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Study of von Willebrand Factor, ADAMTS-13 and some biochemical parameter as Predictor Markers for Severity of Covid-19 in Iraqi Patients

A Thesis

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بسم الله الرحمن الرحيم

﴿قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ﴾

صدق الله العلي العظيم

(سورة البقرة : الآية 32)

Dedication

…. To the Messenger Mohammed ….

and Infallible Imams, Peace be upon them.

And Especial for our Promised Hope,

…. Imam Al-Hujjah...

That Hope that Lights our Paths and the Invisible Hand that Takes Care of Us.

….To My Father….

To those who were praying to the secret of my success and

Affection surgeons Balm

…. My Mother…..

To my life-long companion (husband) ….. Hussein

My Child ….. Haider …..

To the pure hearts and loyal hands who assisted me in Life

My Sisters

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Supervisor's Certification

We certify that this thesis M. Sc. entitled:

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was presented by **(Haneen Saeed Mohsen Al-Mosawi)** and made under our supervision at the Department of Chemistry and Biochemistry, College of Medicine, University of Kerbala, as a partial requirement for the degree of Master in **Clinical Chemistry**.

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In the view of the available recommendation, I forward the present thesis for debate by the examining committee.

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Summary

Coronavirus disease 2019 (Covid-19) is a respiratory disease with heterogeneous manifestations ranging from asymptomatic illness in some, to systemic inflammation, multiorgan failure, and a rapid death in others.

The first stage of disease manifests as an upper respiratory infection followed by pneumonia when the virus invades the respiratory epithelium via binding to reseptors. And a second, more severe, phase may be manifested as multiorgan damage, including respiratory, cardiac, hepatic, and renal injury. At this stage, the receptors on the endothelium can also be involved, causing direct damage to blood vessels and inducing a coagulopathy.

Systemic inflammation and coagulopathy are characteristic hallmarks of this phase. “Covid-19 coagulopathy” manifests mainly as a prothrombotic state affecting both large and small blood vessels, and presenting as arterial, venous, and microangiopathic thrombotic events. The causes of coagulopathy associated with coronavirus disease 2019 (Covid-19) are poorly understood.

Therefore, the aimed of this study to investigate levels of von Willebrand factor (VWF), and adisintegrin and metalloproteinase with a thrombospondin type 1 motif, member-13 (ADAMTS-13) biomarkers, intravascular hemolysis, coagulation, and organ damage in Covid-19 patients and study their association with disease severity and mortality and to a decisive role in identifying patients at risk of developing fatal complications of this case, thus helping to save the lives of many patients.

The case-control study design included collecting the sera of 90 samples from patients and healthy people during the period from January 2021 to May 2021, where the patients were divided into two groups:

The first group: patients with the Corona virus, whose samples were collected from Al-Amal hospital, Al-Shifaa Center in Najaf city, and from Al-Hussein Teaching Hospital in Karbala and it consisted of three groups, the first group mild symptoms, the second group were used mechanical ventilation

(adverse clinical outcome) as sever group symptoms . The results were compared with 30 samples apparently healthy people as a control group.

The serum level of vWF, ADAMTS-13, and vitamin K were determined by ELISA technique, and the level of lipid profiles were determined by enzymatic and colorimetric methods. Ferritin, and D- dimer were determined, by ichroma, CBC automated by auto Hematology Analyzer. Levels of LDL-C, and VLDL-C, data of Atherogenic index (AIP), Catelli's risk index-I (CRI-I), CRI-II, and body mass index (BMI) were calculated by especial equations.

The results of this study found a high significant increase ($p \leq 0.000$) in the levels of D-dimer, ferritin, neutrophils, high sensitivity CRP, TG, VLDL-C, and data of atherogenic index in Covid-19 patients group when compared with healthy group. While, the results showed a significant decrease in the levels of HDL-C, TC, and platelet levels when compared with the healthy group.

These results illustrated that the patients who died had significantly lower ADAMTS-13 activity and vitamin K levels, significantly elevated D-dimer, and ferritin. Significantly elevated vWF levels compared with patients discharged alive.

Linear regression analysis for ADAMTS-13, vWF and Vit. K levels pointed out significant correlation with age, BMI, ferritin, D-dimer, CRP, and NLR in Covid-19 patients groups. Level of serum vWF revealed significant negative correlations with ADAMTS-13, Vit. K, LYMP%, HDL-C , and TC levels. However ADAMTS-13 demonstrated significant positive correlations with TG ,NLR,D-dimer,VLDL-C and levels in patients group.

This study concluded that improve prevention strategies, including those for the mild and sever case by uses of vWF, ADAMTS-13 and Vit. K as predicator markers for Covid-19 severity and their complications.

Thus, in addition to elevated vWF may warrant further work-up including ADAMTS-13 activity and vWF and activity levels since these patients may be at increased risk of mortality and might serve as a simple prognostic measure of thrombotic risk in Covid-19 and potentially a significant contributor to disease

pathogenesis and may benefit from more aggressive therapy .Also, that vitamin K deficiency could support cytokine storm by increasing pro-inflammatory cytokines which is involved in the building up of the inflammatory response recruiting both cellular and humoral components. Vitamin K is well-known to play an essential role in the coagulation system. Highlighting a role of vitamin K, probably via coagulation modulation. Altogether, potential mechanisms linking Covid-19 with coagulopathy in which vitamin K may exert its modulating role in coagulation related with disease pathogenesis Besides, it can also contribute to those events involved in vascular calcification leading to thrombosis and disseminate intravascular coagulation (DIC), which feature the microvascular damage observed in Covid-19 patients.

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Abbreviations

Abbreviations	Full nomenclature
ACE-2	Angiotensin converting enzyme II
ADAMTS-13	A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13
ADP	Adenosine diphosphate
AF	Atrial fibrillation
ARDS	Acute respiratory distress syndrome
ATP	Adenosine triphosphate
BMI	Body mass index
BP	Blood pressure
CBC	Complete blood count
CDC	Centers for Disease Control and Prevention
CHD	Coronary heart disease
CO	Cholesterol oxidase
Covid -19	Coronavirus Diseases-19
CRI-1	Castelli Risk Index
CRI-11	Coronary risk index
CRP	C-reactive protein
CRS	Cytokine release syndrome
CV	Cardio vascular
CVD	Cardio vascular disease
DAMPs	damage-associated molecular patterns
DHAP	dihydroxy acetone phosphate
DIC	disseminated intravascular coagulation
DILI	drug-induced liver injury
DM	Diabetes mellitus
EVs	extracellular vesicles

FFA	Free fatty acids
G-3-P	Glycerol-3-phosphate
GK	Glycerol kinase
GPO	Glycerol-phosphate oxidase
H2O2	Hydrogen peroxide
HF	Heart failure
ICU	Intensive care unit
IL-6	Interleukin-6
LDH	Lactate dehydrogenase
LDL-C	Low density lipoprotein-cholesterol
LPL	Lipoprotein lipase
MERS	East Respiratory Syndrome (MERS
NETs	neutrophil extracellular traps
O.D	Optical Density
PAI-1	plasminogen activator inhibitor-1
PAMPs	pathogen-associated molecular patterns
PCR	polymerase chain reaction
POD	Peroxidase
RAS	renin-angiotensin system
RBD	receptor-binding domain
SARS	Severe acute respiratory syndrome
SD	Standard deviation
TC	Total Cholesterol
TF	tissue factor
TG	Triglycerides
TSP-1	thrombospondin type 1
VLDL	Very low density lipoprotein
vWF	von Willebrand Factor

WBC	White blood cell
WHO	World Health Organization

Chapter ONE

Introduction and Literatures of Review

1. Introduction

The novel coronavirus (Covid-19) that emerged in Wuhan, China in December 2019 quickly spread within Hubei province and has now reached all provinces in China and was exported to >20 countries by 30 January 2020. Covid-19is thought to be primarily transmitted by respiratory droplets with a similar incubation time and generation time as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) **(Zhu, Zhang et al. 2020).** SARS was frightening at the time, maybe even more frightening compared to Covid-19 given its much more frequent progression to severe disease and death. But the world was able to completely interrupt human-to-human transmission, halt the epidemic and Covid-19 is now eradicated. In the absence of vaccines and antivirals, this remarkable achievement was only possible because of rigorous implementation of traditional public health measures **(Wilder-Smith and Freedman 2020).**

The range of clinical responses to (SARS-CoV-2) infections is extremely broad. Although most patients with coronavirus disease 2019 (Covid-19) present with a mild upper respiratory tract infection and then recover, some infected patients develop pneumonia, acute respiratory distress syndrome, multi-organ failure, and death. Clues to the pathogenesis of severe Covid-19 may lie in the systemic inflammation and thrombosis observed in infected patients. We propose that severe Covid-19 is a microvascular disease in which coronavirus infection activates endothelial cells, triggering exocytosis, a rapid vascular response that drives microvascular inflammation and thrombosis **(Klok, Kruip et al. 2020).**

Both arterial and venous thromboembolisms are common in patients with severe Covid-19.The incidence of venous thromboembolic events in patients with Covid-19 admitted to intensive care units ranges from 20% to 35%, and

deep venous thrombosis has been identified in 70% to 100% of patient who died from Covid-19 **(Varga, Flammer et al. 2020).** Furthermore, arterial thrombosis resulting in stroke or myocardial infarction occurs in up to 4% of patients with Covid-19 hospitalized in intensive care units. Patients with severe Covid-19 often have laboratory findings consistent with a hypercoagulable state, suggesting widespread thrombosis and fibrinolysis, as well as elevated levels of D-dimer, and factor VIII. These patients also manifest a hyperinflammatory state, or "cytokine storm," characterized by elevated levels of inflammatory markers such as C-reactive protein and interleukin-6, which have been linked to severity of pneumonia and mortality**(Lowenstein and Solomon 2020).**

Endothelial injury is an underlying mechanism that might link inflammation and thrombosis in severe Covid-19.Autopsy study has suggested that both endothelial inflammation and microvascular thrombosis are prominent, with inflammatory cells attached to the endothelium of small vessels in lung, kidney, heart, and liver **(Escher, Breakey et al. 2020).** Moreover, von Willebrand factor (vWF), which is released from endothelial cells after vascular injury, which is also released from activated endothelial cells**(Lowenstein and Solomon 2020).**

1.1. History of Coronavirus

The World Health Organization (WHO) named the latest virus as SARS-CoV2 as the cause of 2019 novel coronavirus infectious disease (Covid-19), Outbreaks that caused worldwide severe acute respiratory syndrome (SARS) in

2002-2003 and the Middle East Respiratory Syndrome (MERS) **(Zhu, Ge et al. 2020).**

Four corona viruses namely HCoV-HKU1, βCoV 1 (also called bovine coronavirus),human coronavirus HCoV-NL63 (responsible for human common cold), human coronavirus 229E (HCoV-229E) , and organ culture 43(OC43) have been in circulation in humans**(Berry, Gamieldien et al. 2015),** and generally cause mild respiratory disease. In 2012 demonstrated the possibility of animal-to-human and human-to-human transmission of newly emerging Coronaviruses**(Kumar 2021).**

Obtaining the full genome of SARS-CoV-2 is a key to understanding its evolution and function. On January 10, 2020, the draft genome sequence of SARS-CoV-2 was first released on **(Fan, Su et al. 2020)**.

One day later, 5 additional SARS-CoV-2 sequences, gathered from different patients, were deposited in the Global Initiative on Sharing All Influenza Data (GSAID) database, which is primarily used for sharing data on influenza viruses. Based on these shared data, genetic evolutionary analyses from different laboratories have shown that SARS-CoV-2 is a Betacoronavirus belonging to the Sarbecovirus subgenus of the Coronaviridae family, which is distinct from SARS-CoV **(Tang, Comish et al. 2020).**However, like SARS-CoV and MERS-CoV, bats may be the natural origin of SARS-CoV-2. SARS-CoV-2 has 86.9% to 96% nucleotide sequence similarity to multiple strains of bat SARS-like coronaviruses, which are on the same lineage (B) but are located on different branches **(Lu, Zhao et al. 2020)**. It has been proposed that wild animals, such as civets and camels, further serve as

the intermediate host for SARS-CoV and MERS-CoV, respectively **(Cui, Li et al. 2019).**

The intermediate host required for SARS-CoV-2–mediated human disease is unknown. One early hypothesis is that snakes may be a bridge between bats and humans for SARS-CoV-2 infection **(Ji, Wang et al. 2020)**. Although there is no direct evidence that coronaviruses could adapt to cold-blooded hosts thus far, as shown in Figure (1-1) **(Mallapaty, 2020).**

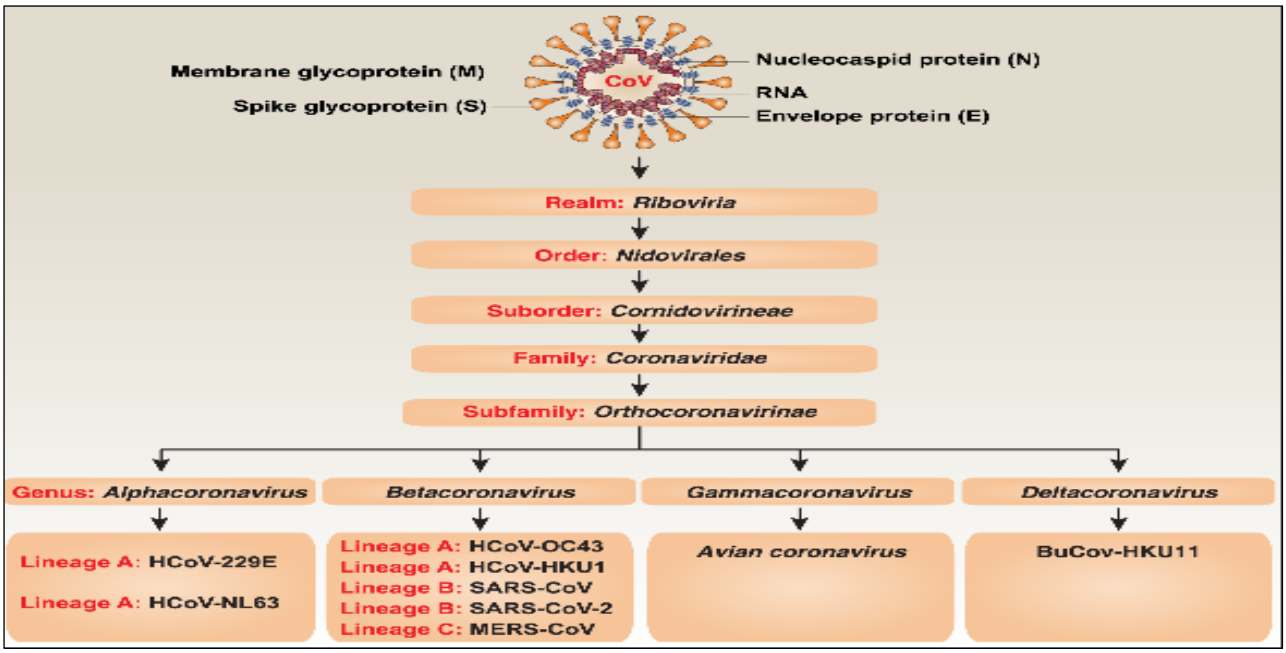

Fig. 1-1: Different human CoVs have different natural and intermediate hosts. BuCoV-HKU11, bulbul coronavirus HKU11; HCoV, human coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; SARS-CoV, severe acute respiratory syndrome coronavirus(Tang, Comish et al. 2020).

1.1.1. Structure of the Covid-19

The corona viral genome normally encodes four structural proteins including spike (S) protein, nucleocapsid (N) protein, membrane (M2) protein, and envelope (E) protein. S protein contains the receptor-binding domain (RBD) and mediates the attachment of viruses to the surface receptors in host cells, as well as subsequent fusion between the viral and host cell membranes,

to facilitate viral entry into host cells. Multiple binding and neutralization epitopes have been identified in the S proteins of CoVs, which makes S protein an essential antigen for vaccine design **(Qiu, Mao et al. 2020).**

Structurally, SARS-CoV-2 has four main structural proteins as shown in Figure (1-2) **(Tang, Comish et al. 2020).**It is including encode viral structural proteins spike (S) glycoprotein, small envelope (E) glycoprotein, membrane (M) glycoprotein, and nucleocapsid (N) protein, and also several accessory proteins. The spike or S glycoprotein is a trans membrane found in the outer portion of the virus. S protein forms homotrimers protruding in the viral surface and facilitates binding of envelope viruses to host cells by attraction with angiotensin-converting enzyme 2 (ACE2) expressed in lower respiratory tract cells**(Astuti 2020)**.This glycoprotein is cleaved by the host cell fur in-like protease into 2 sub units namely S1 and S2. Part S1 is responsible for the determination of the host virus range and cellular tropism with the receptor binding domain make-up while S2 functions to mediate virus fusion in transmitting host cells. Protein (N) is also heavily phosphorylated and suggested to lead to structural changes enhancing the affinity for viral RNA **(Hong, Jhun et al. 2021)**.

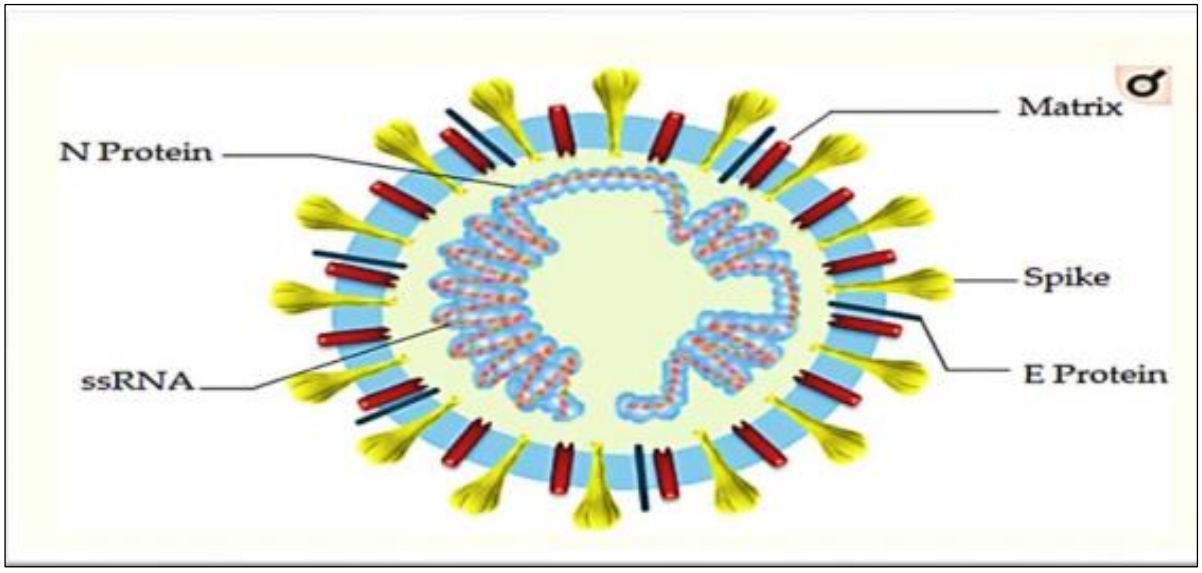

Fig. 1-2: Schematic representation of the taxonomy of Corona viridae (Astuti 2020).

1.1.2. Types of Corona Viruses

Covid-19 are single-stranded RNA viruses that belong to the order Nidovirales, family Coronaviridae, and subfamily Coronavirinae and have been classified into four major groups: α-CoVs, β-CoVs, γ-CoVs, and δ-CoVs with 17 subtypes.CoVs primarily infect wild animals including mammals and birds. The primary target cells of coronaviruses compromise the respiratory and gastrointestinal region epithelial cells and cause various diseases such as upper and lower respiratory tract infections and respiratory syndromes **(Qiu, Mao et al. 2020)**.

Their cell features and delivery through fomites, airborne, or fecal–oral routes. The currentCovid-19, which causes an acute respiratory disease case, is closely related to SARS-CoV, that is, within the genus β-coronavirus **(Yoshioka-Maeda, Iwasaki-Motegi et al. 2020)**. Since there is little epidemiological and pathogenic information about this virus, a genetic analysis of this virus is very similar to SARS-CoV and is sometimes referred to as SARS-CoV-2. Among them, severe acute respiratory syndrome (SARS) Covid-19 and Middle Eastern respiratory syndrome (MERS) Covid-19can cause serious respiratory syndrome in humans. For instance, the outbreak of SARS in 2003 led to a pandemic with 8906 infected cases and 774 deaths reported worldwide Meanwhile, the outbreak of MERS confirmed 2229 cases globally, including 791 associated deaths **(Wang, Rosen et al. 2019).**

Latest bioinformatic analysis indicated that Covid-19 is phylogenetically close to SARS-CoV and bat CoV (BCoV). The genomes of 2019-nCoV and SARS-CoV share more than 79% sequence similarity on average, and their S proteins share 76.47% identity **(Wu, Xu et al. 2020)**.Yet the antigenicity similarity between them remains unknown and is urgently needed for vaccine design. Cross-reactive epitopes (CREs) are shared or similar epitope regions on the antigen surface among viruses that can be bound or neutralized by the same

antibodies. Desirably, if any CREs were identified, previous antibodies for other CoVs might be reused to facilitate Covid-19intervention **(Chen, Zhou et al. 2020).**

Although most of the human coronavirus infections are mild and asymptomatic, the pandemics of the two β-coronaviruses, that is, SARS-CoV (β-coronavirus, subgenus Sarbecovirus, 2002–2003, case fatality rate 10%) **(Fouchier, Kuiken et al. 2003)** and MERS-CoV (Betacoronavirus, subgenus Merbecovirus, 2012, case fatality rate 35%) **(Zaki 2012),** and have caused vast fatal human pneumonia, especially in the immunocompromised people, the cardiopulmonary patients, and the old ones and adolescents **(ECDC, 2020; WHO, 2002b).** Covid-19infected humans due to eating animals infected with bat coronavirus, that is, Himalayan palm civets, Chinese ferret badgers and raccoon dogs sold for food, can cause animal-to-human and human-to-human transmission. The most relevant animal reservoirs of human MERS-CoV are dromedary camels that caused human–human infections, especially in healthcare environments, in Saudi Arabia, 2012 **(Goli 2020).**

1.1.3. Clinical Spectrum of Covid-19 and Symptoms with Signs

The clinical spectrum of Covid-19can range from asymptomatic infection to mild upper respiratory tract illness to severe interstitial pneumonia with respiratory failure and even death. It is estimated that non-severe patients with no symptoms or mild symptoms could represent ~30–60% of all infections **(Mizumoto, Kagaya et al. 2020).** Compared to severe cases, asymptomatic infection and mildly symptomatic infection often go unrecognized since the majority of affected individuals are not sick enough to seek medical help and cannot be identified by screening methods, such as temperature check. A few studies have shown that high viral loads can be detected in some patients with Covid-19 early in their illness, when their symptoms were mild **(Woelfel, Corman et al. 2020).**

Moreover, another asymptomatic patient was found to shed a similar amount of virus as that shed by symptomatic patients. Therefore, asymptomatic infection may be highly contagious and potentially lead to viral spread. Some transmission models also suggested that a substantial number of undocumented infections leading to mild, limited, or no symptoms may facilitate the rapid dissemination of Covid-19 **(Gostic, Gomez et al. 2020).** However, there is little information regarding the clinical features of asymptomatic and mildly symptomatic infection. Moreover, details of the chest computed tomography (CT) scans and virology course in these patients have not yet been well-documented or described **(Li, Shi et al. 2020).**

Symptoms are experienced by patients. People with mild Covid-19might experience cough, sore throat, high temperature, diarrhoea, headache, muscle or joint pain, fatigue, and loss of sense of smell and taste. Symptoms of Covid-19pneumonia include breathlessness, loss of appetite, confusion, pain or pressure in the chest, and high temperature (above 38 °C)**.**

Signs are evaluated by clinical examination, and include lung sounds, blood pressure and heart rate. Often, people with mild symptoms visit their doctor (primary care physician) for an initial diagnosis. People with more severe symptoms might visit a hospital outpatient or emergency department. Depending on their symptoms and signs, patients may be sent home to isolate, may receive further tests or be hospitalized **(Struyf, Deeks et al. 2020).**

1.1.4. Risk Factors for Severe and Critically Covid-19 Patients

In a series of multivariable-adjusted analyses based on Covid-19 patient, higher disease severity was found to be associated with demographic factors: **(Wolff, Nee et al. 2020, Zhang, Cao et al. 2021).**

1. Old Age and Male Gender

2.Diabetes and Obesity, Hypertension, Allergy and Asthma

3.Possible Mechanisms Contributing toIncreased Severityby ChronicObstructive Pulmonary Disease(COPD), Interstitial Lung

4.Chronic Liver Diseases

5.Patients with Coexisting Chronic Kidney Diseases Patients with Cancers and Hematologic Malignancies

6.Pregnancy

7.Viral Load

8.Ethnicity

1.1.5.Effect of Covid-19 on Cardio vascular System and Hematological Changes

Although the respiratory system is the principal target for Covid-19 as described above, it can affect other major organ systems such as the gastrointestinal tract (GI), hepatobiliary, cardiovascular, renal, and central nervous system Covid-19 induced organ dysfunction, in general, is possibly explained by either one or a combination of the proposed mechanisms such as direct viral toxicity, ischemic injury caused by vasculitis, thrombosis, or thrombo-inflammation, immune dysregulation, and renin-angiotensin-aldosterone system (RAAS) dysregulation **(Conti, Ronconi et al. 2020)**.

The exact mechanism of cardiac involvement in Covid-19 is unknown, it is likely multifactorial. ACE-2 receptors are also exhibited by myocardial cells implicating direct cytotoxicity by theCovid-19 on the myocardium leading to myocarditis. Proinflammatory cytokines such as IL-6 can also lead to vascular inflammation, myocarditis, and cardiac arrhythmias **(Cascella, Rajnik et al. 2021).**

Acute coronary syndrome (ACS) a well-recognized cardiac manifestation of Covid-19and is likely due to multiple factors that include but not limited to Covid-19associated hypercoagulability, the release of proinflammatory cytokines, worsening of preexisting severe coronary artery disease, stress cardiomyopathy, and associated hemodynamic derangement which may reduce coronary blood flow, reduced oxygen supply resulting in the destabilization of coronary plaque micro-thrombogenesis or worsening of preexisting severe coronary artery disease **(Guo, Fan et al. 2020, Hua, O'Gallagher et al. 2020).**

Coronavirus disease -19 has a significant effect on the hematological and hemostatic system. The mechanism of leukopenia, one of the most common laboratory abnormalities encountered in, is unknown. Several hypotheses have been postulated that include ACE-2 mediated lymphocyte destruction by direct invasion by the virus, lymphocyte apoptosis due to proinflammatory cytokines, and possible invasion of. Thrombocytopenia is uncommon in Covid-19and is likely due to multiple factors that include virus-mediated suppression of platelets, formation of autoantibodies, and activation of coagulation cascade that results in platelet consumption **(Abou-Ismail, Diamond et al. 2020).T**hrombocytopenia and neutrophilia are considered a hallmark of severe illness **(Coopersmith, Antonelli et al. 2021).**

Although it is well known that Covid-19 is associated with a state of hypercoagulability, the exact mechanisms that lead to the activation of the coagulation system are unknown and likely attributed to the cytokine-induced inflammatory response. The pathogenesis of this associated hypercoagulability is multifactorial and is probably induced by direct viral-mediated damage or cytokine-induced injury of the vascular endothelium leading to the activation of platelets, monocytes, and macrophages, increased expression of tissue factor, von Willebrand factor, and Factor VIII that results in the generation of thrombin and formation of fibrin clot formation **(Amgalan and Othman 2020).**

Other mechanisms that have been proposed include possible mononuclear phagocytes induced prothrombotic sequelae, derangements in the renin-angiotensin system (RAS) pathways, complement-mediated microangiopathy **(Abou-Ismail, Diamond et al. 2020).**

1.1.6 Sepsis-induced coagulopathy and disseminated intravascular coagulation

The pathophysiology of bacterial SICS and disseminated intravascular coagulation (DIC) has been extensively studied. Since "inflammation" and "coagulation" are the common keywords in SIC/DIC, it is helpful to consider prior studies regarding SIC/DIC. The mechanism of procoagulant responses in bacterial sepsis is complex, and various factors, including pathogen-associated molecular patterns (PAMPs) and host-derived damage-associated molecular patterns (DAMPs), are known to trigger the proinflammatory responses and activate systemic coagulation As shown in Figure (1-3) **(Iba, Levy et al. 2020).** Since inflammation and coagulation are both essential host defense mechanisms, the responses increase in proportion to disease severity and can potentially injure the host in which defense mechanisms include proinflammatory cytokines such as interleukin (IL)-1β, IL-6, tumor necrosis factor-α (TNFα), and complement system proteins, all of which can induce coagulopathy. In addition, tissue factor expression on monocytes/macrophages, neutrophil activation, and neutrophil extracellular traps (NETs) produce activation of thrombosis **(Chang 2019).** This thrombo-inflammatory response, together with extracellular vesicles, causes endothelial damage that further increase thrombin generation **(Wang, Luo et al. 2018)**. In SIC/DIC, fibrinolysis is often suppressed due to the over-production of plasminogen activator inhibitor-1 (PAI-1), with progressive fibrin clot formation within the tissue microcirculation leading to organ dysfunction **(Iba, Levi et al. 2020) .** To detect this type of coagulation disorder, a decrease in the platelet count and increase in

prothrombin time (PT)—the two laboratory parameters used in the SIC score—are the most useful indicators. There is a lack of increase in D-dimer levels with increasing SIC/DIC severity due to suppression of fibrinolysis, also called fibrinolytic shutdown as shown in Figure (1-6) **(Iba and Levy 2018).**

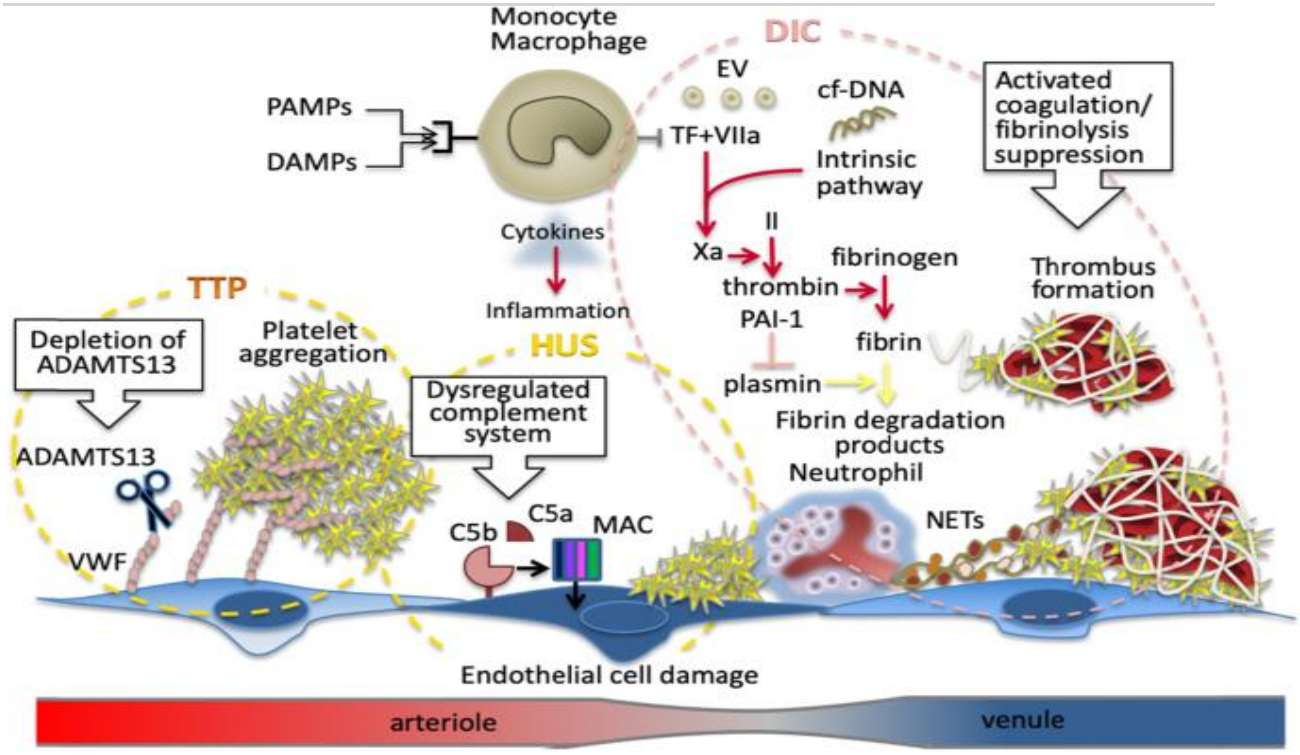

Fig. (1-3): Mechanism of endothelial cell damage(Iba, Levy et al. 2020).

In Covid-19, the D-dimer level is commonly high and usually greater than five times the upper limit of the normal range. Also, in SIC/DIC, anticoagulant proteins such as antithrombin decrease significantly because of increased vascular permeability and other mechanisms **(Iba, Levy et al. 2020).** Thrombus formation in disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, and hemolytic uremic syndrome. In bacterial sepsis, immune cells such as monocyte and macrophages are activated by pathogen-associated molecular patterns (PAMPs) and host-derived damage-associated molecular patterns (DAMPs). The immune cells initiate coagulation cascades through expressing tissue factor (TF) and releasing extracellular vesicles (EVs). The activated neutrophils and neutrophil extracellular traps (NETs) are also

involved in coagulation. Degradation of fibrin, the end product of coagulation activation, is suppressed by increased levels of plasminogen activator inhibitor-1 (PAI-1). In thrombotic thrombocytopenic purpura (TTP), increased high multimers of von Willebrand factor (vWF) caused by ant-ADAMTS13 antibodies stimulate platelet aggregation **(Madoiwa 2015).**

1.1.7. von Willebrand Factor

The von Willebrand Factor (vWF) is a large multimeric hyper-adhesive glycoprotein circulating, present in blood plasma, endothelial cells, megakaryocytes, and platelets. It plays a major role in hemostasis, mediating platelet adhesion to vascular injury sites. It also binds and protects coagulation factor VIII (FVIII) from degradation **(Terraube, O'donnell et al. 2010).** Recent studies showed that vWF is also involved in inflammation, linking thrombosis and inflammation. Inflammation can provoke thrombosis through the vWF-dependent pathway, which includes endothelial activation, the secretion of vWF into the bloodstream, vWF activation and interaction with platelets, and subsequent platelet adhesion to a vessel wall **(Kawecki, Lenting et al. 2017, Chen and Chung 2018).** vWF is considered a risk factor of arterial thrombosis and might contribute to the development of adverse events in atherosclerosis and other cardiovascular diseases **(Van Belle, Vincent et al. 2019, Fan, Wang et al. 2020).**

1.1.7.1. Secretion and Chemical Characteristics

The production of vWF occurs selectively in megakaryocytes (MKs) and endothelial cells as ultra-large vWF (ULvWF) multimers, stored in specific intracellular organelles: a) α-granules in MKs and platelets, and, b) Weibel-Palade bodies (WPBs) in endothelial cells .

The mature of vWF molecule is composed of 40 to 100 monomers. vWF polymerization requires a multistep process: vWF monomers undergo an initial dimerization in the endoplasmic reticulum (ER), followed by a complex

multimerization in the Golgi apparatus and post-Golgi compartment **(Budde, Pieconka et al. 2006).** Multimers of UL-vWF are packaged into α- granules in helicoidally structures. This conformation enables a well-ordered, rapid secretion of vWF multimers in bloodstream, mimicking an unrolling chain through plasma membrane pores **(Stockschlaeder, Schneppenheim et al. 2014).** vWF is secreted in both via constitutive and regulated manner from WPBs in endothelial cells. Platelet activation is necessarily for vWF secretion from α-granules and no constitutive release has been demonstrated. A certain amount of vWF molecules is not secreted freely circulating in plasma, and remains anchored to the endothelial surface, probably interacting with P-selectin **(Zhang, Zhou et al. 2009).**

1.1.7.2. Function of von Willebrand Factor

von Willebrand Factor plays a pivotal role in mediating adhesion and aggregation of platelets and acting as a carrier for factor VIII, protecting it from degradation, cellular uptake, or binding to the surface of activated platelets and endothelial cells at sites of vascular injury and by protecting factor VIII from rapid clearance in the circulation of high shear rates (eg, in coronary arteries that have stenotic or ruptured atherosclerotic plaque lesions) **)Brehm, König et al. 2020)**. Recent studies have provided evidence indicating that vWF regulates not only hemostasis and thrombosis but also the processes of angiogenesis, smooth muscle cell proliferation, tumor cell metastasis, and immune cell regulation **(Patmore, Dhami et al. 2020).**

But, vWF can also be regarded as prothrombotic factor, particularly if high vWF concentrations are present in the blood stream. Recently described the prothrombotic properties of the common vWF variant**.** It plays a significant role in premature and repeated events of myocardial infarction (MI) in Ludwigshafen Risk and Cardiovascular Health Study **(Huck, Chen et al. 2020).** Atrial fibrillation (AF) is the most commonly sustained cardiac arrhythmia and

is associated with a high risk of stroke and thromboembolism **(Roldán, Marín et al. 2011).**

1.1.7.3. Clinical Importance of von Willebrand Factor

The deficiency of von Willebrand Factor causes a bleeding phenotype (von Willebrand disease, vWD) of variable severity, depending on the molecular defect of the multidomain and multifunctional protein induced by mutations. In addition to the numerous loss-of-function mutations, a number of gain-of-function (GOF) mutations have been identified in the vWF gene. The latter are located in the A1 domain and cause bleeding events, due to spontaneous binding and crosslinking of platelets in the circulation. The results are thrombocytopenia but also proteolytic degradation of the high molecular weight functionally highly active vWF multimers by the vWF-specific protease ADAMTS-13 **(Patmore, Dhami et al. 2020).**

Elevated of vWF levels are associated with cardiovascular disease, increased risk of ischemic heart disease, and the occurrence of acute ischemic stroke; these high levels of vWF are associated with the risk of stroke, even in the general population **(Luo, Ni et al. 2012).** In animal models of atherosclerosis, both the inhibition and deletion of vWF successfully reduced inflammation, plaque size and platelet adhesion, while the deletion of ADAMTS13 had opposite effects. Human clinical data on the impact of vWF inhibition on atherosclerosis are not available **(Jin, Tohyama et al. 2012).**

1.1.8. A disintegrin and Metalloprtease with a Thrombospondin Type 1 Motif, Member-13(ADAMTS-13)

A Disintegrin and Metalloproteinase with a Thrombospondin Type 1 Motif, Member 13 (ADAMTS-13) is a member of the ADAMTS type 1 repeats family of metalloproteases. It is a multidomain protein with Ca^{2+}- and Zn^{2+}-dependent metalloprotease (M), disintegrin-like (D), thrombospondin-1 repeat (T), Cys-

rich (C), and spacer (S) domains, followed by 7 T domains and 2 CUB (complement components C1r and C1s, sea urchin protein Uegf, and bone morphogenetic protein-1) domains. ADAMTS-13 limits the growth of von Willebrand factor (vWF)–platelet aggregates by cleaving a cryptic Tyr^{1605}-Met^{1606} bond in the vWF A2 domain that is exposed under conditions of high fluid shear stress, thereby releasing adherent platelets. Congenital or acquired ADAMTS-13 deficiency prevents the regulation of vWF-dependent platelet accumulation and causes microvascular thrombosis that characterizes thrombotic thrombocytopenic purpura **(Roose, Vidarsson et al. 2018)**. Interactions between vWF and the proximal MDTCS domains of ADAMTS13 have been studied extensively. When subjected to tensile stress in solution, bound to a surface, or on endothelial cell surfaces **(Akiyama, Takeda et al. 2009).**

1.1.8.1. Chemical Structure of ADAMTS-13

A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13is a metalloprotease containing 1,427 amino acid residues predominantly secreted by hepatic stellate cells **(Haegele, Fuxsteiner et al. 2018).** It has been reported that endothelial cells also express ADAMTS-13 mRNA and protein.as shown in Figure (1-4) **(Koupenova, Clancy et al. 2018).**

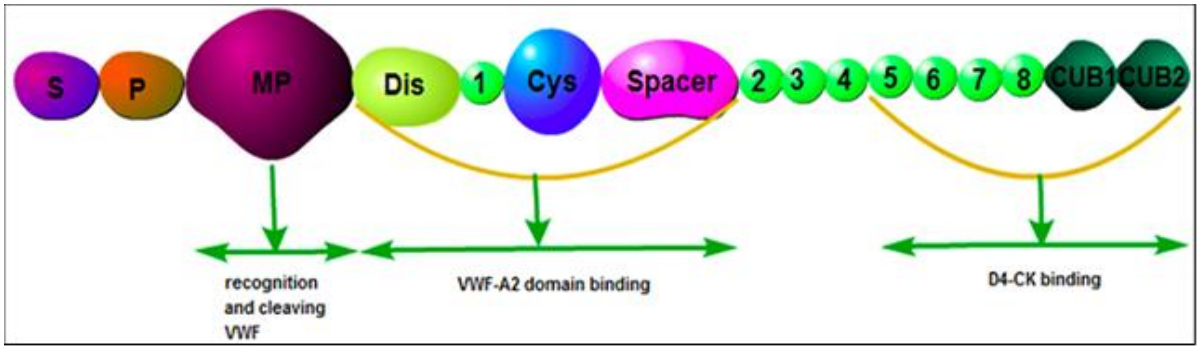

Fig. (1-4): Structure of ADAMTS13. S, signal peptide domain; P, short propeptide domain; MP, metalloprotease domain; Dis, disintegrin-like domain; 1, thrombospondin-1 repeat (TSP1) domain; Cys, characteristic Cysteine -rich domain; Spacer, spacer domain; CUB, CUB domains (Koupenova, Clancy et al. 2018)

ADAMTS-13 is the cleaving protease of vWF, which is a large multimeric glycoprotein. vWF is released by endothelial cells in the form of ultra-large multimers (UL-vWF) of varying sizes, with the molecular weight upto 20,000 kDa. ADAMTS-13 cleaves the Y1605-M1606 bond with inthe UL-vWF A2 domain Figure (1-5) **(Chen, Cheng et al. 2019).**

1.1.8.2. Physiological Functions of ADAMTS-13

The physiological function of ADAMTS-13 is based on its multi-domain structure consisting of a signal peptide domain, a short propeptide domain, a metalloprotease domain, a disintegrin-like domain, a thrombospondin-1 repeat (TSP1) domain, a characteristic Cys-rich domain, a spacer domain, and two CUB domains. The C-terminal regions of ADAMTS proteases are more variable with an additional seven thrombospondin type 1 (TSP-1) repeats and two CUB (the Complement components C1r ADAMTS-13 and C1s, sea urchin protein Uegf, and Bone morphogenetic protein-1) domains for ADAMTS-13 **(Zander, Cao et al. 2015) (Roose, Vidarsson et al. 2018).**

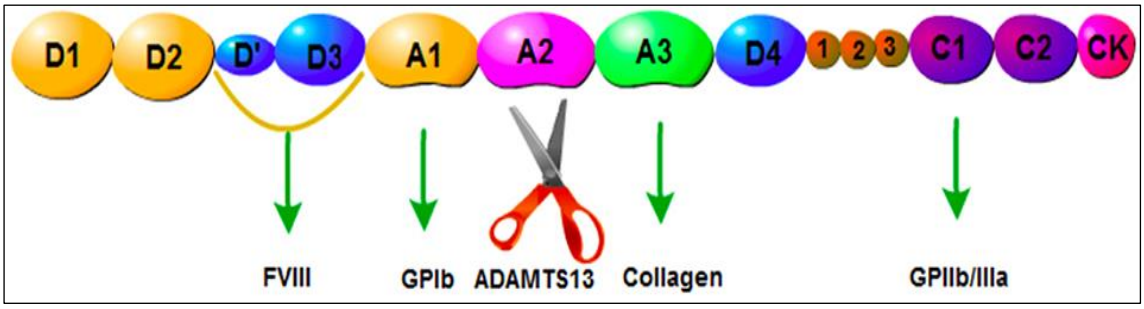

Fig. (1-5): Structure of VWF. D1,D2: pro-vWF, D'D3: FVIII, A1: GPIbα protein addition site, A2: ADAMTS-13 cleaving site, A3: collagen (Chen, Cheng et al. 2019).

Dysfunction of the ADAMTS-13-vWF axis leads to vWF accumulation and adhesion of platelets, which is the first step of thrombosis and inflammation **(Witsch, Martinod et al. 2018),** as shown in Figure (1-5). ADAMTS-13 has a plasma half-life of 2 to 3 days, with 3% to 5% of ADAMTS-13 circulating bound to vWF **(Feys, Anderson et al. 2009).** There are no data regarding the clearance of the protease, nor any known inhibitor of its function. Therefore, the regulation of ADAMTS-13 is likely to be at the substrate level (vWF), with 3 factors known to regulate its activity **(Masias and Cataland 2018):**

A. Fluid shear stress, found in the microcirculation, which allows vWF to unfold and expose its A2 domain for ADAMTS-13 binding;

B. Factor VIII, which enhances the ADAMTS-13 proteolytic activity by affecting the A1A2A3 domain-domain interaction when ADAMTS-13 binds vWF under shear forces **(Bonazza, Rottensteiner et al. 2015)**;

C. Platelet glycoprotein 1bα (GP1bα), which increases ADAMTS-13 function under static or shear conditions by exposing the vWF A2 domain when it binds to the A1 domai.

1.1.8.3. Medical Importance of ADAMTS13

A severe functional ADAMTS-13 deficiency causes the blood accumulation of platelet-hyperadhesive ultralarge vWF multimers, leading to the formation of platelet-rich microthrombi within small arterioles **(Sadler 2015)**. In most cases, the mechanism for ADAMTS13 severe deficiency is acquired via autoantibodies to ADAMTS-13, as demonstrated by positive anti-ADAMTS13 IgG in ~75% of TTP during an acute phase **(Mariotte, Azoulay et al. 2016)** as shown in Figure (1-6).

In physiologic conditions, ultralarge vWF multimers released from endothelial cells are cleaved by ADAMTS-13 in smaller vWF multimers, less adhesive to platelets. In TTP, because of the absence of functional ADAMTS-

13 (either absent by congenital defect or inhibited by specific autoantibodies), ultralarge vWF multimers are released into the blood and bind spontaneously to platelets to form aggregates within the arterial and capillary microvessels. The vWF–platelet aggregates are large enough to form microthrombi inducing tissue ischemia, platelet consumption, and microangiopathic **(Zhu, Muia et al. 2019).**

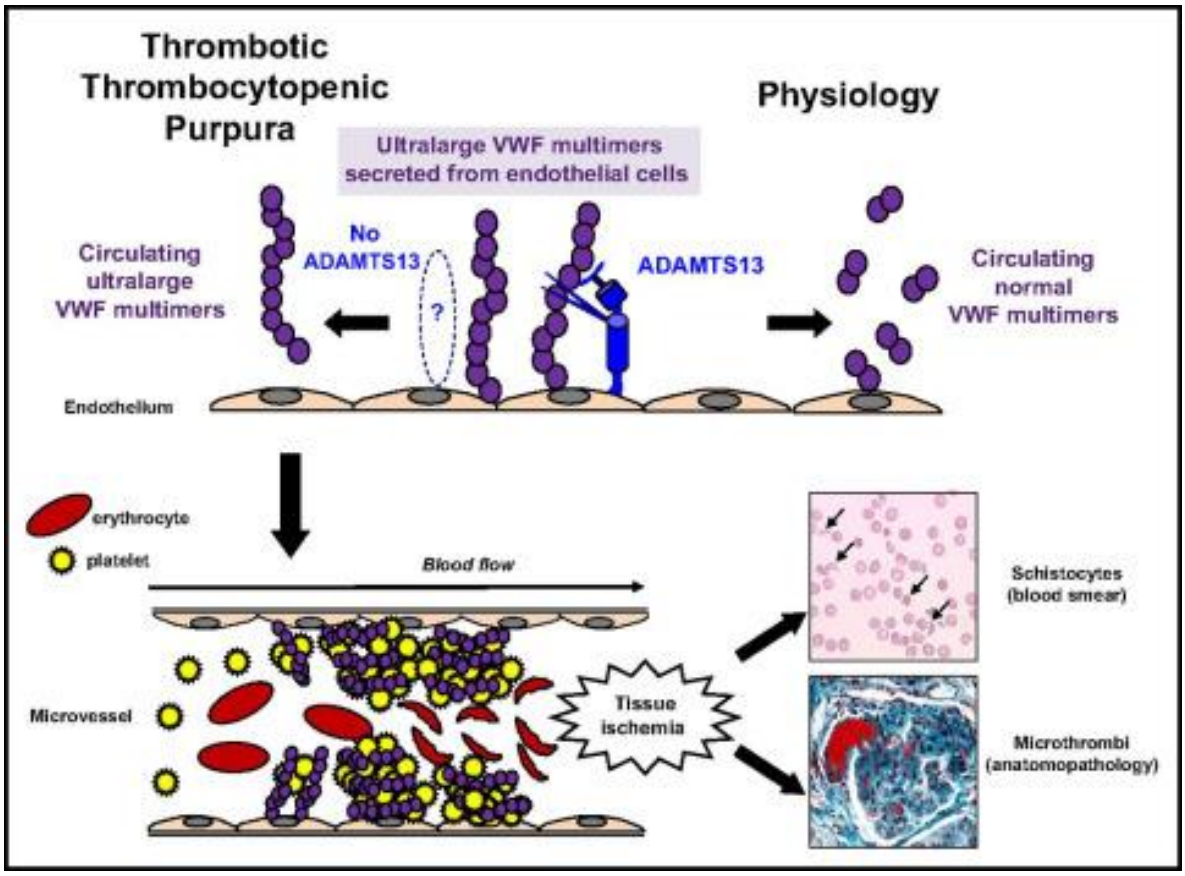

Fig. (1-6): Pathophysiology for Thrombotic thrombocytopenic purpura(TTP) (Joly, Coppo et al. 2017)

1.1.9. Vitamin K

Vitamin K , one of the fat-soluble vitamins, is essential for synthesizing several proteins – factor II (prothrombin), factors VII, IX, and X involved in regulating blood clotting (coagulation) **(Maresz 2015)**. Natural vitamin K is found in two different forms: K1 (phylloquinone), an effective form of dietary vitamin K and mainly found in green leafy vegetables, and K2 **(Holmes, Hunt**

et al. 2012). They are primarily of microbial origin, and sources are especially from fermented foods like cheese, curds, and animal livers. It is noteworthy to understand that these menaquinones are synthesized by human intestinal microbiota. The third form of vitamin K is K3 (menadione), which is synthetically or artificially produced. The dietary reference intake of vitamin K recommended by Food and Nutrition Board (FNB) at the Institute of Medicine of the National Academies is 120µg for adult males and 90 µg for adult females **(Samad, Dutta et al. 2021).**

1.1.9.1 Structure, Uptake and Distribution of Vitamin K

Naturally, vitamin K exists as two vitamers: K1 and K2. Structurally K1 belongs to the phylloquinone family whereas K2 chemical structure. Vitamin K1 represents the predominant form that can be found in daily diet **(Schurgers and Vermeer 2000)**, and is mainly present in green vegetables and fruits .In mammalian cells in absence of bacteria, vitamin K1 was shown to be able to convert into vitamin K2 MK-4 isoform. Normally K2 form is primarily bacterial in origin, and is produced in the human intestines. It can also be found in fermented food, meat and cheese **(Marles, Roe et al. 2017).**

Overall, as much as 95% of extrahepatic vitamin K comes from dietary menaquinones, not phylloquinones. A healthy adult consumption of vitamin K should be around 1 µg/day/kg and specifically 50 to 600 µg/day for vitamin K1 and 5 to 600 µg/day for vitamin K2 Although, low amounts in µg are sufficient already to maintain the daily body requirements due to an efficient vitamin K recycling system developed in mammals, study has shown that majority of healthy adults are sub-clinically deficient for vitamin K in their circulation. Contrarily to vitamin K1, which is rapidly removed from the circulation and mainly remains in the liver, K2 form is known to be equally distributed between the circulation and the extra-hepatic tissues .Thus, K2 is thought to provide a rapid and localized protective response whilst action of K1 is found to be more

widespread. Commercially, there are two vitamin K2 forms available, named MK-4 and MK-7. MK-4 has a relatively short half-life of up to three hours, whilst MK-7 can remain stable for up to 3 days. Vitamin K1 and MK-4 present similar properties whereas vitamin K2 larger isoforms (for example MK-7,8,9,10) are proposed to also possess function beyond coagulation **(Halder, Petsophonsakul et al. 2019).**

Indeed, the presences of large side chains confer potential hydrophilic properties that are different from the K1 and MK-4 forms. Since vitamin K, even given at high doses has no reported side effects, its potential prophylactic benefits supplementation may be advisable **(Kaneki, Hedges et al. 2001).**

1.1.9.2. Roles of vitamin K

Under normal conditions, coagulation system is balanced towards the anticoagulation state . Vitamin K is an essential "switch" in balancing coagulation and anticoagulation process **(Espana, Medina et al. 2005)**. Indeed, vitamin K acts as a cofactor in the activation of extra-hepatic and hepatic vitamin K-dependent proteins (VKDPs) including pro-thrombin and clotting factors VII, IX, X, major factors involved in blood coagulation. On the other hand, vitamin K can also trigger key anticoagulants via VKDPs for producing proteins C, S and Z **(Danziger 2008).** In the presence of vitamin K, the glutamate (Glu) residues present on these proteins are carboxylated into gamma-carboxyglutamic acid (Gla) by γ-glutamyl carboxylase (GGCX) enzyme, enzyme that uses vitamin K as a cofactor for its activity. Glu is modified into Gla on the coagulation factors of which these proteins display a higher affinity for calcium enabling them to form calcium bridges and bind to the surface membrane phospholipids prior to clot assembling **(Schurgers and Spronk 2014).**

It is important to note that vitamin K does not start the clotting process; it only enhances the coagulation system to work effectively. While vitamin K involvement in coagulation is well established, it is also a key component of the anticoagulation response. This response is facilitated through the activation of protein C, S and Z. Vitamin K-dependent protein C activation can inhibit clotting factors V and VIII which are responsible for clot generation **(Espana, Medina et al. 2005).**

Beyond its essential role in coagulation, vitamin K is suggested to possess immune-modulatory functions as well as preventing vascular calcification. Studies have shown that K2 form has more potent anti-inflammatory effect when compared to K1.K2 acts as an immunosuppressive compound to modulate expression of a multitude of pro-inflammatory cytokines such as TNF, IL-1α, IL-1β and suppresses IL-6 release **(Espana, Medina et al. 2005).**

It can also impair T cell activation and proliferation. Besides, vitamin K has been shown to activate extra-hepatic VKDPs such as the Matrix Gla-protein (MGP), Osteocalcin and Gla-rich protein (GRP) .MGP is mainly expressed in cartilage and vasculature and involved in ECM remodeling responsible for preventing vascular calcification and thus plays a fundamental role in vascular health.It has been suggested that vitamin K dependent MGP plays an important role in elastin degradation in the lungs phenomenon that is accelerated in pulmonary disease (Piscaer, Wouters et al. 2017). Furthermore, vascular calcification is often observed in chronic kidney disease patients, patients who have been reported to be more prone to develop severe form of Covid -19 highlighting the importance of vitamin K and MGP **(T 2021).**

1.1.10. Mechanisms of Coagulation in Covid-19

The pathogenesis of the Covid-19 induced coagulopathy has not yet been fully elucidated, but the mechanisms may overlap in some part to those of bacteria-induced septic coagulopathy/DIC as shown in Figure (1-7) **(Wang, Shi et al. 2020).**

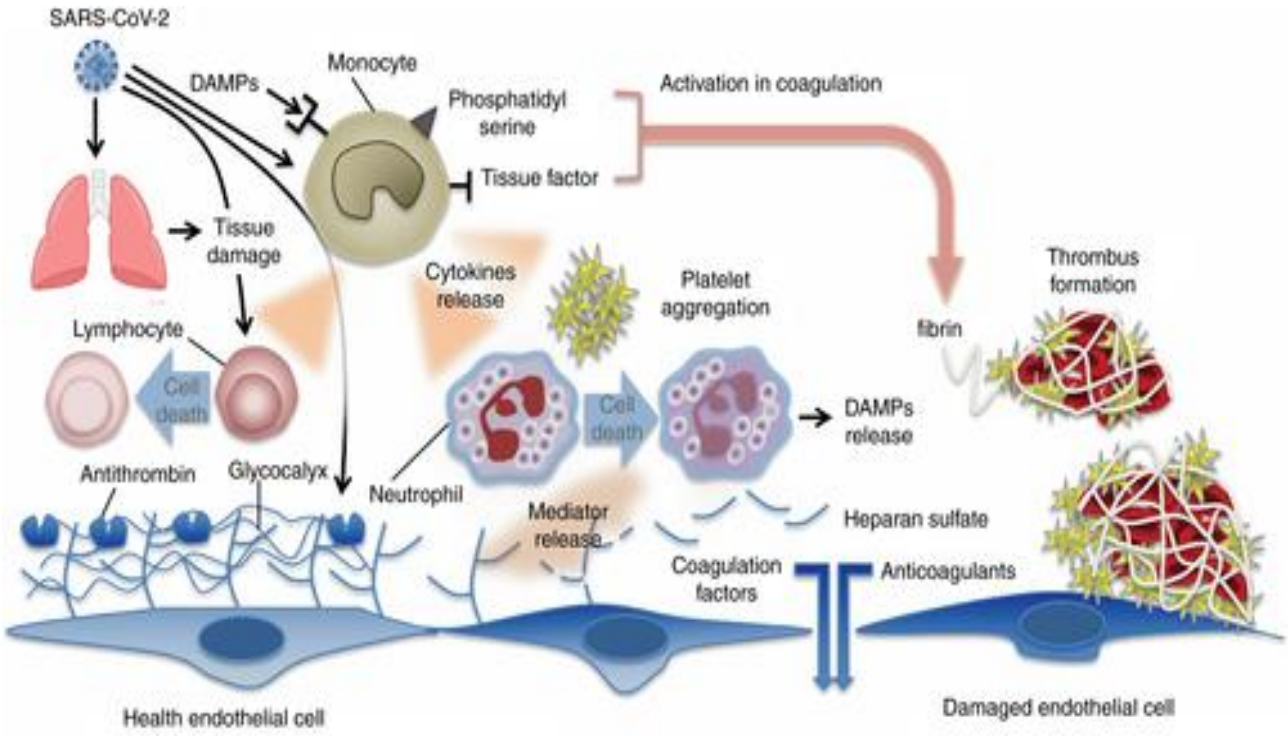

Fig. (1-7): Mechanisms of coagulation activation in Covid-19 (Wang, Shi et al. 2020).

The excess production of proinflammatory cytokines, increased levels of damage-associated molecular patterns, the stimulation of cell-death mechanisms and vascular endothelial damage are the major causes of coagulation disorder in any severe infection.The elevated levels of fibrin-related biomarkers, and prolonged PT and aPTT are often recognized in Covid-19, but the degree is less prominent compared with the bacterial sepsis-induced coagulopathy/DIC **(Baseler, Chertow et al. 2017).** The involvement of proinflammatory cytokines and chemokines such as tumor necrosis factor (TNF)-α, IL-1β, are reported in Covid-19, increased inflammatory cytokines and chemokines recruit immune cells to the infected tissues, primarily for the host defense, but also

results in the damage of the host **(Wang, Shi et al. 2020).** This mechanism is the same as bacterial infections; however, the response of the lymphatic system is more prominent with viral infections **(Zhang, Zhou et al. 2020).** The immune activation stimulates the expression of tissue factor on monocytes/macrophages and vascular endothelial cells. The coagulation cascades are initiated mainly by the tissue factor on the cellular surface. Different from coronavirus SARS infection, coagulopathy in the Ebola infection is characterized by marked thrombocytopenia, fibrin deposition, and prolongation of PT and aPTT **(Falasca, Agrati et al. 2015)**. Together with consumptive coagulopathy, the thrombus formation in the microvascular contributes to tissue ischemia and organ dysfunction. Hemorrhagic symptoms are commonly seen in Ebola infection, and the organ damage is dominant in the liver and vascular system that are unusual in Covid-19**.**

Both pathogens (viruses) and damage-associated molecular patterns (DAMPs) from injured host tissue can activate monocytes. Activated monocytes release inflammatory cytokines and chemokines that stimulate neutrophils, lymphocytes, platelets, and vascular endothelial cells. Monocytes and other cells express tissue factor and phosphatidylserine on their surfaces and initiate coagulation. Healthy endothelial cells maintain their anti-thrombogenicity by expressing glycocalyx and its binding protein antithrombin. Damaged endothelial cells change their properties to procoagulant following disruption of the glycocalyx and loss of anticoagulant proteins **(Iba, Levy et al. 2020).**

Aims of the Study:

The aims of the study include:

1. To evaluate the discriminative ability of von Willebrand Factor levels as thrombo-inflammatiry markers for predicting, monitoring, and a prognosis of severity patients with and without the severe or fatal forms of Covid -19
2. To evaluate the ADAMTS-13, and Vitamin K levels as essential predictive biomarkers in severe Covid-19 patients.
3. To study the biochemical changes in sera of Covid -19 such as lipid profiles, D-dimer, ferritin and C-reactive protein.
4. To study the correlation between the thrombo-inflammatory markers with Covid -19 symptoms severity and mortality.
5. To study the correlations between von Willebrand Factor level and each of ADAMTS-13, and Vitamin K levels and various biochemical parameters determined in severe Covid-19 patients.

Chapter Two

Subjects , Materials and Methods

2. Subjects, Materials and Methods

2.1. Subjects

The current case-control study included 90 patients and healthy subjects,Sample collection was conducted during the period from January 2021 toMay 2021 and they are classified as shown in Figure (2-1).

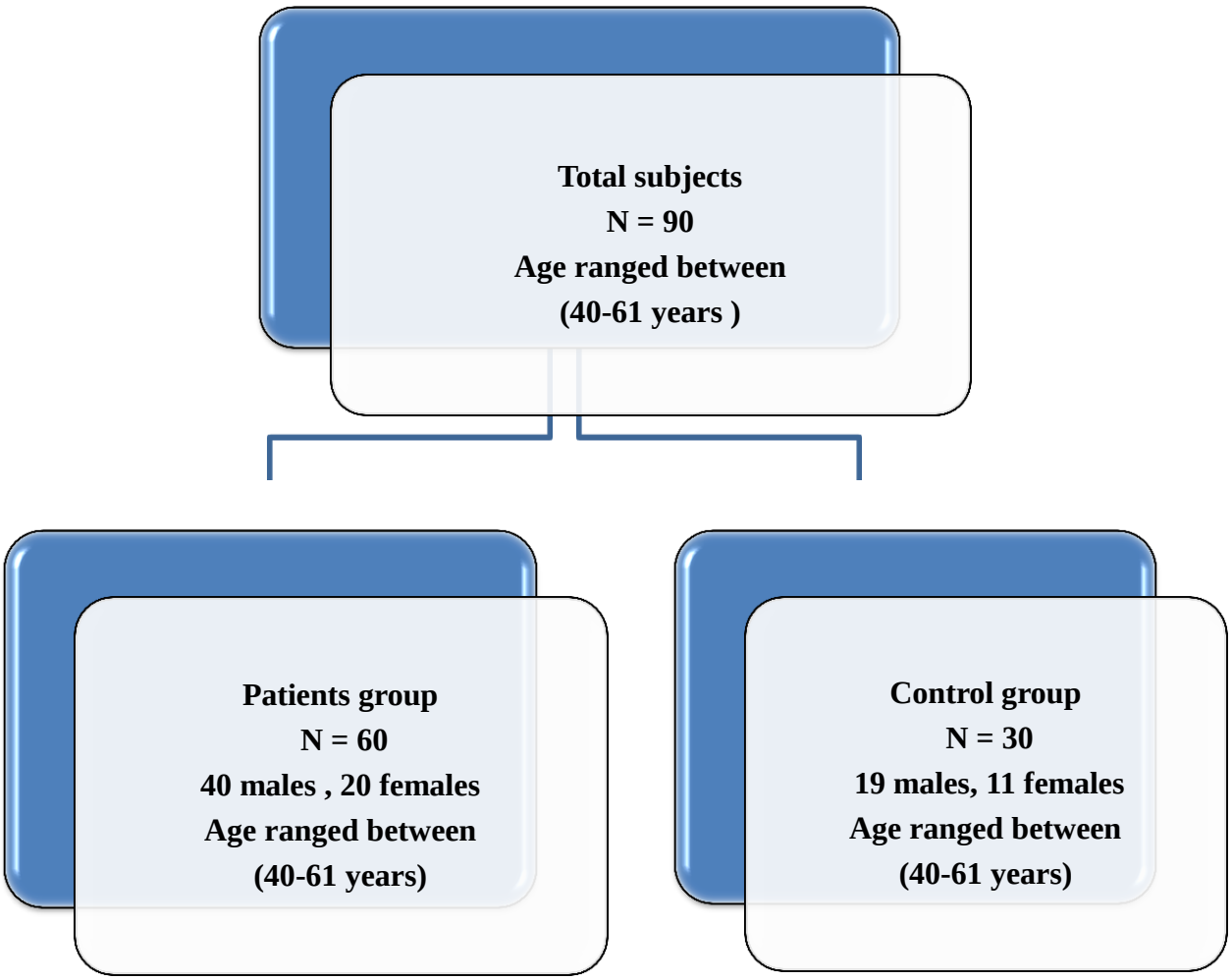

Fig. (2-1): Scheme subjects groups of Covid-19

2.1.1. Patients

The participated in the study included 60 patients, of whom infected with Covid-19 a admitted to each of Al- Amal Hospital ; Al-Shifaa Center (Najaf / Iraq), and Al-Hussein Teaching Hospital / Al-Hussein Medical City, Karbala Health Directorates / Karbala – Iraq. They are diagnosed by quantitative by RT-PCR and chest X-ray or CT scan at the 7-12 day from symptoms on set, with age ranged between (40-61) years and it consisted of three categories Covid-19 patients were collected at admission and the disease severity was assessed using Murray scores **(Murray, *et al.* 1988)**.The patients were considered to have mild / moderate Covid-19 depending upon fever, respiratory manifestations and radiological indicative of pneumonia. Patients were considered to have severe Covid-19 if any of the following changes was present:

(i) Respiratory distraction (≥ 30 / min)
(ii) Resting oxygen saturated $\leq 90\%$ or
(iii) Arterial oxygen (PaO_2) / fraction of inspired oxygen ≤ 300 mmHg .or
(iv) Respiratory failure requiring mechanical ventilation and require intensive care unit. Moreover, patient dead considered as Non-survived.

This investigation was approved by local medical ethics and all participants, information consent before the onset of study. The patients were registered and handed over a file for recording their data such as name, age, gender, weight, height.

Exclusion criteria:

The study excluded patients with any chronic or inmmune diseases like diabetes mellitus, infection and inflammation, and receiving long term oral corticosteroid ,anti-IL-6 or anti-TNF therapy and patients had history of vasculitis connective tissue disease. Patients suffering from cancer and kidney diseases of, smoker and also thyroid gland diseases.

2.1.2. Control

Thirty apparently healthy subjects were selected as control group. Their age and gender were comparable to that of patients. None of them was anemic or has an obvious systemic disease or any chronic diseases.

2.1.3. Blood Samples Collection

Five milliliters of venous blood were drawn from each the patients and control group by medical syringes, and 2 ml was but into EDTA tubes in order to use for CBC. Others 3ml blood were place in gel tubes and then left at room temperature for a period of ten minutes to fifteen minutes for coagulation, then centrifuged (at 3000 x g) for 10 minutes for serum sepration. The sera were divided into three Eppendorf tubes and stored at (-20 Cº) until time of biochemical estimation.

2.2. Materials

2.2.1. Instruments and Tools

Various instruments and laboratory tool are used to perform this study as shown in table (2-1).

Table (2-1): Instruments and Tools used in this study

No.	Instruments	Company	Country
1	Centrifuge	Kubota	Japan
2	Different glassware	Different sources	China
3	Disposable syringe	Enteplin	Egypt
4	ELISA microplate reader	Biotek	USA
5	Refrigerator	Nikai	Japan
6	Incubator	Kubota	Kubota

7	Different size micropipettes	Dragon	German
8	Deep freeze	Nikai	Japan
9	Shaker	Finepcr	Switzerland
10	Spectrophotometer	CECIL	France
11	Spin react Spincell3	Spin react	SPAIN

2.2.2. Chemicals and Kits

Various biochemical kits were used in this study. Table (2-2) shown the kits used with their sources.

Table (2-2): Chemicals and kits used in this study with their suppliers

No.	Chemicals	Company	Country
1	Complete blood count	Biolabo	France
2	Ichroma™ Ferritin	Boditech	Korea
3	Ichroma™ D-Dimer	Boditech	Korea
4	Total cholesterol kit	Biolabo	France
5	HDL-Cholesterol kit	Biolabo	France
6	Triglyceride kit	Biolabo	France
7	C- Reactive protein Kit	Melsin	China
8	von Willebrand Factor kit	Melsin	China
9	ADAMTS-13/vWF-cp kit	BT- LAB	China
10	Vitamin K kit	Melsin	China

2.3. Methods

2.3.1.Calculation of Body Mass Index(BMI)

Body mass index was used to define obesity. The range of BMI (18.5-24.99 kg/m^2) that set it WHO but it does not accurately indicate the degree of fatness **(Al-Khazraji 2010)**. In the present study, WHO classification will be used for adults as shown in Table (2-3) the Body Mass Index was measure by dividing weight in kilograms by length of individual in square meter: **BMI = (weight in kg) / (height in meters^2) .**

Table (2-3): Estimation of body mass index(Sorkin, Muller et al. 1999).

Weight status	BMI (kg/m^2)
Under weight	< 18.5 kg/m^2
Normal weight	18.5 to 24.9 kg/m^2
Over weight	25.0 to 29.9 kg/m^2
Obese	≥ 30.0 kg/m^2

2.3.2. Determination of Serum Ferritin Concentration

Principle

The test used a sandwich immunodetection method; the detector recombinant protein in buffer binds to antibody in a sample, forming recombinant protein. Antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized antigen on a test strip. The more antibody in the sample forms the more recombinant protein antibody complex and leads to stronger intensity of fluorescence signal on detector recombinant protein, which is

processed by Instrument for ichroma™ tests to show ferritin concentration in the sample.

Procedure

1. Thirty of the sample (human serum/plasma/control) was transferred using .The pipette was transferred to a tube containing detection buffer.
2. The cap of the detection buffer tube was closed and the sample was mixed well by shaking it about 10 times.
3. Seventy five µl of the sample mixture was withdrawn and the sample was loaded into a test cartridge.
4. The test cartridge loaded with the sample was left at room temperature for 10 minutes.
5. The ferritin identification code has been put into place.
6. The "Select" button was pressed on the device for ichroma™ tests to start the scanning process.
7. Instrument for ichroma™ tests will start scanning the sample.loaded cartridge immediately.
8. The test result was read on the display of the ichroma™ test instrument.

Interpretation of test result

An instrument for ichroma™ tests calculates the test result automatically and displays the ferritin concentration of the test sample in terms of ng/ml.

Reference range

Women: 20 - 250 ng/ml

Men: 30 - 350 ng/ml

Working range: 10 - 1,000 ng/ml.

2.3.3. Determination of Plasma D-Dimer Concentration

Principle

The test uses a sandwich immune detection method; the detector antibody in buffer binds to antigen in sample, forming antigen antibody complexes, and

migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip. The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody, which is processed by instrument for ichroma™ tests to show D-Dimer concentration in sample.

Procedure

1. Ten µl of plasma using a transfer pipette was transferred to a tube containing the detection buffer.
2. Closed the lid of the detection buffer tube and mixed the plasma thoroughly by shaking it about 10 times.
3. Seventy five µl of a plasma mixture and dispense it pipetted out into the sample well on the cartridge.
4. The sample-loaded cartridge was left at room temperature for 12 minutes.
5. Scanned the sample-loaded cartridge, inserted it into the cartridge holder of the Instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.
6. Pressed ‘Select’ button on the Instrument for ichroma™ tests to started the scanning process.
7. Instrument for ichroma™ tests will started scanning the sample-loaded cartridge immediately.
8. Read the test result on the display screen of the Instrument for ichroma™ tests

Interpretation of test result

The instrument for ichroma™ tests calculates the test result automatically and displays the d-Dimer concentration of the test sample in terms of ng/ml (FEU, Fibrinogen equivalent units).

Reference value : 500 ng/ml. Working range 50~10,000 ng/ml .

2.3.4. Determination of High Sensitivity C-Reactive Protein Concentration

Principle

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human CRP. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human CRP and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human CRP, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of Human CRP.

Reference value 0.5 - 200 mg/l .

Procedure

1. One hundred µl of standard or sample were added to each well. Incubated for 90 minutes at 37 °C
2. Aspirate the liquid, immediately added 100µl Biotinylated antibody working solution was added to each well. Incubated for 60 min at 37 °C.
3. Aspirated and washed for 3 times.
4. One hundred µl of HRP conjugate was added. Incubated for 30 minutes at 37 °C
5. Ninety µl of substrate reagent was added. Incubated for 15 minutes at 37 °C.

6. Fifty µl of stop solution was added. Determined the Abs value at 450 nm immediately.

Calculation

The concentration was calculated from the calitration curve. The standard curve has demonstrated a direct relation between hs-CRP concentration and the corresponding absorbance. The results have appeared automatically by the ELISA reader program from the calibration curve.

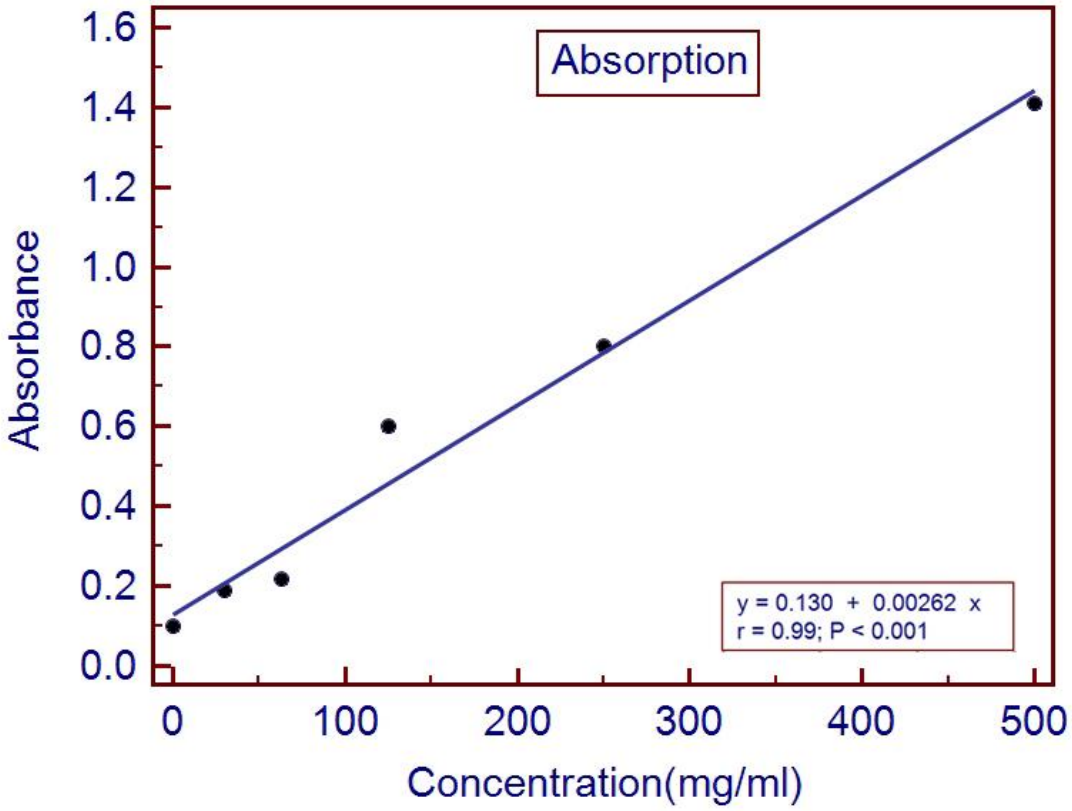

Fig. (2-1): Standard curve of serum –CRP(mg/L)

2.3.5. Determination of Complete Blood Count

When performing a complete blood count (CBC), we use an auto hematology analyzer consisting of three solutions that will be mentioned in detail, as well as accessories such as a shaker to mix the sample and a printer to print the results.

Principle

STEL 3-Lyser is used on STEL 3 Auto hematology analyzer for white blood cell counting, differentiating and hemoglobin measurement. In the process of blood cell counting, red blood cells are dissolved by lyse and release hemoglobin. Hemoglobin reacts with Lyse; the reaction produces a solution with particular color, so concentration of the solution can be determined by colorimetric method. At the same time, white blood cells release cytoplasm and shrink; size of solid content, such as, nucleus and granules left in different types of white blood cells is different. Based on electrical non-conductivity of blood cells, auto hematology analyzer can classify white blood cells into three groups, such as, lymphocytes, intermediate cells and neutrophils by detecting changes in electrical resistance when suspended particles in electrolyte solution passing through the counting hole.

Reagent Composition

Quaternary ammonium salt<0.15%, NaCl<0.15%, Stabilizer<0.12%

Principle

In the process of blood cell counting, STEL 3-Diluyent is a kind of balanced electrolyte solution with electrical conductivity. Based on electrical non conductivity of blood cells, STEL 3 auto hematology analyzer can perform complete blood count by detecting changes in electrical resistance when suspended particles in electrolyte solution pass through the counting hole.

Reagent Composition

NaCl <0.6% , Stabilizer <0.1% and buffer solution

Principle

STEL 3-Cleaner is used on STEL 3 Auto hematology analyzer for cleaning and washing tubing system, removing blood residual and other particles to ensure blood cell counting.

Reagent Composition

Buffer solution<0.3%, Protease<0.2%

2.3.6. Determination of Serum Total Cholesterol Concentration

The total serum cholesterol (TC) was determined by the enzymatic colorimetric method for the quantitative in vitro diagnostic measurement using a kit.

Principle

This method is for the measurement of the total serum cholesterol, which involves the use of three enzymes: cholesterol esterase (CE), cholesterol oxidase (CO) and peroxidase (POD). In the presence of the former, the mixture of phenol and 4-aminoantipyrine (4AA) is condensed by hydrogen peroxide to form Quinonimine dye proportional to the concentration of cholesterol in the sample.

Cholesterol esterase

Cholesterol ester + H_2O ----------►Cholesterol + Free Fatty acids

Cholesterol oxidase

Cholesterol + O_2 ----------► Cholesterol-4-en-one 3 + H_2O_2

Peroxidase

$2H_2O_2$ + phenol + 4-amino-antipyrine----------►Quinonimine(pink) +$4H_2O$

Table (2-4) show reagents used of the determination of serum total cholesterol

Reagent	Content	Concentration
Reagent 1Buffer	Phosphate buffer Chloro-4-phenol Sodium Chelate Triton ×100 Preservative	100 mmol/l 5 mmol/l 2.3 mmol/l 1.5 mmol/l
Reagent 2Enzymes	4-amio-antipyrine 4AA Cholesterol Esterase CE Cholesterol Oxidase CD Peroxidase POD	0.25mmol/l ≥1.70 IU/L ≥ 100 IU/L ≥ 1200 IU/L
Reagent3 Standard	Cholesterol	200 mg/dl

Procedure

1. Three sets of tubes serum, standard and blank) were prepared for (TC) as the following table (2-5).

Tubes / Solutions	Blank	Standard	Sample
Working reagent	1 ml	1 ml	1 ml
Demineralized water	10 µl	--------	------
Standard	--------	10 µl	-------
Serum	---------	--------	10 µl

2. The tubs were mixed and incubated for at least 5 min at 37 ºC.
3. The absorbance of samples and standards were measured at wave length 500 nm against blank.

Calculation

$$\text{The total cholesterol (mg/dl)} = \frac{\text{Abs. of sample}}{\text{Abs. of standard}} \times \text{Conc.of standard(200 mg/dl).}$$

Reference Values: < 200 mg/dl.

2.3.7. Determination of Serum Triglycerides Concentration

Serum triglycerides were determined by the enzymatic colorimetric method for the quantitative in vitro diagnostic measurement using a kit.

Principle:

The method is based on the enzymatic hydrolysis of serum or plasma triglyceride to glycerol and free fatty acids (FFA) by lipoprotein lipase (LPL). The glycerol is phosphorylated by adenosine triphosphate (ATP) in the presence of glycerol kinase (GK) to form glycerol-3-phosphate (G-3-P) and adenosine diphosphate (ADP). G-3-P is oxidized by glycerol-phosphate oxidase (GPO) to form dihydroxy acetone phosphate (DHAP) and hydrogen peroxide. A red chromogen is produced by peroxidase (POD) catalyzed coupling of 4aminoantipyrine (4-AA) and phenol with hydrogen peroxide (H_2O_2) proportional to the concentration of triacylglycerol in the sample. Serum triacylglycerol is measured by using an enzymatic method based on the following reactions:

$$\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{Glycerol + Free fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{Glycerol Kinase}} \text{Glycerol-3-phosphate + ADP}$$

GPO

Glycerol-3-phosphate + O_2 ----------► Dihydroxyacetonephosphate + H_2O_2

Peroxidase

H_2O_2 + 4-Chlorophenol + 4-AA ----------► Quinoneimine + H_2O

Table (2-6): Show reagents used of the determination of serum triglycerides

Reagent	Content	Concentration
Reagent 1Buffer	PIPES Magnesium chloride Chloro-4-phenol Preservative	100 mmol/l 9.8 mmol/l 3.5 mmol/l
Reagent 2 Enzymes	4-aminoantipyrin(4-AA) Adenosine triphosphate Na (ATP) Lipase Peroxidase (POD) Glycerol-3-phosphate oxidase (GPO) Glycerol Kinase (GK)	0.5 mmol/l 1.3 mmol/l ≥1000 IU/l ≥1700 IU/l ≥3000 IU/l ≥660 IU/l
Reagent 3 Standard	Glycerol equivalent to triglyceride 200 mg/dI	200 ml/dl

Procedure

Three sets of tubes (serum, standard and blank) were prepared as the following table (2-7).

Tubes / Solutions	Blank	Standard	Sample
Working Reagent	1 ml	1 ml	1 ml
Demineralized water	10 µl	--------	--------
Standard	--------	10 µl	--------
Serum	--------	-------	10 µl

2. The solutions were mixed and incubated for at least 5 min at 37 ºC.
3. The absorbance of samples and standards were measured at wave length 500 nm against blank.

Calculation

$$\text{Triglyceride (mg/dl)} = \frac{\text{Abs. of sample}}{\text{Abs. of standard}} \times \text{Conc. of standard (200 mg/dl)}$$

Reference Values: 35-160 mg/dl.

2.3.8. Determination of Serum High Density Lipoprotein-Cholesterol Concentration

Serum HDL-C was determined by the colorimetric method for the quantitative in vitro diagnostic measurement using kit.

Principle

This technique uses a separation method based on the selective precipitation of VLDL-C, LDL-C and chylomicrons from specimens by phosphotungestic acid

and MgCl2, HDL-Cholesterol obtained in after centrifugation is then measured with the Total Cholesterol reagent. Serum HDL-C is measured using the method of Burstein **(Woo, Simon *et al.* 1980).**

Reagents

Table (2-8): Reagents of (HDL-C) determination.

Reagent	Content	Concentration
HDL-Cholesterol R1	Phosphotungstic acid Magnesium Chloride	13.9 mmoL/L 570 mmoL/L

Procedure

A. Precipitation

1. In order to achieve HDL supernatant, 0.5 ml of the serum was mixed with 50 µl of HDL reagent.
2. The solution were mixed well, incubated for 10 min in room temperature, then mixed again and centrifuged for 10 min, at 4000 rpm.
3. After centrifugation, the clear supernatant from precipitate was separated to determine the HDL cholesterol concentration.

B. HDL-C Determination

1. Three sets of tubes (serum, standard and blank) were prepared for HDL-C as in the following table (2-9).

Tubes / Solution	Sample	Standard	Blank
Cholesterol reagent	1 ml	1 ml	1ml
HDL-C supernatant	25 µl	-------	------
Standard (HDL)	-------	25 µl	------
Demineralized water	--------	-------	25 µl

2. The solutions were mixed and incubated at least for 5 min at 37 ºC.
3. The absorbance of the sample and standard were measured at wavelength 500 nm against blank.

Calculation

$$\text{HDL-C concentration} = \frac{\text{Abs.of sample}}{\text{Abs.of standard}} \times \text{Conc. of standard (100 mg/dl)}$$

Reference Values: 35 – 55 mg/dl.

2.3.9. Calculation the Low Density Lipoprotein-Cholesterol and Very Low Density Lipoprotein-Cholesterol Concentration

The estimation of very low-density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein cholesterol (LDL-C) Serum level of LDL-C was calculated by Friedwald formula. Friedwald in 1972 had postulated a formula to calculate LDL-C value, which based on the assumption that VLDL-C, is present in serum at a concentration that equals to one fifth of triglyceride concentration **(Kawada, Fujita *et al.* 2004)**. **Therefore:**

LDL-C = TC- [HDL-C +VLDL]

VLDL =TG/5

Reference Values

Normal value of LDL-cholesterol less than 100 mg/dl

Suspicious: 150 mg/dl

Elevated: 190 mg/dl.

2.3.10.Determination ofserum ADAMTS-13Cleaving Protease activity.

Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human ADAMTS-13/vWF-cp antibody. ADAMTS-13/vWF-cp present in the sample was added and binds to antibodies coated on the wells. Then, biotinylated human ADAMTS-13/vWF-cp Antibody was added and binds to ADAMTS-13/vWF-cp in the sample. Then Streptavidin-HRP was added and binds to the Biotinylated ADAMTS-13/vWF-cp antibody. After incubation, unbound Streptavidin-HRP is washed away during a washing step. Substrate solution was then added and color develops in proportion to the amount of human ADAMTS-13/vWF-cp. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

Procedure

1. All reagents, standard solutions and serum were prepared as instructed. Brought all reagents to room temperature before used. The assay was performed at room temperature.
2. Fifty µl standards were added to standard well. Standard solution contains biotinylated antibody.
3. Forty µl serum was added to sample wells and then added 10µl anti-ADAMTS-13/vWF-cp antibody to sample wells, then added 50µl streptavidin-HRP to sample wells and standard wells. Mixed well, and covered the plate with a sealer to incubate for 60 minutes at 37 °C.
4. Removed the sealer and washed the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash

buffer, overfilling wells with wash buffer. Blot the plate on to paper towels or other absorbent material.

5. Fifty µl substrate solution A was added to each well and then added 50µl substrate solution B to each well. Incubated plate covered with a new sealer for 10 minutes at 37°C in the dark.
6. Fifty µl Stop Solution was to each well, the blue color will changes into yellow immediately.
7. Determined the absorbance of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Calculation

The activity was calculated from the calibration curve. The calibration curve has demonstrated a direct relation between concentration and the corresponding absorbance. The results have appeared automatically by the ELISA reader program from the **calibration** curve.

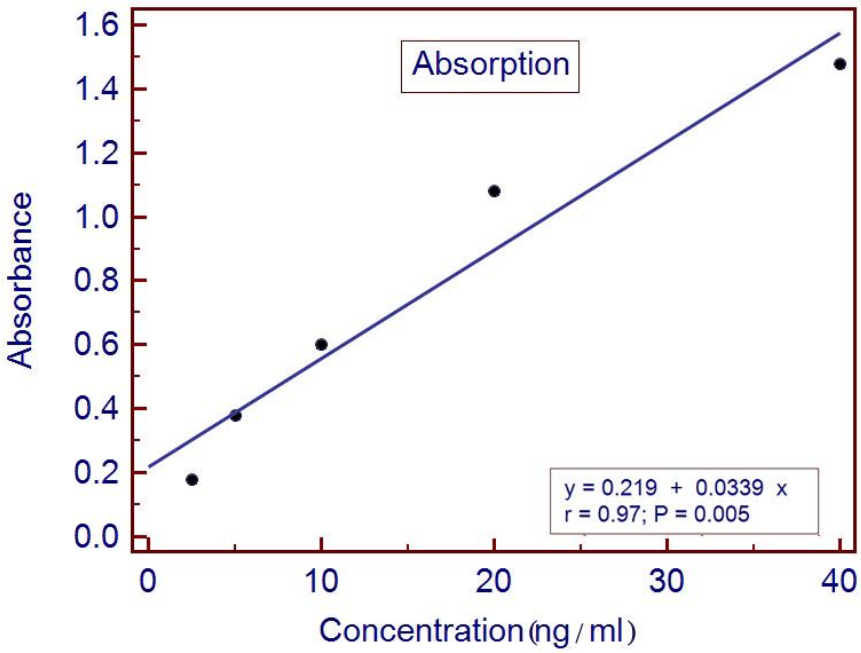

Fig. (2-2): Calibration curve of ADAMTS-13 (ng/ml) activity level

2.3.11. Determination of Serum Levels of von Willebrand Factor Concentration

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human vWF antibody. vWF present in the sample was added and binds to antibodies coated on the wells. Then, biotinylated human vWF Antibody was added and binds to vWF in the sample. Then Streptavidin-HRP was added and binds to the biotinylated vWF antibody. After incubation, unbound Streptavidin-HRP is washed away during a washing step. Substrate solution was then added and color develops in proportion to the amount of vWF. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

Procedure

1.All reagents Standards and Serum were Prepared as instructed. Brought all reagents to room temperature before used. The assay was performed at room temperature.

2. 50 µl standards were added to standard well. Standard solution contains biotinylated antibody.

3. 40 µl serum was added to sample wells and then added 10µl testing Sample to sample wells and standard wells.

4.Added 50µl streptavidin-HRP to sample wells Mixed well, and covered the plate with a sealer to incubate for 60 minutes at 37 °C.

5. Each wells wereAspirated and washed, repeating the process four times for a total of five washes. Washed by filling each well wth Wash Solution (400µl) used a squirt bottle, manifold dispenser ar autowasher. Camplete removal af liquid at aach step is Esseritial to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6. AchromogenTetramethyl benzidin(TMB) solution A 50µl and chromopen splution B50µl were added to each well Gently mix and incubate for 15 minutes at 37"C. Protect from light.

7. 5oµl Stop Solution was added to each well. The color in the wells should change from blue to yellow. If the color in the wels la green or theTolor change does not appear uniform, peotty tup the plate to ansure thorough maing. B.

8. the Optical Density (O.D.) was readed at 450 nm using a microtiter plate reader within 15 minutes.

Calculation

The concentration was calculated from the calibration curve. The calibration curve has demonstrated a direct relation between vWF concentration and the corresponding absorbance. The results have appeared automatically by the ELISA reader program from the calibration curve.

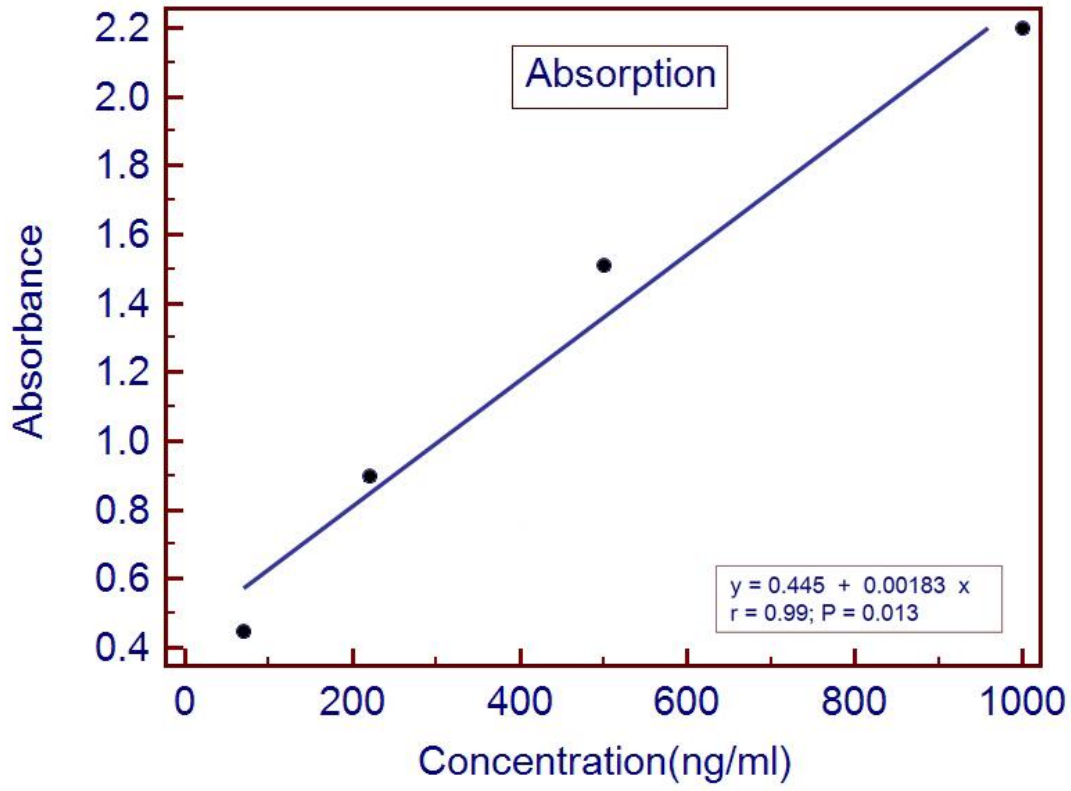

Fig. (2-3) Calibration curve calibration curve of vWF Concentration in (ng/ml)

2.3.12. Determination of serum human Vitamin K Concentration

Principle

The microtiter plate provided in this kit has been pre-coated with antibody. Added standard, samples and HRP conjugated antibody to wells. After incubation and washing to remove the uncombined enzyme, added chromogen(TMB) solution A and B. The color of the liquid will changed into blue. At the effect of acid, the color finally becomes yellow. The color changed is measured spectrophotometrically at a wavelength of 450 nm.

Procedure

1. All reagents were prepared before starting assay procedure. all standards and samples be added to the microelisa stripplate.
2. Fifty µl of standard was added to set Standard wells, testing sample wells.
3. Ten µl of serum was added to sample wells then 40 µl of sample diluent was added to testing sample well.
4. A volume of 100 µl of HRP-conjugate reagent were added to each well, covered with an adhesive strip and incubated for 60 minutes at 37 °C.
5. Each well was aspirated and washed, repeating the process four times for a total of five washes. Washed by filling each well with Wash Solution (400 µl) using a squirt bottle, manifold auto washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting. Inverted the plate and blot it against clean paper towels.
6. Fifty µl chromogen solution and chromogen solution B 50ul was added to each well. Gently mixed and incubated for 15 minutes at 37 °C.
7. Fifty µl stop solution was added to each well. The color in the wells should changes from blue to yellow.

8. Read the absorbance at 450 nm using a microtiter plate reader within 15 minutes.

Calculation

The concentration was calculated from the calibration curve. The calibration curve has demonstrated a direct relation between VK concentration and the corresponding absorbance. The results have appeared automatically by the ELISA reader program from the calibration curve.

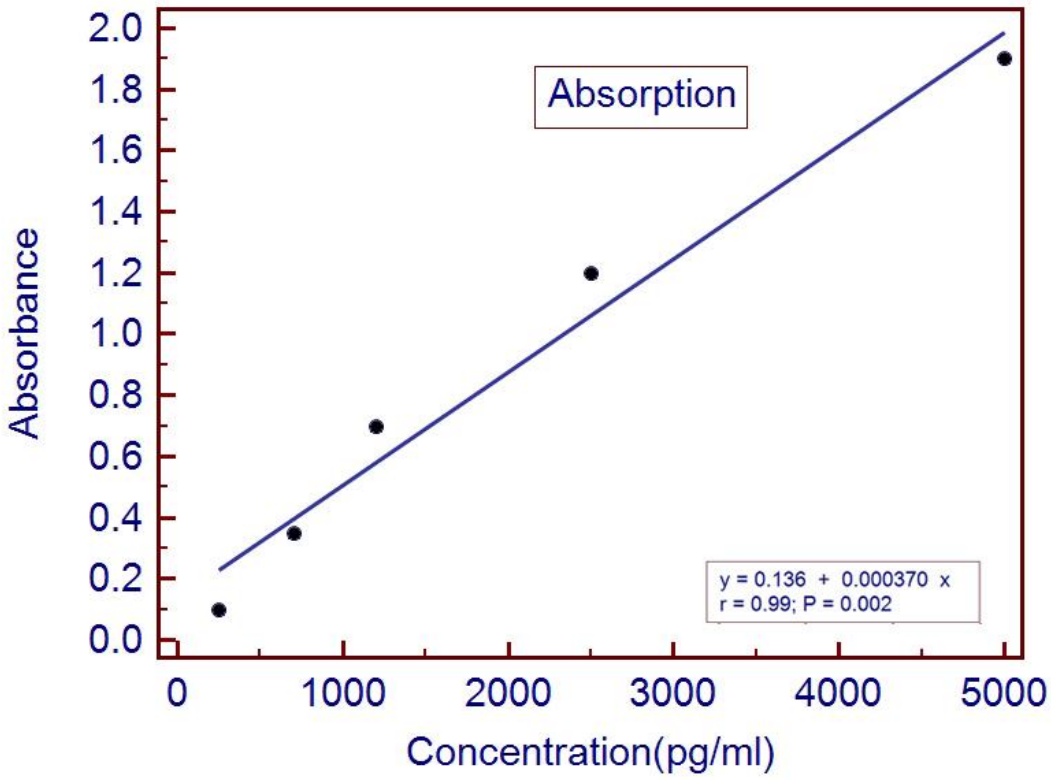

Fig. (2-4): Calibration curve of Vitamin K Concentration in (pg/mL)

2.4. Statistical Analysis

Data (represented as Mean ± SD) were analyzed by using the Statistical Package for the Social Sciences (SPSS) (version 23). Independent t- test was used to evaluate significant differences between healthy and patients groups. Pearson correlation coefficient test was applied to mention the statistical relationship (association) between any two variables in present study.

The levels of significance of 5% ($p \leq 0.05$) and 1% ($p \leq 0.01$) were obtained to represent the strength of evidence in support of significant differences between variables.

Chapter Three

Results and Discussion

3. Results and Discussion

3.1. Anthropometrics and Characteristics of Patients and Control groups

The base line characteristics of the study groups are presented in Table (3-1) The total of samples were 90 samples and subject groups consisted of 60 patients with Covid-19, and 30 apparently healthy adults as a control group to compared with patients groups. In variables of age, there is no significant difference between the studied groups. There is a significant difference between patients and control in BMI.

Table (3-1): General characteristics of the patients and control group

Parameters	Covid-19 patients group Mean ± SD N = 60	Healthy group Mean ± SD N = 30	*P*-value
Gender, F/M	20/40	11/19	-----
Age, (years)	56.89 ± 6. 32	56.48 ± 5.41	N.S
BMI, (kg/m^2)	28.82 ± 4.31	24.46 ± 3.11	0.001
SBP, (mmHg)	124.73 ± 7.31	123.69 ± 7.02	N.S
DBP, (mmHg)	70.15 ± 10.27	70.83 ± 6.18	N.S

N.: Number of subject. MI: Body mass index, Data represented as Mean ±SD, SD: Stander deviation, SBP: Systolic blood pressure, DBP: Diastolic blood pressure F: female, M: male, N.S.: Non-significant.

In Table (3-2) a total of Covid-19 patients were included in this study 33 mild, 15 severe and 12 dead cases .The age and BMI of deceased (death) cases was significantly higher when compared with the other cases.

Table (3-2): Demographic characteristics of Covid-19 patients groups

	Parameters Covid-19 patients group			***P*-value**
	Deceased N = 12	**Severe N = 15**	**Mild N = 33**	
Age, (years)	61.91 ± 3.42	60.82 ± 3.11	46.21 ± 7.50	a=0.09 b=0.00 c=0.00
BMI, (kg/m^2)	33.19 ± 3.35	30.07 ± 2.18	23.71 ± 5.01	a=0.01 b=0.00 c=0.00
SBP, (mmHg)	131.62 ± 2.41	130.42 ± 1.63	123.56 ± 3.12	a=0.07 b=0.001 c=0.001
DBP, (mmHg)	78.03 ± 2.15	77.12 ± 1.31	78.71 ± 1.79	a=0.06 b=0.06 c=0.06

BMI: Body mass index, Data represented as Mean ± SD, SD: Stander deviation, N.: Number of subject. SBP: Systolic blood pressure, DBP: Diastolic blood pressure, a= Deceased × Severe, b= Deceased × Mild, c= Severe × Mild.

The aging immunity, there are several other factors related to aging that could be reasons for higher mortality and morbidity in the elderly. The average number of comorbid conditions steadily increased with age and elderly Covid-19 patients had a significantly higher performance score than young and middle-aged patients **(Kim, Whitaker et al. 2020).** Another well-recognized feature of aging immunity is chronic subclinical systemic inflammation, also known as inflammation. Inflammation is a key pathogenic mechanism in Covid-19; hence, inflammation has been estimated to contribute to the poorer outcome in elderly patients with Covid-19 **(Bonafè, Prattichizzo et al. 2020).**

Sex differences in both the innate and adaptive immune systems have been previously reported and may account for the female advantage in Covid-19. Within the adaptive immune system, females have higher numbers of CD4+ T cells more robust CD8+ T cell cytotoxic activity, and increased B cell production of immunoglobulin compared to males **(Ciarambino, Para et al. 2021).**

The male gender is also characterized by an intrinsic tendency to meta-inflammation Compared to the female sex. The cumulative affect of the old it likely results in age, male gender, and obesity a case of progressive meta-inflammation that leads to dysregulation and it distorts the immune system in the face of a perfect inflammatory cell storm. In agreement with this the concept of targeted inflammatory treatment strategies a response such as IL-6 blockade **(Mauvais-Jarvis 2020)**. Or implantation from mesenchymal stem cells are showing some promising initial results in preventing a cytokine storm **(Zhang, Wang *et al.* 2020).** Advanced age, male sex, and the presence of multiple comorbidities have been clearly identify as major risk factors for the development of severe Covid-19 **(Yan, Zhou et al. 2021).**

This fact could be influenced by both the physiological aging process and, especially, the greater prevalence in older adult patients of frailty and comorbidities that contribute to a decrease in functional reserve that reduces intrinsic capacity and resilience and hinders the fight against infections **(Bonanad, García-Blas et al. 2020).**

In immunopathology, vulnerability to an infection in the elderly is usually explained by immunosenescence **(Barbé-Tuana, Funchal et al. 2020).** Immunosenescence is quite complicated. Briefly, in old age, the production of naïve T and B cells decreases, and the function of innate immune cells is impaired; hence, cells involved in the innate immunity do not get activated efficiently during an infection, and progression to an adaptive immune response does not occur in a coordinated manner **(Aiello, Farzaneh et al. 2019).**

Obesity increases the duration of virus shedding of influenza a virus. Based on currently available information and clinical expertise, the centers for disease control and prevention has identified severe obesity (i.e., BMI around 40 kg/m²) as a common clinical risk factor for worse prognosis and higher mortality in patients with coronavirus disease 2019 (Covid-19) infection. Furthermore, any degree of obesity (BMI 30 kg/m²) has been associated with poor prognosis in patients with Covid-19 **(Kompaniyets, Goodman et al. 2021).**

Hypertension was the most common comorbidity found in hospitalized patients with Covid-19infection. Previous studies have reported a higher risk of all-cause mortality in Covid-19 patients with concomitant hypertension **(Zhong, Zhao et al. 2020)**. Hypertension was also the most common comorbidity in ICU patients in Lombardy, Italy (49%) and hospitalized Covid-19 patients in New York, USA (56.6%) **(Lam, Chow et al. 2020)**.

The current results suggest that high SBP and unstable SBP/DBP control were independently associated with greater risks of adverse outcomes, including mortality, ICU admission, and heart failure, in all Covid -19 patients. The findings provide important evidence for the clinical management of Covid-19 patients**) Umishio, Ikaga et al. 2021)**.

Therefore, health care institutions should also play close attention to the mild patients, identify progresses early, and provide appropriate treatment to reduce mortality. As reported in a recent article **(Zhu, Zhang et al. 2020).** Severe Covid-19 patients were older and were more likely to have underlying disease, which is consistent with our study. Non severe patients who progressed to severe cases were older and had a higher probability of having underlying diseases. In terms of the symptoms, patients who progressed from non-severe to severe were more likely to have shortness of breath. Several published articles have also reported a higher rate of shortness of breath in severe Covid-19 patients **(Wang, Hu et al. 2020).** Although these exacerbated patients felt dyspnea at an early stage, their blood oxygen saturation did not reach the

standard of severe cases, which may be explained by the compensatory effect of faster breathing.

3.2. Biochemical Markers and Covid-19 Patients

3.2.1. Role of Lipid Profile Levels as a Risk Index in Covid-19

In table (3-3), the data observed demonstrated a significant increase in serum levels of TG, VLDL-C, CRI-1, CRI-11, AIP, except levels of HDL-C, TC, ;and LDL-C which a lower significantly in patients infected with coronavirus group compared with healthy group.

Table(3-3):Comparisons of lipid profiles between theCovid-19 and healthy group

Parameters	Covid-19 Patients group Mean ± SD N = 60	Healthy group Mean ± SD N = 30	P-value
TG, (mg/dl)	242.78 ± 60.21	110.4 ± 57.66	0.000
TC, (mg/dl)	110.98 ± 21.34	124.5 ± 36.27	0.000
LDL-C, (mg/dl)	39.63 ± 7.88	61.38 ± 14.09	0.000
VLDL-C, (mg/dl)	48.55 ± 10.28	21.51 ± 9.01	0.000
HDL-C, (mg/dl)	15.17 ± 5.69	45.11 ± 14.05	0.000
CRI-1 (TC/HDL-C)	4.91 ± 1.17	2.30 ± 0.68	0.000
CRI-11(LDL-C/HDL-C)	1.77 ± 0.87	1.119 ± 0.50	0.01
AIP (log TG/HDL-C)	1.025 ± 0.43	0.401 ± 0.19	0.000

Data represented as Mean ± SD: Standard deviation, TG: Triglyceride, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low density lipoprotein-cholesterol VLDL.C: Very Low-Density Lipoprotein- Cholesterol, TC: Total cholesterol, CRI-1: (TC/HDL) Castelli Risk Index, CRI-11: (LDL/HDL) Coronary risk index, AIP :(log TG/HDL) Atherogenic index. Significant at P≤0.01, ns = not significant, independent t-test, df = 88.

Table (3-4) shows the categories of Covid-19 patients groups. The TG, TC and VLDL-C and athrogenic index of non-survived cases higher significantly when compared with the other cases.

Table (3-4): Comparison of the serum lipid profiles in various group patients studied.

Parameters	Covid-19 patients group			*P*-value
	Deceased N = 12	Severe N = 15	Mild N = 33	
TG, (mg/dl)	251.03 ± 80.11	246.61 ± 73.03	230.73 ± 71.6	A= 0.01 B= 0.01 C= 0.01
TC, (mg/dl)	120.03 ± 30.12	115.13 ± 10.11	97.83 ± 5.18	A= 0.02 B= 0.001 C= 0.001
LDL-C, (mg/dl)	45.24 ± 5.18	24.42 ± 3.97	21.01 ± 2.18	A= 0.02 B= 0.001 C= 0.001
VLDL-C, (mg/dl)	50.20 ± 14.13	49.32 ± 16.28	46.146 ± 12.03	A= 0.06 B= 0.05 C= 0.07
HDL-C, (mg/dl)	20.71 ± 6.89	22.90 ± 7.34	24.82 ± 2.02	A= 0.07 B= 0.05 C= 0.03
CRI-1 (mg/dl)	5.79 ± 1.63	5.02 ± 1.01	3.94 ± 1.18	A= 0.02 B= 0.001 C= 0.001
CRI-11(LDL-C/HDL-C)	2.37 ± 0.79	1.87 ± 1.06	1.082 ± 1.14	A= 0.001 B= 0.001 C= 0.05
AIP (log TG/HDL-C)	1.083 ± 0.68	1.032 ± 0.49	0.968 ± 0.20	A= 0.06 B= 0.01 C= 0.01

Data represented as mean ± SD, TG: Triglyceride, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low density lipoprotein-cholesterol VLDL ; C: Very Low-Density Lipoprotein- Cholesterol, TC: Total cholesterol ; F: female ; M: Male ; A= p value (non-survived + severe) ; CRI: (LDL-C/HDL-C coronary risk index ; AIP: (log TG/HDL-C) atherogenenic index ; B= p value (non-survived + moderate) ; C = p value (severe + moderate)

Interestingly, the studies by Wei *et al*. in 2020 have specifically shown that low HDL-C levels were associated with severe Covid-19 disease. However, the heterogeneity reported in these two studies, which included mild, severe and critical patients and ICU and non-ICU patients, does not allow for direct comparisons with our specific severe Covid-19 ICU **(Wei, Hu et al. 2020) .**

Cholesterol is also a signaling molecule regulates its synthesis, the cell cycle and can modify proteins, due to its versatile roles and involvement in numerous physiological processes, the organism must maintain the cholesterol homeostasis unless its excess could be potentially toxic. This is achieved by sophisticated regulation of de novo synthesis of cholesterol **(Praksch, Sandor et al. 2019).**

In particular, they are involved in fusion of the viral membrane to the host cell, viral replication, as well as endocytosis and exocytosis (Sumi and Harada 2020). Cholesterol and lipid rafts play an especially fundamental role in the early stage of cell infection **(Fecchi, Anticoli et al. 2020).** Thus that the cholesterol may play an essential role not only in viral replication and internalization, but also in immune activation **(Chu, Chan et al. 2020).**

Viral infections cause alterations of lipid biomarkers in their hosts, leading to disrupted cholesterol rafts. This helps them in infiltrating the host defenses. Also, the depressed lipid parameters can be a predictor for the severity of Covid-19 disease, however, in current study HDL-C, LDL-C, and TC were significantly depressed, In contrast to these investigations, a preprint reports significantly increased levels of LDL-C compared to control groups and a positive relationship of Covid-19 severity with elevated lipid profile alterations **(Malik, Ishaq et al. 2021).**

In Covid-19, a surge in pro-inflammatory cytokines confers the presence of systemic inflammation. HDL-C can deactivate this inflammatory cascade by inhibiting the activation of monocytes and neutrophils while maintaining an

antioxidant function, allowing for the removal of oxidized lipids and neutralizing oxidative factors. This, in turn, mitigates inflammatory response in the host cells **(Stasi, Franzin et al. 2021).**

During systemic inflammation, HDL-C can be oxidized **(Roccaforte, Daves et al. 2020).** In this study it has been shown that HDL-C, TC levels in Covid-19 patients were significantly lower during active infection than in healthy subjects. These decreased levels were due to the involvement of these lipoproteins in regulation of immune cells during Covid-19 infection.

Lipoproteins play an important role in vasodilatation and in reducing LDL oxidation, thrombosis, apoptosis, inflammation and infection. Besides being an anti- inflammatory lipoprotein with protective effects against lipid oxidation, HDLs negatively regulate T-cells activation and expression of inflammatory mediators in macrophages and dendritic cells **(Voloshyna, Hussaini et al. 2012).**

In serum of patients with Covid-19, the sharply elevated values of pro-inflammatory cytokines indicate the presence of systemic inflammation **(Gruber, Patel et al. 2020)**. HDLs inhibit the expression of adhesion molecules by the endothelium in response to inflammatory cytokines and inhibit the adhesion of monocytes to the endothelium. They also inhibit the activation of monocytes, reduce their secretion of pro-inflammatory cytokines, inhibit activation and diagenesis of neutrophils **(DeGoma, degoma et al. 2008).**

During a cytokine storm in Covid-19, HDL and LDL-C are oxidized, leading to an up-regulation of immune activation based on the immunomodulatory mechanism of HDL, consider immune regulation in Covid-19 as the primary cause for decreased lipid levels in Covid-19, HDL and LDL-C **(Feingold 2020).** These direct anti-inflammatory and an enhancement of antioxidant functions, which would allow removal of oxidized lipids and neutralization of some oxidative mediators, thus further mitigating the local inflammatory response.

showed that the incubation of endothelial cells with TNFα is effective to stimulate the expression of adhesion molecules which mediates adhesion and diapedesis of monocytes and other leukocytes within the arterial wall **(degoma, Leeper et al. 2008)**.

3.2.2. Comparison of Complete Blood Count, D-dimer and Ferritin Levels in Covid-19 Patients and Apparently Control Group

In table (3-5) revealed the data analysis which showed a significantly higher presence in serum D-dimer, ferritin levels the percentage of neutrophils on lymphocytes and platelets, There was a increase in the number of neutrophils and an decrease in lymphocytes.

Table (3-5): Complete blood count, D-dimer and Ferritin in Covid-19 Patients as compared with control group.

Parameters	Covid-19 patients group Mean ± SD N = 60	Healthy group Mean ± SD N = 30	P-value
PLT%	250.74 ± 57.33	257.3 ± 21.11	0.05
NEUT%	84.30 ± 7.84	45.31 ± 9.83	0.000
LYM%	10.21 ± 2.81	23.85 ± 6.91	0.000
NLR	8.26 ± 3.69	1.79 ± 0.87	0.000
D-Dimer (ng/ml)	3450.20 ± 1800.17	269.69 ± 88.96	0.0001
Ferritin (ng/ml)	1081.93 ± 471.46	106.7 ± 47.81	0.0001

Data represented as mean ± SD: Standard deviation, LYM: Lymphocyte, NEUT: neutrophil, NLR: Neutrophil/ Lymphocyte ratio, PLT: Platelet.

In Table (3-6) the values of neutrophil, D-dimer and Ferritin levels in deceased (death) and severe cases showed a higher significent when compared with the mild case.

Table (3-6): Comparison between levels of complete blood count, D-dimer and ferritin levels in various groups of coronavirus patients.

Parameters	Covid-19 patients groups			P-value
	Deceased N = 12	Severe N = 15	Mild N = 33	
PLT%	157.73 ± 60.31	279.99 ± 40.11	314.45 ± 55.70	a=0.000 b=0.00 c=0.001
NEUT%	93.03 ± 4.78	81.01 ± 9.84	78.81 ± 9.79	a=0.001 b=0.000 c=0.02
LYM%	10.10 ± 2.13	10.29 ± 2.37	10.24 ± 3.11	a=0.06 b=0.06 c=0.08
NLR	9.21 ± 2.86	7.87 ± 1.65	7.69 ± 4.00	a=0.000 b=0.000 c=0.06
D-Dimer, ng/ml	5602.6 ± 2348.30	4065.1 ± 2220.15	683.4 ± 301.81	a=0.000 b=0.000 c=0.000
Ferritin, ng/ml	700.29±1409.71	1187.34 ± 692.50	648.79 ± 399.94	a=0.000 b=0.000 c=0.000

Data represented as Mean ± SD: standard deviation ; LYM: lymphocyte ; NEUT: neutrophil ; NLR: neutrophil/ lymphocyte ratio ; PLT: Platelet; a= p-value (deceased + severe) ; b = p-value (deceased + mild) ; c= p-value (severe + mild).

This study focused on the value of inflammatory markers and immune cell counts to predict the severity of Covid-19.

Corona virus disease 2019 is a systemic infection with a significant impact on the hematopoietic system and hemostasis. Lymphopenia may be considered as a cardinal laboratory finding, with prognostic potential. Neutrophil/lymphocyte ratio and peak platelet/lymphocyte ratio may also have prognostic value in determining severe cases **(Guéant, Guéant-Rodriguez et al. 2021).**

During the disease course, longitudinal evaluation of lymphocyte count dynamics and inflammatory indices, including LDH, CRP and IL-6 may help to identify cases with dismal prognosis and prompt intervention in order to improve outcomes. Biomarkers, such high serum ferritin have also emerged as poor prognostic factors. Furthermore, blood hypercoagulability is common among hospitalized Covid-19 patients. Elevated D-Dimer levels are consistently reported, whereas their gradual increase during disease course is particularly associated with disease worsenin **(Santos, Morales et al. 2020).**

Covid-19 infected patients, whether hospitalized or ambulatory, are at high risk for venous thromboembolism, and an early and prolonged pharmacological thromboprophylaxis with low molecular weight heparin is highly recommended. Last but not least, the need for assuring blood donations during the pandemic is also highlighted **(Cheung, Quiwa et al. 2020).**

In the current results for Covid-19 patients group generally present with decreased lymphocyte numbers in peripheral blood, which is particularly pronounced in severe cases T cells appear to be the most affected lymphocyte subset. In agreement with these observations, found decreased numbers of lymphocytes in the present of Covid-19 patients. In addition to lymphopenia, Covid-19 patients typically display increased concentrations of inflammatory cytokines in serum, especially in severe cases **(Brito, Lima et al. 2021).**

Due to the high risk of thrombosis, heparin prophylaxis has been recommended for all adolescents and adults hospitalized with Covid-19.

Heparin-induced thrombocytopenia (HIT), a well-known clinical entity occurring 5–14 days after heparin exposure, is an important differential for patients presenting with thrombocytopenia after hospitalization **(Bonafè, Prattichizzo et al. 2020).**

Patients who received ICU care had numerous laboratory abnormalities. The abnormalities in CBC for Covid-19infection may be associated with cellular immune deficiency, coagulation activation, myocardia injury, hepatic injury, and kidney injury. The strong association between patients' clinical outcome and increase of neutrophil counts was a biologically and clinically relevant finding of present study and the reasons for that are manifold **(Wenling, Junchao et al. 2020)**.

Extremely high ferritin levels are the hallmark of hyper ferritinemic syndromes, which is an umbrella term for macrophage activation syndrome, catastrophic antiphospholipid syndrome, adult onset Still's disease, and septic shock **(Colafrancesco, Alessandri et al. 2020)** .

Ferritin estimation and significence in Covid-19 infections whether its pneumonia, haemophagocytic lymphohistiocytosis, lymphopenia, cytokine storm or simple bleeding disorders after progression of Covid-19 infections, several significant studies reported the importance of Ferritin as one of the bio-markers that's been elevated considerably whenever there is disease severity and/or progression. It was well reported that patients admitted to Intensive care units (ICUs) after developing Covid-19 complications exhibit more severe alterations of pro-inflammatory markers such as PCT, DD, CRP, IL and Fer **(Leisman, Ronner et al. 2020)**.

In general, highly elevated ferritin levels portend poor prognoses in hospitalized patients. There are multiple studies correlating elevated ferritin levels and other pro-inflammatory markers in Covid-19 with poor outcomes **(Zhang, Zhao et al. 2020).** Previous efforts in treating Covid-19 include

trialing various anti-inflammatory biologic agents to inhibit this robust immune response **(Singh and Singh 2020).**

Serum ferritin, a feature of hemophagocytic lymphohistiocytosis, which is a known complication of viral infection, is closely related to poor recovery of Covid-19 patients, and those with impaired lung lesion are more likely to have increased ferritin levels **(Huang, Li *et al.* 2020).** The development and progression of cardiovascular disease and elevated levels of fibrinogen and D-dimer have been associated with an increased risk of cardiovascular disease **(Bennett, Lane et al. 2008).**

An increased risk of venous thromboembolism (VTE) in patients with Covi-19 pneumonia admitted to intensive care unit (ICU) has been reported. Whether Covid-19 increases the risk of VTE in non-ICU wards remains unknown. In patients admitted with Covid-19 pneumonia and elevated D-dimer levels, the incidence of asymptomatic DVT is similar to that described in other series? Higher cut-off levels for D-dimer might be necessary for the diagnosis of DVT in Covid-19 patients **(Demelo-Rodríguez, Cervilla-Muñoz et al. 2020).**

The mechanisms responsible for the association of hyperferritinemia and disease severity in patients with Covid-19 are unclear, but there are several possibilities for this phenomenon included proinflammatory cytokines such as interleukin-Iβ (IL-lβ), tumor necrosis factor-a (TNF-α), and IL-6 may increase ferritin synthesis **(Gómez-Pastora, Weigand et al. 2020).** Hence,speculated that Covid-19-induced production of proinflammatory cytokines (i.e., IL-6, TNF-α), which are known to be elevated in Covid-19, might promote ferritin synthesis early in inflammation. Also, the cellular damage derived from inflammation can promote the leakage of intracellular ferritin, thus elevating serum ferritin **(Lin, Long et al. 2020).**

In acidosis, the microvascular environment and increased production of reactive oxygen species (ROS) might liberate iron from ferritin, and it is this

unliganded iron that can participate in Haber-Weiss and Fenton reactions, creating hydroxyl radicals, causing further cellular damage,8 and worsening tissue injury, thus causing a vicious cycle of inflammation. Similarly, one study found that the assembly of Middle East Respiratory Syndrome (MERS) coronavirus nanoparticles is related to chaperonemediated ferritin ()**Jayaraj, Kumarasamy et al. 2020)**.

Serum levels elevation upon admission was common and was associated with both increased disease severity and in-hospital mortality. D-dimers are one of the fragments produced when plasmin cleaves fibrin to break down clots. The assays are routinely used as part of a diagnostic algorithm to exclude the diagnosis of thrombosis **(Linkins and Takach Lapner 2017).**

However, any pathologic or non-pathologic process that increases fibrin production or breakdown also increases plasma D-dimer levels though D-dimer elevation has been observed in articles describing the clinical features of Covid-19, whether the level of D-dimer is a marker of severity has not been examined **(Yao, Cao *et al*. 2020).** Level of D-dimer levels and disease severity stratified by the area of affected lungs on chest CT, oxygenation index, as well as clinical staging according to the interim guideline. In addition, a higher percentage of D-dimer elevation was seen in the present study than previously reported D-dimer is a byproduct of fibrin degradation **(Mishra, Pathak et al. 2020)**. Moreover, some authors have suggested a particularly high frequency of thromboembolic events, including fatal pulmonary embolism **(Artifoni, Danic et al. 2020).** Use of heparin was associated with reduced mortality in Covid-19 patients, suggesting that thromboembolism prophylaxis is critical in the management of Covid-19 **(Fei, Tang et al. 2020).**

3.2.3. Comparisons between vWF and ADAMTS-13 Levels in Patients and Apparently Healthy Groups

The data analysis demonstrated in table (3-7) a significant increase in the levels of serum vWF and decreased level of serum ADAMTS13 in Covid-19 patients compared with the healthy group.

Table (3-7): Comparisons of serum lvWF, and ADAMTS-13 levels between Covid-19 patients group and healthy groups

Parameters	Patients group N = 60 Mean ± SD	Healthy group N = 30 Mean ± SD	*P*-value
vWF (ng/ml)	355.20 ± (44.45)	205.07 ± (20.98)	0.0001
ADMTS-13 (ng/ml)	19.92 ± (9.44)	37.10 ± (7.48)	0.0001

**** Significant at P≤0.01, independent t-test, df=88.**

In Table (3-8) the values of vWF, ADAMTS-13 levels in deceased and severe cases shown a higher significant of vWF levels when compared with the mild case.

To the best of our knowledge, this results is the first study conducted in Iraq in group of COVID-19 patients showing a statistically significant association between serum levels of vWF, and ADAMTS-13 with the In some patients, Covid-19infection. In normal blood vessels, the endothelial cell (EC) monolayerlining blood vessel functions to prevent pathological thrombosis. Data from autopsy study in Covid-19have identified marked EC apoptosis, together with loss of EC tight junction integrity in the pulmonary microvasculature **(Wichmann, Sperhake et al. 2020).**

Table (3-8): Comparison of serum vWF, and ADAMTS-13 levels in various Covid-19 patients with healthy group.

Parameters	Covid-19 patients group			P-value
	Deceased N = 12	Severe N = 15	Mild N = 33	
vWF (ng/ml)	392.01 ± 35.22	354.11 ± 21.54	320.01 ± 24	a= 0.1 b= 0.01 c= 0.03
ADAMTS-13 (ng/ml)	15.09 ± 2.49	17.98 ± 4.35	23.78 ± 10.71	a= 0.01 b= 0.01 c= 0.01

Data represented as Mean ± SD: standard deviation ; a= p-value (deceased + severe) ; b = p-value (deceased + mild) ; c= p-value (severe +mild)

In vivo, vWF biosynthesis is limited to EC and megakaryocytes **(Lenting, Christophe et al. 2015).** The vWF was synthesized within megakaryocytes is stored within the a-granules of their platelet progeny. Conversely, vWF synthesized in EC is either constitutively secreted into the plasma, or else stored within Weibel–Palade bodies (WPB). This stored vWF can then besecreted following EC activation, thereby facilitating tethering of platelets and leucocytes to the vessel wall. Importantly, ultra-large vWF multimers have been shown to play a critical role in the pathogenesis underlying microvascular occlusion in several conditions including thrombotic thrombo cytopenic purpura (TTP), cerebral malaria and sickle cell disease **(Larkin, de Laat et al. 2009).**

In some patients, Covid-19infection leads to a severe bilateral pneumonia and significant hypoxia that is refractory to standard treatments. Coagulation activation is a hallmark of severe Covid-19 **(Vahabi, Salehi et al. 2021).**This coagulopathy develops at a relatively early stage. Consequently, D-dimer levels are often significantly elevated at time of initial presentation and represent an

independent marker for poor clinical outcome **(Fogarty, Townsend et al. 2020).**

Consistent with coagulation activation, high ratesof deep vein thrombosis and pulmonary embolism have beenassociated with severe Covid-19infection, particularly in patients requiring intensive care unit (ICU) support **(Klok, Kruip et al. 2020)**. In addition to these macrovascular complications, accumulating evidence suggests that microvascular occlusion within the lungs plays a critical role in Covid-19 pathogenesis. Importantly, post-mortem studies have shown widespread microthrombi throughout the pulmonary vasculature in patients with fatal Covid-19 **(Wichmann, Sperhake et al. 2020).**

The importance of ADAMTS-13 activity is evidenced by the severe condition of patients with thrombotic thrombocytopenicpurpura (TTP), which is characterized by a deficiency in, or the presence of neutralizing antibodies to, ADAMTS-13. The thrombotic manifestations in patients are related to circulating ultra large vWF multimers, which spontaneously bind and aggregate platelets **(Joly, Coppo et al. 2017).**

Covid-19infection is characterized, among other features, by a prothrombotic state with high D-dimer, Clinical observations have also highlighted that these patients have elevated vonWillebrand factor (vWF) and decreased level of ADAMTS-13. It affects primarily the respiratory system, but can also involve the cardiovascular, gastrointestinal and haematological systems. Autopsy reports of Covid-19cases have revealed alveolar exudative inflammation, epithelium proliferation, interstitial inflammation and hyaline membrane formation in the lungs. Pathological lesions were also seen in other organs such as heart, vessels, liver and kidney **(Yao, Li et al. 2020).** The most characteristic finding in non survivors with Covid-19was diffuse alveolar damage, which was accompanied by extensive microvascular thrombosis in lungs and extra-pulmonary organs **(Zhang, Sun et al. 2020)**. Megakaryocytes have been

identified within the small vessels and alveolar capillaries identified by CD61 and vWF immunostains **(COVID 2020).**

The rate of venous thromboembolism (VTE) in critically ill hospitalized patients is 10% **(Cade 1982).** However, early studies show that the incidence of VTE in patients with severe novel coronavirus pneumonia can be as high as 25% **(Cui, Chen et al. 2020).** Early case reports have described an association between Covid-19and venous thromboembolic events **(Danzi, Loffi et al. 2020).**

The founded treatment with prophylactic heparin (lower molecular weight heparin or unfractionated heparin) was associated with a reduced 28-day mortality in severe Covid-19 **(Pavoni, Gianesello et al. 2020).**

Recently published a multicenter prospective in France, assessing thrombotic risk in Covid-19 patients, which showed that factor VIIIc was considerably increased in Covid-19 patients **(Tang, Bai et al. 2020).** Interestingly, examined those admitted to ICU and ventilated, there was no statistically significant difference in vWF and factor VIIIc levels between ventilated versus non-ventilated patients. The current results extend these high vWF levels to those who are receiving general and high dependency care **(Ladikou, Sivaloganathan et al. 2020).** Also reported that Covid-19 is characterized by hyper-coagulability with a severe inflammatory state. Their results confirmed higher mean levels of vWF and factor VIIIc, 529 U/dl and 297 U/dl respectively **(Panigada, Bottino et al. 2020).**

It remains unclear whether this observed coagulopathy is the case for other infections, such as sepsis or viral pneumonias, as well. High levels of D-Dimers have been previously associated with 28-daymortality in patients presenting to the emergency department with sepsis who investigated vWF and related parameters in severe sepsis and septic shock **(Ladikou, Sivaloganathan et al. 2020)**. Their results showed that the median vWF antigen was significantly higher in patients compared to controls ($P<0.001$); however, this did not

correlate with disease severity, organ dysfunction or outcome **(Kremer Hovinga, Zeerleder et al. 2007).**

Von willebrand factor has three main functions: binding to collagen in the wounded sub-endothelial matrix, binding to glycoprotein-1bonplatelets, and carrying then subsequently delivering coagulation factor VIII (FVIII) to the surface of activated platelets bound to wounded endothelium **(Sadler 2005).**

Whether the increased vWF reported in Covid-19 is a result of increased production, abnormal and/or increased release, or decreased destruction is unclear. Since ADAMTS13 ,a vWF-cleaving protease, plays a key role in regulating both vWF quantity and multimer size, analysis of this enzyme would be important in elucidating the pathophysiology of Covid-19coagulopathy **(Escher, Breakey et al. 2020, Martinelli, Montagnana et al. 2020).**

In addition, ADAMTS-13 activity is not expected to significantly decrease in acute inflammation, the majority of Covid-19patients had decreased ADAMTS-13activity, indicating a profound endothelial dys-regulation or an intrinsic ADAMTS-13 activity deficiency. Possible mechanisms of ADAMTS-13 activity deficiency include decreased production, inhibition, or consumption of ADAMTS-13. About 12% of patients in prior had ADAMTS-13 activity levels less than 30% but none had detectable anti-ADAMTS-13 antibodies **(Sweeney, Barouqa et al. 2021).**

Indeed, elevated vWF antigen and activity levels, D-dimer levels, FM levels along with moderately reduced ADAMTS-13 activity levels is a repertoire of hallmarks shared by critical illnesses that result in severe microvascular endothelial cell injuries **(Schwameis, Schörgenhofer et al. 2015, Smeets, Fijnheer et al. 2018).**

Thrombocytopenia is not common in Covid-19and was not directly associated with low ADAMTS-13 activity levels. Also, lack of severe ADAMTS-13 activity deficiency (only two patients had ADAMTS-13 activity <10%) and lack of anti-ADAMTS-13 antibodies in patients excludes thrombotic thrombocytopenic purpura (TTP). Prior studies found a high D-dimer levels

with LDH activity and the occasional finding of fibrin strands in peripheral blood suggests that high D-dimer levels may be a direct product of small vessel thrombosis (arterial and venous), which have been documented in Covid-19autopsies **(Ackermann, Verleden et al. 2020).** Microvascular thrombosis leads to ischemic end-organ damage, most commonly affecting kidneys, but other organs can also be affected. Covid-19primarily manifests as respiratory failure, however, renal and cardiovascular complications are common in Covid-19,high D-dimer levels, coagulation factor consumption, platelet consumption, and anemia along with multiple organ damage are hallmarks of DIC **(Schutte, Thijs et al. 2016).**

ADAMTS-13 treatment ameliorates inflammatory responses, demyelination and disease course. Therefore, our study suggests that ADAMTS-13 may represent a potential therapeutic strategy for MS patients **(Lu, Liu et al. 2020).** Serum level of vWF is an interesting target for primary or secondary prevention of cardiovascular diseases particularly in high-risk patients **(Firbas, Siller-Matula et al. 2010).** Numerous epidemiologic studies have established that an excess of vWF predicts an increased risk for both stroke and stroke mortality **(Catto, Carter et al. 1997).** Conversely, deficiency of vWF may protect against cardiovascular disease or stroke **(Seaman, Yabes et al. 2015).** The von Willebrand disease patients with average vWF levels of 24% had a 35% to 67% reduced risk for ischemic stroke when compared to controls, suggesting that partial inhibition of vWF could be protective in the stroke-prone population **(Sanders, Eikenboom et al. 2013).** The underlying patho-physiologic mechanism of large artery atherosclerosis stroke is likely shear and vWF dependent platelet thrombus formation in the setting of atherosclerotic stenosis **(Denorme and De Meyer 2016).** Importantly, vWF and platelet rich thrombi are more resistant to thrombolysis and are associated with poorer outcome after revascularization in stroke patients **(Douglas, Fitzgerald et al. 2020).** At one end extracellular vWF binds to exposed collagen, that found in ruptured plaques, via its A3 domain, and at the other end vWF uses its A1 domain to

bind to platelet, thus serving as a bridge between collagen and platelets **(Gardiner and Andrews 2014).** Therefore, vWF is a key player in mouse models of acute stroke **(De Meyer, Schwarz *et al.* 2010).**

Notably, the effects of P2Y12-receptor and GPIIb/IIIa inhibitors or aspirin, particularly when measured under pathophysiological relevant high shear rates, are compromised in conditions where vWF levels are increased **(Spiel, Derhaschnig et al. 2012).**

Von willebrand factor release during endotoxemia partly antagonized the inhibitory effect of prasugrel. Similar results were obtained for aspirin or nitric oxide coupled aspirin **(Derhaschnig, Schweeger-Exeli et al. 2010).** Furthermore, the desmopressin induced vWF release accelerated the normalization of the prolonged CADP-CT by GPIIb/IIIa inhibitors (plus L-aspirin. This means that the efficacy of GPIIb/IIIa inhibitors, similar to aspirin or prasugrel, will be limited in cases of increased circulating vWF. Similar to previous observations, the apparent platelet inhibition by aspirin was mitigated in the presence of physiological calcium concentrations **(Kovacevic, Buchtele et al. 2020).**

Finally, the obtained results concluded that a new associative data supporting that endotheliopathy and dys-regulated immune responses are involved in respiratory through microvascular damage in patients with severe Covid-19.The marker of endothelial damage such as vWF and level of ADAMTS-13 increased in Covid-19 hospitalized patients. These markers were even higher levels of vWF and lower level of ADAMTS-13 in intensive care unit patients and correlated with severity and mortality. In patients with severe Covid-19 the degree of endotheliopathy at intensive care unit admission is associated with different organ dysfunctions even after adjustment for other risk factors, age, sex, and body mass index further underlining the direct involvement of endotheliopathy in organ failure. The vWF released from damaged endothelial cells interacts with neutrophil extracellular traps released from neutrophils and

could play a major role as a scaffold for thrombus formation in Covid-19 pathophysiology.

3.2.4. Comparison of Vitamin K Levels between Covid-19 Patients and the Apparently Control Grops.

As revealed in table (3-9) a significant decrease in the level of vitamin K in Covid-19 patients group compared with the control group.

Table (3-9): Comparison of Vitamin K level between Covid-19 patients group and healthy group

Parameters	Covid-19 patient group Mean ± SD N = 60	Healthy group Mean ± SD N = 30	P-value
Vit.K, (pg/ml)	614.32 ± 106.76	1198.9 ± 151.59	0.001

Data represented as Mean ± SD: standard deviation

In Table (3-10) Categories of Covid-19patients found low levels in non-survived and severe cases compared with mild case.

Table (3-10): Comparison of serum vitamin K level in various Covid-19 patients groups

Parameters	Covid-19 patients groups			P-value
	Deceased N = 12	Severe N -= 15	Mild N = 33	
Vit.K, (pg/ml)	**608.46 ± 103.32**	**611.40 ± 118.13**	**623.84 ± 112.79**	**a=0.4 b= 0.5 c= 0.4**

Data represented as Mean ± SD: standard deviation ; a= p-value (Deceased + Severe) ; b = p-value (Deceased + Mild) ; c= p-value (Severe +Mild).

Prevalent coagulopathy and thromboembolism are observed in severe Covid-19patients with 40% of Covid-19mortality being associated with cardiovascular complications. Abnormal coagulation parameters are related to poor prognosis in Covid-19patients.Victims also displayed presence of extensive thrombosis in infected lungs. Vitamin K is well-known to play an essential role in the coagulation system. The present study revealed an existing association between vitamin K deficiency and Covid-19severity, highlighting a role of vitamin K. The potential mechanisms linking Covid-19with coagulopathy in which vitamin K may exert its modulating role in coagulation related with disease pathogenesis and explore the potential benefits of using vitamin K against Covid-19 to improve disease outcome. One of the predominant theories favors the concept of a "cytokine storm" in which the immune response is exacerbated through the induction of an excessive pro-inflammatory cytokine response driving lung injury **(Coperchini, Chiovato et al. 2020).** It was reported that presence of a high viral load causes massive destruction of lung tissues, in turn leading to hyperinflammation causing acute respiratory distress syndrome (ARDS) **(Song, Li et al. 2020).** In addition to respiratory symptoms, a growing body of evidence also shows that the virus can specifically infects endothelial cells affecting thus the normal process of coagulation **(Connors and Levy 2020).**

Severe Covid-19 patients were found to possess coagulopathy characterized by abnormal coagulation parameters **(Connors and Levy 2020)**, widespread presence of blood clots **(Panigada, Bottino et al. 2020),** as well as arterial and venous thromboembolism **(Lodigiani, Iapichino et al. 2020) (Middeldorp, Coppens et al. 2020).**

bb Furthermore, preliminary data from several studies seem to indicate that anticoagulant therapy is associated with lower mortality in Covid-19bpatientsb **(Tang, Bai et al. 2020).**Vitamin K is an essential component preventing blood clotting and a major player of the coagulation system of which a link between

vitamin K deficiency and the worst Covid-19outcomes was recently revealed **(Dofferhoff, Piscaer et al. 2020).**

Covid-19 respiratory symptoms are heterogeneous and may sometimes lead to serious complications. Similar to other severe respiratory diseases, severe forms of Covid-19 induce pneumonia, acute lung injury (ALI), ARDS and sepsis leading to multiple organ failure and death **(Zaim, Chong et al. 2020).** Studies have shown that the respiratory symptoms can worsen with development of ARDS occurring as fast as 9 days post onset **(Huang, Wang et al. 2020).** Damage to the lungs characterized by a pulmonary ground glass opacification was observed by computed tomography (CT) scan in even asymptomatic cases indicating that the plethora of complications arising from Covid-19is still far from being fully understood **(Guan, Ni et al. 2020).**

Cytokine storm is considered to be one of the major causes of ARDS and multipleorgan failure **(Chousterman, Swirski et al. 2017)**, and plays a crucial role in the process of disease aggravation (Shimabukuro-Vornhagen, Gödel et al. 2018). The cytokine storm is the result of an exacerbated immune response resulting in the excessive production of pro-inflammatory cytokines. Whilst it is revealed that Covid-19 infection could alter both the innate and adaptive immunity **(Giamarellos-Bourboulis, Netea et al. 2020, Wang, Nie et al. 2020)**, respiratory epithelial cellsand myeloid cells are thought to play an important role in orchestrating innate immunity in the airway **(Yoshikawa, Hill et al. 2009).** Infiltration of a large number of inflammatory immune cells is observed in the lungs from severe Covid-19patients **(Xu, Shi et al. 2020),** with majority being macrophages and neutrophils **(Barnes, Adrover et al. 2020)**. Such increase in infiltration and accumulation of immune cells (macrophages, neutrophils) enhance the probability of rupture of atherosclerotic plaques potentially leading to cardiovascular complications. Lung infiltration of macrophages has been reported in Covid-19 infection **(Tian, Hu et al. 2020).** Pro-inflammatory cytokines such as IL-6 **(Yan, Yang *et al.* 2020)**. Interleukin-1 **(Conti, Ronconi et al. 2020)** and TNF **(Li, Xu et al. 2020)**, are thought to be

produced by macrophages, reported to be hyper-induced during Covid-19 infection and are found to be positively correlated with disease severity relating to cytokine storms **(Wang, Jiang *et al.* 2020).**

Patients with severe Covid-19present with abnormal coagulation parameters which are associated with poor disease prognosis. Likewise, Covid-19patients present with higher than normal levels of fibrinogen **(Arachchillage and Laffan 2020),** resulting from a high level of IL-6 in the serum. Interleukin-6 is known to stimulate the production of fibrinogen by hepatocytes **(Schmidt-Arras and Rose-John 2016).**

In addition, plasma levels of the procoagulation protein, von Willebrand factor is also increased in Covid-19 patients **(Panigada, Bottino et al. 2020).** Levels of D-dimer and fibrin degradation product, which can reflect the occurrence of thrombosis and is associated with a diagnosis of disseminated intravascular coagulation (DIC), are found to be significantly enhanced in severe Covid-19cases **(Arachchillage and Laffan 2020).** Although, the prevalence of DIC in Covid-19 is still in debate **(Connors and Levy 2020),** pulmonary microthrombi formation is clearly observed in Covid-19 **(Atallah, Mallah et al. 2020)**. Pulmonary embolism, strokes and heart attacks can be a direct consequence of thrombosis. Indeed, pulmonary embolism is observed in 50% of Covid-19 patients admitted to ICU, adequate oxygenation and ventilation are recommended for Covid-19 patients with ARDS **(Liu, Liu *et al.* 2020).**

Studies demonstrate that Covid-19 can infect endothelial cells, cells which represent one third of the total cells in lungs **(Zeng, Pappas et al. 2012)**, and hence can contribute directly to thrombosis via endothelial cell lysis. Damage to the endothelial wall exposes the subendothelial collagen that is involved in platelet adhesion, activation and ultimately coagulation **(Farndale, Sixma et al. 2004).** Secretion of factors involved in coagulation by the endothelial cells is also altered **(Frantzeskaki, Armaganidis et al. 2017).**The idea of using anticoagulant therapy in Covid-19patients to lower the mortality is well

established **(Tang, Bai et al. 2020)**. In fact, the coagulation process is a balance between procoagulation and anticoagulation factors that require a strict control. Dys-regulation towards either ends could lead to thrombophilia or coagulopathy. Protein C and protein S are among the key players in this process **(Esmon, Vigano-D'Angelo et al. 1987)**. Interestingly, a low protein C activity is found in severe and aged Covid-19 patients favoring a hypercoagulability state **(Tabatabai, Rabin et al. 2020).** Under normal conditions, coagulation system is balanced towards the anticoagulation state **(Dahlbäck 2000)**.Vitamin K is an essential "switch" in balancing coagulation and anticoagulation process **(Espana, Medina et al. 2005)**. Indeed, vitamin K acts as a cofactor in the activation of extra-hepatic and hepatic vitamin K-dependent proteins (VKDPs) including pro-thrombin and clotting factors VII, IX, X, major factors involved in blood coagulation. On the other hand, vitamin K can also trigger key anticoagulants via VKDPs for producing proteins C, S and Z **(Danziger 2008)**. Beyond its essential role in coagulation, vitamin K is suggested to possess immune-modulatory functions as well as preventing vascular calcification. Studies have shown that K2 form has more potent anti-inflammatory effect when compared to K1 **(Reddi, Henderson et al. 1995)**. K2 acts as an immunosuppressive compound to modulate expression of a multitude of pro-inflammatory cytokines such as TNF, IL-1, IL-1 and suppresses IL-6 release **(Ohsaki, Shirakawa et al. 2010, Pan, Maresz et al. 2016).** It can also impair T cell activation and proliferation. Besides, vitamin K has been shown to activate extra-hepatic VKDPs such as matrix -Gla protein(MGP) **(Willems, Vermeer et al. 2014)**. MGP is remodeling responsible for preventing vascular calcification(Schurgers, Uitto et al. 2013).And thus plays a fundamental role in vascular health **(Munroe, Olgunturk et al. 1999)**. It has been suggested that vitamin K dependent MGP plays an important role in elastin degradation in the lungs phenomenon that is accelerated in pulmonary disease **(Piscaer, Wouters et al. 2017)**. Furthermore, vascular calcification is often observed in chronic kidney disease patients **(Kiechl, Werner et al. 2006)**, patients who have been

reported to be more prone to develop severe form of Covid-19highlighting the importance of vitamin K and MGP. Impaired coagulation function has been demonstrated in Covid-19patients **(Han, Yang et al. 2020)**. Findings from several recent studies have further suggested that anticoagulant therapy is beneficial and can lower the mortality in Covid-19patients **(Kollias, Kyriakoulis et al. 2020)**. Furthermore, patients with pre-conditions such as diabetes, hypertension and cardiovascular disease which are known to be associated with vitamin K deficiency **(Campbell 2017)** are prompt to develop a more severe Covid-19disease (Zhou, Yu et al. 2020). This is particularly evident in patients suffering from chronic kidney disease (CKD), a population characterized by enhanced number of severe Covid-19 cases.

Recently researchers observed the link between individuals with vitamin K status and Covid-19outcomes. Coagulopathy is one of the primary features of poor outcomes in patients who develop sepsis from an infection. Similarly, coagulopathy has been observed in severe Covid-19patients and is associated with poor prognosis, as observed by Tang *et al* in 183 consecutive patients **(Tang, Bai et al. 2020).** Also, low vitamin K level appears to be associated with increased elastin degradation **(Piscaer, van den Ouweland et al. 2019),** preferably degrading the lung tissue, resulting in breathing difficulty in Covid-19patients. Since Covid-19patients with severe disease are associated with co-morbidities such as cardiovascular diseases, type II diabetes, or hypertension, which are linked to reduced vitamin K levels, it is hypothesized that low vitamin K levels might be associated with severity in Covid-19 **(Yang, Yu *et al.* 2020).** Furthermore, a study conducted by Dofferhoff *et al* measuring the level of desphospho-uncarboxylated matrix Gla protein (DP-ucMGP, inversely related to vitamin K status) and comparing between 123 Covid-19 patients and 184 controls concluded that reduction of vitamin K levels in Covid-19patients than the controls and is related to poor prognosis **(Dofferhoff, Piscaer et al. 2020).**

The CKD population serves as a valuable indicator when addressing potential consequences of poor vitamin K status, a status that represents an aggravating risk factor in Covid-19 **(Dofferhoff, Piscaer et al. 2020).** A serious hypercoagulable state has been observed in many severe Covid-19cases and associated with poor prognostic outcome **(Guan, Ni et al. 2020).** Contrarily to severe IAV cases, multiple blood clots are observed in the lungs at the site of Covid-19 infection **(Ackermann, Verleden et al. 2020).** As mentioned earlier, Covid-19can directly infect endothelial cells which are known to express significant amount of receptor ACE2 **(Varga, Flammer et al. 2020)**. Endothelial cells play a direct role in coagulation. Indeed, they secrete coagulation inhibitors like protein S as well as provide receptors for anticoagulant proteins present in the blood that interfere with clot formation(like protein C) **(Esmon, Vigano-D'Angelo et al. 1987).**

A low prophylactic dose of low molecular heparin (LMWH), an anticoagulant, was suggested to be given to all Covid-19patients requiring hospitalization as long as no contraindications such as active bleeding was recorded **(Thachil, Tang et al. 2020)**. While studies have shown beneficial effect of LMWH on Covid-19patients in terms of reduce mortality **(Tang, Bai et al. 2020)**, in clinical practice severely infected patients still continue to clot and fail to response adequately to both prophylactic and therapeutic doses **(Thachil 2020)**.This might be resulting from the fact that Covid-19patients present with low levels of anti-thrombin and higher levels of fibrinogen, which contribute to heparin resistance **(Barrett, Moore et al. 2020).**

Indeed, hyperfibrinogenemia was clearly demonstrated in patients with severe Covid-19and was shown to reduce significantly LMWH efficacy to reduce clot formation (Harr, Moore et al. 2014). Furthermore, due to the risk of venous thromboembolism, pulmonary embolism and renal insufficiency resulting from Covid-19the use of unfractioned heparin (UFH) might be a better choice of anticoagulant **(Turshudzhyan 2020)**. Indeed, patients who present with pulmonary embolism and receive LMWH are at an increased risk of

bleeding that cannot be stopped further supporting the use of UFH. Direct oral anticoagulant (DOAC) drugs are currently broadly administered as anticoagulant treatments. This novel class of anticoagulant act directly on selective blood clotting factors to prevent formation of blood clots. However, their use in Covid-19 patients remain controversial. Indeed, up to now there is very limited clinical data on safety or efficacy of DOAC in Covid-19patients **(Schutgens 2021)**.

The need to switch the patient receiving vitamin K antagonist (VKA) to direct oral anticoagulants (DOAC) for coagulation therapy to reduce laboratory testing frequency monitoring during this pandemic. Nevertheless, it is not applicable for patients with mechanical heart valves or anti-phospholipid syndrome **(Thachil, Tang et al. 2020)**. Since there is a risk of vitamin K deficiency in patients admitted to ICU, administering vitamin K supplements to patients during admission to ICU might help reduce the risk of vitamin K deficiency and further complications **(Crowther, McDonald et al. 2002).**

Meanwhile, ample evidence suggests a direct impact on the cytochrome P450 pathway which is observed in both antiviral treatment (remdesivir, dexamethasone), as well as Covid-19 disease. DOACs are also known to alter the same P_{450} pathway **(Driggin, Madhavan et al. 2020).** Thus, combined antiviral and anticoagulant treatment using DOAC might cause drug-drug interactions resulting in potential decrease or increase in anticoagulation activity. A recent study in Italy on Covid-19patients where DOAC treatment was simultaneously administered with antiviral drugs showed that all patients presented with alarming increase of DOAC at plasma levels **(Testa, Prandoni et al. 2020)**. Approximately 40% of deaths from Covid-19 infection are related to cardiovascular complications **(Akhmerov and Marbán 2020)**. Interestingly, through activation of MGP, vitamin K can prevent development of arterial calcification **(Dalmeijer, Van der Schouw et al. 2013)**, a process known to cause cardiovascular disease **(Spronk, Soute et al. 2003)**, as well as maintain arterial elasticity **(Braam, Hoeks et al. 2004)**. Vitamin K dependent MGP

protects elastic fibers against mineralization, fibers which are fundamental parts of the extracellular matrix (ECM) and play a crucial role in lung fibrosis **(Enomoto, Suda et al. 2013).** Low vitamin K status is found to be associated with increased elastin degradation in pulmonary disease **(Piscaer, van den Ouweland et al. 2019)**.

A possible link to low levels of vitamin K and severe cases of Covid-19was lately reported **(Klok, Kruip et al. 2020).** The diverse and distinct roles of vitamin K in modulating blood clotting, elastin degradation, immunomodulation, and managing vascular health, together with the low toxicity of vitamin K in humans makes vitamin K an attractive remedy using prophylactically as supplement or therapeutically to improve Covid-19disease outcomes **(Kudelko, Yip et al. 2021).**

3.3. Correlations Analysis

3.3.1. The Correlations between Serum vWF and ADAMTS-13 Levels with other Clinical Parameters Studied in Covid-19 Patient Group

In table (3-11) illustrated data analysis was used to verify the relationship of the biochemical with the levels of vWF and ADAMTS-13 in Covid-19 patients group. The correlation of vWF level shown a significant positive correlation with age, BMI, TG, D-dimer, NEUT%, VLDL-C, ferritin levels. However, significant negative correlations of TC, and ADAMTS-13, LDL-C,PLT,LYM%, vit. K levels in Covid-19 patients group.

Table (3-11): The Correlations between serum vWF level and clinical parameters in Covid-19 patients group.

Parameters	r	P-value
Age, (years)	0.379	0.05
BMI, (kg/m²)	0.386	0.04
SBP (mmHg)	0.151	0.427
DBP (mmHg)	0.112	0.556
TG, (mg/dl)	0.389	0.037
TC, (mg/dl)	-0.334	0.067
LDL-C, (mg/dl)	-0.367	0.046
VLDL-C, (mg/dl)	0.282	0.108
HDL-C, (mg/dl)	-0.304	0.585
CRI-I, (TC/HDL-C)	0.221	0.615
CRI-II, (LDL-C/HDL-C)	0.190	0.717
AIP, (log TG/HDL)	0.102	0.833
PLT%	-0.193	0.736
NEUT%	0.025	0.982
LYM%	-0.369	0.05
NLR	0.362	0.046
D-dimer, ng/ml	0.473	0.001
Ferretin, ng/ml	0.466	0.001
ADAMTS-13, ng/ml	-0.575	0.000

Vit. K, mg/ml	-0.367	0.041

Data represented as r: Correlations ; BMI: Body mass index ; SBP: Systolic blood pressure ; DBP: Diastolic blood pressure ; TG: triglyceride ; HDL-C: High-density lipoprotein cholesterol ; LDL-C: low density lipoprotein-cholesterol ; VLDL.C: Very Low-Density Lipoprotein- Cholesterol ; TC: total cholesterol ; CRI-I: (TC/HDL-C) Castelli Risk Index ; CRI-II: (LDL-C/HDL-C) coronary risk index ; AIP :(log TG/HDL-C) atherogenic index, LYM: lymphocyte ; NEUT: neutrophil ; NLR: neutrophil/lymphocyte ratio ; PLT: Platelet ; r= Pearson correlation coefficient.

As, shown in table (3-12) level of ADAMTS-13 significant positive correlation with lym%,Vit K, HDL-C, TC levels. While, significant negative correlations with age, BMI, TG, atherogenic index, NLR, D-dimer, ferritin, vWF, levels in Covid-19 patients group.

Table (3-12): Correlations between serum ADAMTS-13 level and other clinical parameters in Covid-19 patients group.

Parameters	r	P-value
Age, (years)	-0.391	0. 2
BMI, (kg/m²)	-0.486	0.043
SBP, (mmHg)	0.266	0.340
DBP, (mmHg)	0.217	0.405
TG, (mg/dl)	-0.406	0.007
TC, (mg/dl)	0.374	0.046
LDL-C, (mg/Dl)	-0.378	0.040
VLDL-C, (mg/dl)	-0.305	0.138
HDL-C, (mg/dl)	0.347	0.061
CRI-I, (TC/HDL-C)	-0.323	0.065
CRI-II, (LDL-C/HDL-C)	-0.267	0.714

AIP, (log TG/HDL-C)	-0.200	0.807
PLT%	0.298	0.063
NEUT%	-0.325	0.082
LYM%	0.385	0.042
NLR	-0.372	0.044
D-dimer, ng/ml	-0.546	0.000
Ferritin, ng/ml	-0.452	0.001
vWF, ng/ml	-0.575	0.000
Vit. K, mg/ml	0.397	0.001

Data represented as r : Correlations ; BMI: Body mass index ; SBP: Systolic blood pressure ; DBP: Diastolic blood pressure ; TG: triglyceride ; HDL-C: High-density lipoprotein cholesterol ; LDL-C: low density lipoprotein-cholesterol ; VLDL.C: Very Low-Density Lipoprotein- Cholesterol ; TC: total cholesterol ; CRI-I: (TC/HDL-C) Castelli Risk Index ; CRI-II: (LDL-C/HDL-C) coronary risk index ; AIP :(log TG/HDL-C) atherogenic index ; LYM: lymphocyte ; NEUT: neutrophil ; NLR: neutrophil/lymphocyte ratio ; PLT : Platelet ; r= Pearson correlation coefficient.

Covid-19-associated coagulopathy has been broadly discussed in the literature. This coagulopathy overlaps with disseminated intravascular coagulopathy (DIC) in critically ill patients with circulatory collapse and multi-organ failure. However, they usually do not meet the ISTH criteria for DIC **(Wada, Thachil et al. 2013, Thachil, Tang et al. 2020).** There is an association between inflammation and coagulation. A noteworthy cytokine release, mainly IL-6, induces tissue factor overexposure in endothelial cells and monocytes, triggering thrombin generation. This cytokine storm correlates with poor outcome **(Hu, Huang *et al.* 2021).** In patients with Covid-19, as confirmed this study, there is a peculiar coagulopathy derived from the systemic inflammatory response associated with endothelial dysfunction **(Katneni, Alexaki et al. 2020).**

The higher level of D-dimer in Covid-19 patients than in Covid-19 outpatients. This was significantly higher in patients requiring intensive care than in ward patients. Consequently, D-dimer predicts poor outcome as the highest levels are present in critically ill patients **(Tang, Li et al. 2020)**. Also like to remark that D-dimer was significantly increased in Covid-19 inpatients than in non- Covid-19inpatients. Although D-dimer increases in hospitalized patients, it could be a hemostatic parameter of clinical worsening in Covid-19, implying hospitalization **(Wu, Cai et al. 2020).** Levels of vWF and ADAMTS-13 are expressed by endothelial cells. Dysregulation of the vascular endothelium in acute hyperinflammation setting induces excess vWF released by endothelial cells. ADAMTS13 activity decreases in proportion to the inflammatory response **(Katneni, Alexaki et al. 2020).** According to this contrast between vWF and ADAMTS-13 activity could be a consequence of inhibition and/or deficiency of ADAMTS-13 activity **(Marco and Marco 2021).**

The interaction between vWF and ADAMT-13 in Covid-19 despite playing an important role in the maintenance of hemostasis and prevention of undesirable thrombosis, vWF-ADAMTS-13 interactions haven't received much attention in the evaluation of Covid-19 pathophysiology, specifically VTE. Importantly, reduced ADAMTS-13 activity has been shown to correlate with increased inflammation in multiple systems **(Liu, Zhao et al. 2017, Takaya, Kawaratani et al. 2018).**

The development of coagulopathy is associated with poor prognosis. A procoagulant status in patients with pneumonia has been reported **(Connors and Levy 2020, Ranucci, Ballotta et al. 2020)**. An increase in D-dimer level is one of the most common hematological abnormalities found in hospitalized patients. Although older patients with comorbidities are known to have higher D-dimer levels, this abnormality in the context of Covid-19promotes mortality due to Covid-19-derived complications **(Ranucci, Ballotta et al. 2020).** Tang

et al. described elevated D-dimer levels in non-survivors vs. survivors in Covid-19 **(Tang, Li et al. 2020)**. As a consequence, patients with markedly increased D-dimer levels should be strictly followed-up and consider hospitalization despite not having severe symptoms. Fibrinogen levels remain elevated in the context of acute infection and predict mortality **(Zhang, Cao *et al.* 2020).** Thachil *et al.*, instead of the prothrombotic role, a protective function for fibrinogen as an acute-phase reagent, the fibrinogen level is increased in patients with Covid-19to protect the host **(Thachil 2020)**. Covid-19represents a severe inflammatory disease, and pro-inflammatory markers are released. Endothelial injury associated with a cytokine storm contributes to the thrombo-inflammatory process **(White, MacDonald et al. 2021).** The vWF is a large multimeric glycoprotein stored in endothelial cells and platelets and released in response to different stimuli, such as acute inflammatory processes **(Gragnano, Sperlongano et al. 2017).** ADAMTS-13 is a metalloprotease expressed by hepatic and endothelial cells and is implicated in the cleavage of prothrombotic ultra-large vWF multimers, contributing to the maintenance of hemostatic balance **(Katneni, Alexaki et al. 2020)**. In this setting, acquired non-immune low ADAMTS-13 levels could explain this thrombotic tendency. These findings could be related to ADAMTS-13 consumption because of excess release of vWF, finally leading to thrombosis development. These clinical and laboratory data are compatible with thrombotic microangiopathy **(Adam, Zacharowski et al. 2020, Huisman, Beun et al. 2020).**

Level of ADAMTS-13 was inversely correlated with D-dimer, and vWF, all of which were statistically significant. In addition, vWF presented a significant direct correlation with D-dimer. Of note, the strongest correlations were found between vWF and D-dimer and between, which are normally increased in acute inflammatory processes **(Marco and Marco 2021).** In addition, the inverse correlation between ADAMTS-13 and vWF enhances the microangiopathy state. Moreover, although no statistical differences between groups were found when evaluating platelet count, found a direct correlation between ADAMTS-

13 activity and platelets and an inverse correlation between vWF levels and platelets. Mancini *et al.* has recently published that an alteration in the vWF-ADAMTS-13 axis in Covid-19 patients is related to disease severity. They designed a cross-sectional study including 50 critical admitted to 3 units of different intensity care and recruited 274 healthy volunteers tested for thrombophilia as control group **(Mancini, Baronciani et al. 2021).**

3.3.2. The Correlations between Serum Vitamin K Level and Clinical Parameters Studied in Covid-19 Patients Group.

The linear regression analysis in table (3-13) illustrated a significant positive correlation of clinical parameters studied level HDL-C, TC, PLT%, ADAMTS-13 with vitamin K in Covid-19 patients group. While a significant negative correlation of NEUT%,VLDL-C,LDL-C,TG, D-dimer, NLR, vWF, ferritinlevel with Covid-19 patient.

Table (3-13): The correlations between vitamin K level and clinical parameters in Covid-19 patients group

Parameters	R	p-value
Age, (years)	-0.341	0.064
BMI, (kg/m²)	-0.372	0.050
SBP, (mmHg)	0.283	0.280
DBP, (mmHg)	0.209	0.245
TG, (mg/dl)	-0.356	0.061
TC, (mg/dl)	0.382	0.044
LDL-C, (mg/dl)	-0.339	0.060
VLDL-C, (mg/dl)	-0.245	0.218

HDL-C, (mg/dl)	0.233	0.074
CRI-I, (TC/HDL-C)	-0.369	0.050
CRI-II, (LDL-C/HDL-C)	-0.377	0.050
AIP, (log TG/HDL-C)	-0.356	0.062
PLT%	0.323	0.071
NEUT%	-0.325	0.071
LYM%	0.376	0.040
NLR	-0.370	0.041
D-dimer, ng/ml	-0.487	0.001
Ferritin, ng/ml	-0.467	0.001
vWF, ng/ml	-0.367	0.041
ADAMTS-13, ng/ml	0.397	0.001

Data represented as r : Correlations ; BMI: Body mass index ; SBP: Systolic blood pressure ; DBP: Diastolic blood pressure ; TG: triglyceride ; HDL-C: High-density lipoprotein cholesterol ; LDL-C: low density lipoprotein-cholesterol ; VLDL.C: Very Low-Density Lipoprotein-Cholesterol ; TC: total cholesterol ; CRI-I: (TC/HDL-C) Castelli Risk Index ; CRI-II: (LDL-C/HDL-C) coronary risk index ; AIP: (log TG/HDL-C) atherogenic index ; LYM: lymphocyte ; NEUT: neutrophil ; NLR: neutrophil/ lymphocyte ratio ; PLT: Platelet and r= Pearson correlation coefficient.

Coronavirus disease or Covid-19 is a heterogeneous condition caused by severe acute respiratory syndrome-coronavirus 2 (SARS-COV-2) infection. Clinically, it is generally characterized by an interstitial pneumonia that can lead to impaired gas-exchange, acute respiratory failure and death **(Schenck, Hoffman et al. 2020)**. The pathogenesis is complex and a variable combination of hyper-inflammatory responses and alterations in the coagulation asset have been described in critically hill patients **(Luthra-Guptasarma and Guptasarma , Randazzo, Cuevas-Ferrando et al. 2020).** Understanding the immune-inflammatory mechanisms related to the clinical manifestations of Covid-19can guide the identification of potential pharmacological targets.

Deficient immune response, proposed as an immunologic mechanism prompting to severe Covid-19clinical expression **(Letzel, Pozas et al. 2020) (Khalid, Al-ebini et al. 2021) (Kee, Metz-Zumaran et al. 2021).** Decreased ADAMTS-13 levels were previously identified in the plasma from patients with many conditions, such as systemic inflammation **(Reiter, Varadi et al. 2005, Ono, Mimuro et al. 2006)** , stroke **(Vergouwen, Bakhtiari et al. 2009)** and metabolic syndrome **(Ziliotto, Bernardi et al. 2018)**.Thrombus formation involves coagulation proteins and platelets. The latter, referred to as platelet-mediated thrombogenesis, is predominant in arterial circulation. Platelet thrombogenesis follows vascular injury when extracellular vWF binds via its A3 domain to exposed collagen, and the free vWF A1 domain binds to platelet glycoprotein Ib (GPIb) **(Zhu, Gilbert *et al.* 2020).** The systemic release of pro-inflammatory mediators during the severe phase of Covid-19 inhibit the cleavage of high molecular weight vWF or interfere with the proteolytic interaction with ADAMTS-13, leading to thrombosis **(Katneni, Alexaki et al. 2020).** The D-dimer levels of Covid-19 patients were also elevated. The reasons responsible for the elevated D-dimer levels are only partially explained. It is well known that D-dimer are produced during fibrin breakdown and serve as a marker of fibrinolytic activity. A relationship between pro-inflammatory cytokines and markers of activation of the coagulation cascade, including D-dimer, has been demonstrated in critical patients or patients with sepsis **(Borjini, Fernández et al. 2016).** There is also evidence that under inflammatory conditions, the alveolar haemostatic balance is shifted towards a predominance of prothrombotic activity **(Cheng, Sun et al. 2017).** In addition, pro-inflammatory cytokines may be involved in endothelial injury, and may activate coagulation and inhibit fibrinolysis in patients with severe sepsis **(Wu, Liu *et al.* 2018).** The spike protein of Covid-19binds the angiotensin-converting enzyme 2 receptor on endothelial cells **(Lu , Ferrario, Jessup *et al.* 2005),** resulting in endothelial cell apoptosis and thrombosis **(Boor and Hartmann 2020).** Additionally, endothelial cell apoptosis causes inflammatory cell

infiltration and further increases in the risk of thrombosis **(Connors and Levy 2020)**. Accordingly, the meta-analysis indicated that the ferritin levels in Covid-19patients with thrombotic complications were higher than those in patients without, suggesting the hyper-inflammation state in patients with thrombosis. Several studies indicated that high serum ferritin levels are associated with hypertension **(Choi, Yeum et al. 2015, Jamshidi-Naeini, Bavil et al. 2019).**

Thromboembolism is also prevalent in coronavirus disease 2019 (Covid-19). Vitamin K plays an important role in coagulation and possibly also in lung diseases **(Dofferhoff, Piscaer et al. 2020).**

Chapter Four

Conclusion and Recommendation

4. Conclusions and Recommendations

4.1. Conclusions

1. The clinical features of Covid-19 patients who progressed from non-severe to severe cases. Mild patients with an older age and underlying diseases were more likely to exacerbate. Elevated vWF and reduced ADAMTS-13 levels could be valuable markers to predict the possibility of aggravation of non-severe Covid-19 patients, which could help health care workers identify those patients at an early stage for early treatment.

2. The abnormal changes of vWF, ADAMTS-13 and inflammatory factors likes CRP, NEUT.%, and NLR suggest that aggressive anticoagulant therapy might be needed. ADAMTS 13 activity that was strongly associated with disease severity. Such an imbalance enhances the hypercoagulable state of Covid-19patients and their risk of microthrombosis.

3. The correlations of vWF-ADAMTS-13 in Covid-19 patients could help better define the pathophysiology, clarify the pathogenesis, improve prediction of clinical prognosis, and better guide thromboprophylaxis and treatment of Covid-19 patients, which could help health care workers, identify those patients at an early stage for early treatment.

4. The current results suggested that the serum level of vitamin K severely reduced in Covid-19 patients group which is compatible with the increased thrombogenicity that is frequently observed in severe Covid-19. Vit.K was linked to accelerated elastic fiber degradation and premorbid vascular calcifications, and related with Neutrophle %,, D-dimer , Ferritin, vWF, and ADAMTS-13 as immunopathological damage in coronavirus patients. Vitamin K administration improves Covid-19 outcomes and could be uses

as hall mark of severe Covid-19 and provide a rational for combined therapeutic approaches for medical staff.

5. Serum levels of HDL-C and TC concentrations were lower in Covid-19 patients and ICU admission for severe Covid-19.

6. This study provides evidence for a causal effect of D-dimer, ferritin, CRP level are associated in patients with Covid-19and severity of virus corona by abnormal levels compared with healthy group might represent a valid target for lowering risk Covid-19.

4.2. Recommendations

1. A large sample size and multicenter of studies are needed in order to confirm these results and possibly determine a more accurate cutoff.
2. Histopathologic comparison of Covid-19 thrombi collected from extracorporeal membrane oxygenation with non-Covid-19 specimens shows a specific involvement of neutrophil extracellular traps in Covid-19 thrombogenesis.
3. Evaluate the levels of sex hormones contribute to the observed sex-specific differences inflammatory and coagulation parameters in Covid-19 patients.
4. Determine the relationship and causality between ADAMTS-13 activity, complement, endothelial, and coagulation activation.
5. Study the efficacy of treatments aiming at preventing and/or ameliorating Covid-19 microangiopathy.

Chapter Five

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وجدت نتائج هذه الدراسة زيادة معنوية عالية ($p\leq0.000$) في مستويات D-dimer و ferritin و neutrophilsوحساسية عالية CRP و TG و VLDL-C وبيانات مؤشر تصلب الشرايين في مجموعة مرضى Covid-19 عند المقارنة مع مجموعة اصحاء. بينما أظهرت النتائج انخفاضًا معنويًا في مستويات HDL-C و TC ومستويات الصفائح الدموية عند مقارنتها مع مجموعة اصحاء.

أوضحت هذه النتائج أن مرضى الاعراض الشديده كان لديهم نشاط ADAMTS-13 أقل بشكل ملحوظ ومستويات فيتامين K ، وارتفاع كبير في D-dimer ، وفيريتين. مستويات vWF مرتفعة بشكل ملحوظ مقارنة بالمرضى الذين خرجوا أحياء.تحليل الانحدار الخطي لـ ADAMTS-13 و vWF و أشارت مستويات Vit.K إلى ارتباط كبير مع العمر ومؤشر كتلة الجسم وفيريتين و D-dimer و CRP و NLR في مجموعات مرضى Covid-19 .أظهر مستوى vWF في المصل ارتباطات سلبية معنوية مع ADAMTS-13 و Vit. K و HDL-C و%LYM ، TC ومع ذلك أظهر ADAMTS-13 ارتباطات إيجابية كبيرة مع مستويات TG وNLR و VLDL-C في مجموعة المرضى.

لخصت هذه الدراسة إلى أن تحسين استراتيجيات الوقاية ، بما في ذلك تلك الخاصة بالحالة الخفيفة والشديدة باستخدام vWF و ADAMTS-13 و Vit.K كعلامات توقع لخطورة Covid-19 ومضاعفاتها.وبالتالي ، بالإضافة إلى vWF المرتفع قد يتطلب مزيدًا من العمل الإضافي بما في ذلك نشاط ADAMTS-13 و vWF ومستويات النشاط نظرًا لأن هؤلاء المرضى قد يكونون في خطر متزايد للوفاة وقد يكون بمثابة مقياس تنبؤي بسيط لمخاطر الجلطة في Covid-19 وربما يساهم بشكل كبير في إحداث المرض وقد يستفيد من العلاج الأكثر قوة ، كما أن نقص فيتامين K يمكن أن يدعم عاصفة السيتوكين عن طريق زيادة السيتوكينات المؤيدة للالتهابات والتي تشارك في بناء الاستجابة الالتهابية التي تجند المكونات الخلوية والخلطية. من المعروف أن فيتامينK يلعب دورًا أساسيًا في نظام التخثر. إبراز دور فيتامين K ، ربما عن طريق تعديل التخثر. إجمالاً ، الآليات المحتملة التي تربط بين كوفيد -19 واعتلال التخثر ، حيث يمكن لفيتامين Kأن يمارس دوره التحويلي في التخثر المرتبط بإحداث المرض.إلى جانب ذلك ، يمكن أن يساهم أيضًا في تلك الأحداث التي تنطوي على تكلس الأوعية الدموية مما يؤدي إلى تجلط الدم وينتشر التخثر داخل الأوعية (DIC) تتميز بتلف الأوعية الدموية الدقيقة الذي لوحظ في مرضىCovid-19 .

الخلاصـــة

مرض فيروس كورونا 2019 (كوفيد -19) هو مرض تنفسي له مظاهر غير متجانسة تتراوح من المرض بدون أعراض في البعض إلى الالتهاب الجهازي وفشل العديد من الأعضاء والموت السريع في حالات أخرى.

تتجلى المرحلة الأولى من المرض كعدوى في الجهاز التنفسي العلوي يليها التهاب رئوي عندما يغزو الفيروس ظهارة الجهاز التنفسي عبر الارتباط بالمقاومات. ويمكن أن تظهر المرحلة الثانية الأكثر شدة على أنها أضرار متعددة للأعضاء ، بما في ذلك إصابة الجهاز التنفسي والقلب والكبد والكلية. في هذه المرحلة ، يمكن أيضًا أن تتأثر المستقبلات الموجودة على البطانة ، مما يتسبب في تلف مباشر للأوعية الدموية ويؤدي إلى اعتلال التخثر.

يعد الالتهاب الجهازي واعتلال التخثر من السمات المميزة لهذه المرحلة. يتجلى "مرض تجلط الدم Covid-19"بشكل أساسي في صورة حالة تخثرية تؤثر على الأوعية الدموية الكبيرة والصغيرة على حدٍ سواء ، وتتظاهر بأحداث تخثر شرياني وريدي واعتلال الأوعية الدقيقة. أسباب تجلط الدم المرتبط بمرض فيروس كورونا 2019 (كوفيد -19) غير مفهومة جيدًا.

لذلك ، تهدف هذه الدراسة إلى التحقيق في مستويات عامل فون ويلبراند (VWF) ، والمؤشرات الحيوية العضو 13 (ADAMTS-13) من النوع 1 ، وانحلال الدم داخل الأوعية الدموية ، والتخثر ، وتلف الأعضاء في مرضى كوفيد- 19 مريضا ودراسة ارتباطهم بخطورة المرض والوفيات وله دور حاسم في تحديد المرضى المعرضين لخطر الإصابة بمضاعفات قاتلة لهذه الحالة ، مما يساعد في إنقاذ حياة العديد من المرضى.

تضمن تصميم دراسة الحالات والشواهد جمع مصل 90 عينة من المرضى والأشخاص الأصحاء خلال الفترة من يناير 2021 إلى مايو 2021 ، حيث تم تقسيم المرضى إلى مجموعتين:

المجموعة الاولى: مرضى فيروس كورونا الذين جمعت عيناتهم من مستشفى الامل ومركز الشفاء بمدينة النجف الاشرف ومن مستشفى الحسين التعليمي بكربلاء وتألفت من ثلاث مجموعات المجموعة الاولى اعراض خفيفة, المجموعة الثانية استخدمت التهوية الميكانيكية (نتيجة سريرية معاكسة) والمجموعه الثالثه كمجموعة اعراض شديدة. تمت مقارنة النتائج مع 30 عينة من الأشخاص الأصحاء على ما يبدو كمجموعة ضابطة تم تحديد مستوى المصل من vWF ، ADAMTS-13 ، وفيتامين K بتقنية ELISA ، وتم تحديد مستوى البروفيلات الدهنية بالطرق الأنزيمية واللونية. تم تحديد فيريتين و D-dimerبواسطة ichroma ، CBCآليًا بواسطة محلل أمراض الدم الآلي. تم حساب مستويات LDL-Cو VLDL-C وبيانات مؤشر Atherogenic (AIP) ومؤشر مخاطر Catelli-I (CRI-I) و CRI-II ومؤشر كتلة الجسم (BMI) من خلال معادلات خاصة.

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دراسة عامل von Willebrand و ADAMTS-13 وبعض المتغيرات البايوكيميائية كمؤشرات تنبؤية لشدة Covid-19 لدى المرضى العراقيين

رسالة ماجستير

مقدمة الى مجلس كلية الطب – جامعة كربلاء كجزء من متطلبات نيل شهادة الماجستير في الكيمياء السريرية

بواسطة

حنين سعيد محسن الموسوي

بكالوريوس علوم – كيمياء – جامعة كربلاء (2014)

باشراف

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كلية الطب – جامعة كربلاء كلية العلوم – جامعة الكوفة

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