنية الصحية والطبية كوفة /جامعة الفرات الاوسط التقنية/2016)

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2022/11 م **1444/5 هـ**