Republic of Iraq
Ministry of Higher Education and
Scientific Research
University of Kerbela
College of Medicine
Department of Chemistry and Biochemistry

Vascular endothelial cell adhesion molecules as predictors for severity of COVID-19

A Thesis

Submitted to the Council of the College of Medicine University of Kerbala in Partial Fulfillment the Requirement for the Degree of Master in Clinical chemistry

By

Saja Haider Fadhil

B.Sc. in Clinical laboratories- College of Applied Medical Sciences-University of Kerbela / 2017

Supervised by

Asst.prof.Dr.Shaymaa Z. Nada
PhD in clinical Biochemistry

Asst.prof.Dr. Lamya A. Drwish
MBchB, MRCP (UK)

2021 AD **1443 AH**

Supervisors Certification

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

Vascular endothelial cell adhesion molecule as predictors for severity of COVID-19

Was prepared under our supervision in laboratories at department of Chemistry and Biochemistry- College of Medicine/ University of Kerbala as a partial requirement for the Master Degree in Clinical Chemistry

Dr. Shaymaa Z.Nada

Asst. Prof.in clinical chemistry

Dr.Lamya A.Drwish

Asst.Prof.MBchB,MRCP**(UK)**

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

Dr. Rana Majeed Hameed

Head of Chemistry and Biochemistry Department

College of Medicine – University of Kerbela

Acknowledgement

First of all, I would like to express my deepest gratitude to

Dr. Shaymaa Z.Nada and Dr. Lamya A. Drwish for providing me the opportunity to carry out this study. I also thank them for invaluable advice, patience and inspiring guidance throughout this work.

I would like to extend my warmest thanks with a highly appreciation to all staff of the **al- Hayat Unit , the staff in Biochemistry Laboratory of AL-Hussein Teaching Hospital.**

I would like to express my sincere thanks to **all covid-19patients** for their acceptance in participation in this study and I wish to them fast recovery.

Thanks to the staff of chemistry and biochemistry department of collage of medicine

Thanks for all, saja

Dedicate

To...

The one who gave me and gave me all the blessings... Thank You for Allah

To...

The teacher of mankind and the source of knowledge is our Prophet Muhammad (peace and blessings be upon him and his family)

To ...

The one who lights the way and gives me strength...

Imam Aba AL-Fadl AL-Abbas (P)

To...

The most precious thing I have in this world ... my loving mother

To ....

The first sweetheart of my heart ... my dear father

List of Contents

List of figures

List of Tables

List of Abbreviation

ACE2	Antigen in converting enzyme
ALT	Alanine aminotransferase
ANG	Angiotensin
ARDS	Acute respiratory distress syndromes
AST	Aspartate amino transferase
BMI	Body mass index
CAD	Coronary artery disease
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
CT	computerized tomographic
DC	Dendritic cell
ECs	Extra cellars
ESR	Erythrocyte sediment rate
Icam-1	Intracellular adhesion molecular
Ig	Immunoglobin
IL-6	Interleukin-6
IL-8	Interleukin-8
LDH	Lactate dehydrogenase
MAbs	Monoclonal antibodies
OD	optical density
PBC	Primary biliary cirrhosis
PCNA	Proliferating cell nuclear antigen
PNS	Peripheral nervous system
RAAS	Renin-Angiotensin-Aldosterone Systems
RLU	relative eight units
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SSAO	semicarbazide-sensitive amine oxidase
SSAO	Semicarbazide-sensitive amine oxidase
TNFa	Tumor necrosis factor
Vap-1	Vascular adhesion protein
Vcam-1	Vascular adhesion molecule
VTE	Venous thromboembolism
WBC	white blood cell counts

Summary

Background: The Coronavirus disease 2019 (COVID-19) pandemic continues to spread across the world. Hence, there is an urgent need for rapid, simple, and accurate tests to diagnose severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections.

Aims: The present study was conducted to investigate the impact of serum levels of vascular endothelia cell adhesion molecule (VAP-1and VCAM-1) in severity of COVID-19 in studied subject .

Materials and methods: A cross sectional study for Iraqi individuals with COVID19 in Kerbala province. The severity of COVID -19 varies; therefore, we included 69 patients in mild presentations and 19 cases with severe form of this disease. The number will be according to the sample size equation. Five ml of blood sample was collected and processed and the serum ~~will~~ separated for measuring the biochemical parameters.

Results: The median of VCAM-1 in diabetics was199 (pg/ml) and in non-diabetics 195.75 (pg/ml) (NS), the median VAP-1 in diabetic group was 3690(pg/ml)and in non- diabetics 3678.50(pg/ml) (HS). In another hands, the median of VAP -1 was 3719.50(pg/ml) in hypertensive group compared to 3600.50(pg/ml) in none hypertensive one. In contrast the VCAM-1 median in none hypertensive patients was higher than with hypertension (199,195.75 (pg/ml) respectively). Also, in smokers groups the median of VCAM-1was more than in non-smoker (236 versus 180.30pg/ml) (S), while the median of VAP-1 in smoker patients was greater than in non-smokers (3698, 3550pg/ml respectively) Finally, the median of VCM-1 in patients with mild degree of COVID- 19 Disease was 198.30, which was less than in patients with severe disease 271.31(pg/ml) (S), while the results for - VAP-1 in mild cases was higher than severe cases (3678(pg/ml), 3664(pg/ml respectively).

Conclusion: According to the results of this study we concluded that a serum level of VAP-1 was associated positively with diabetics, hypertension and smoker states in COVID -19 patients.

In addition, serum level of VCAM-1 was also associated positively with diabetics, hypertension and smoker states in COVID-19 patients.

Chapter one

Introduction

&

Literature Review

1. Introduction

1.1. Coronaviruses

Coronavirus are enveloped, positive single-stranded large RNA viruses that not only infect humans, but also a wide range of animals. Coronaviruses were first described in 1966 by Tyrell and Bynoe, who cultivated the viruses from patients with common colds. The morphology of coronaviruses is spherical with a core shell and surface projections resembling a solar corona, they were termed coronaviruses (Latin: corona = crown). Four subfamilies, namely alpha-, beta-, gamma- and delta-coronaviruses exist. alpha- and beta-coronaviruses apparently originate from mammals, in particular from bats, gamma- and delta-viruses originate from pigs and birds, **(Tyrrell *et al.*, 1966).**

The genome size varies between 26 kb and 32 kb. Among the four subtypes of coronaviruses that can infect humans, the beta-coronaviruses may cause severe disease and fatalities, whereas alpha-coronaviruses cause asymptomatic or mildly symptomatic infections. Severe acute respiratory syndrome coronavirus2 (SARS-CoV-2) belongs to the beta-coronaviruses and is closely related to the SARS-Co V virus, **(Velavan *et al.*, 2020)**.

The major four structural genes encode the nuclei-capsid protein (N), the spike protein (S), a small membrane protein (SM) and the membrane glycoprotein (M) with an additional membrane glycoprotein (HE) occurring in the HCoV-OC43 and HKU1 beta-coronaviruses. COID-19 is 96% identical at the whole-genome level to a beta coronavirus, (**Zhou *et al*., 2020)**.

Severe acute respiratory syndrome coronavirus2 (SARS-CoV-2) apparently succeeded in making its transition from animals to humans on the Hanna seafood market in Wuhan, China. However, endeavors to

identify potential intermediate hosts seem to have been neglected in Wuhan and the exact route of transmission urgently needs to be clarified, (**Zhou *et al.*, 2020)**.

The initial clinical sign of the Covid-19-related disease COVID-19 which allowed case detection was pneumonia. The report in describe gastrointestinal symptoms and asymptomatic infections, especially among young children, (**Chan *et al*., 2015).**

In symptomatic patients, the clinical manifestations of the disease usually start after less than a week, consisting of fever, cough, nasal congestion, fatigue and other signs of upper respiratory tract infections. The infection can progress to severe disease with dyspnea and severe chest symptoms corresponding to pneumonia in approximately 75% of patients**,**(**Guan *et al.*, 2020)**.

Pneumonia mostly occurs in the second or third week of a symptomatic infection. Prominent signs of viral pneumonia include decreased oxygen saturation, blood gas deviations, changes visible through chest X-rays and other imaging techniques, with ground glass abnormalities, patchy consolidation, alveolar exudates and interlobular involvement, eventually indicating deterioration. Lymphopenia (refers to a decreased concentration of lymphocytes in blood). appears to be common and inflammatory markers (C-reactive protein and pro-inflammatory cytokines), **(Chan *et al*., 2020)**.

Recent study indicate that patient's ≥60 years of age are at higher risk than children who might be less likely to become infected or, if so, may show milder symptoms or even asymptomatic infection, **(Heneka *et al*., 2020)**.

1.2. Complication of coronavirus

Coronavirus disease 2019 (COVID-19) is an extremely contagious infectious disease caused byCOIVD-19.It infection was first reported in Wuhan, China, and spread quickly and turned into an unprecedented global pandemic. The novel coronavirus affects not only the respiratory-tract, but also other organs in the human, (**Seyed *et al.*, 2020).**

COVID-19 could cause injuries in the lungs, liver, kidney, heart, vessels, and other organs. Respiratory failure and acute respiratory distress syndrome (ARDS) are the most common complications of severe COVID-19 infection; the majority of hospitalized COVID-19 patients suffer from severe lung injuries and fatal multi-organ failure as well as hemolytic anemia. However; super infection, acute liver, kidney, and cardiac injuries, shock, and hypoxic encephalopathy are less common symptoms, **(Wang *et al.*, 2020).**

Some COVID-19 patients may also present signs of tissue damage including rhabdomyolysis (is a condition in which damaged skeletal muscle breaks down rapidly) or hemoptysis, which lead to cellular injury, release of heme proteins, and collection of heme in body tissues. COIVD-19 usually affects the respiratory system; nervous system involvement has also been reported in some the recent study among patients with COVID-19, **(Ghiasvand *et al.*, 2020)**.

Coronaviruses can attack the neural tissue including microglia, astrocytes, and macrophages, and cause nerve injury through direct nerve infection, **(Wagener *et al.*, 2020)**.

The nervous system injuries could manifest as headache, dizziness, seizure, impaired consciousness, acute cerebrovascular disease, and

ataxia. The virus could also affect the peripheral nervous system (PNS) and cause dysfunction, disguise, vision impairment, and neuropathic pain,(**Ghiasvand *et al.*, 2020)**.

COVID-19 could also cause cardiac injuries such as cardiomyopathy and conduction system malfunction. Another Study suggests the direct involvement of cardiac muscles in some patients. Generally, infectious myocarditis is the most common cardiac complication of COVID-19 infection. COVID-19 uses the angiotensin converting enzyme 2 (ACE2) receptors to infect host cells, through which it can cause pneumonia and myocardial injuries. High expression of ACE2 receptors in the lungs and heart could increase the risk of myocardial injuries in COVID-19 patients. ACE2 is also expressed in the intravascular endothelium, intestinal epithelium, and the kidneys; therefore, these organs could be a target for COVID-19 infection. Tachyarrhythmia is also a common cardiovascular complication in COVID-19 patients. Electrocardiography and echocardiography could be used in diagnosing and predicting the prognosis in COVID-19 patients, **(Montalvan *et al.*,2020)**.

Some COVID-19 patients could suffer from earache that may be a sign of sub-acute thyroiditis. Another Study has shown that a few weeks after upper respiratory tract involvement, sub -acute thyroiditis may occur and it might be a late complication in patients with COVID-19 infection. Therefore, thyroid functions should be checked after discharge in patients with COVID-19, **(Heneka *et al.*, 2020)**.

1.3. Hematological Alterations

COVID-19 is not fully known and the effects between virus and the immune system are complicated, it is known that Lymphopenia,

hyper-inflammatory responses, and cytokines play an important role in the pathology of COVID-19**(Yazdanpanah et al., 2020).**

Recent study suggest, that T lymphocytes, particularly $CD4^+$ T and $CD8^+$ T cells, are affected by Covid-19, $CD4^+$ and $CD8^+$ T cells play a fundamental role in controlling viral infections and maintaining cellular, humoral and cytotoxic immune responses. They may play a very important role the pathological process of COVID-19 **(Henry *et al.*, 2020).** $CD4^+$ T cells have numerous roles and are required to support $CD8^+$ T cell responses. Moreover, these cells help B cells to elicit antibody responses **(Ng *et al.*, 2020).** $CD8^+$ T play critical roles in mediation of viral clearance and acute viral respiratory infections in viruses such as respiratory syncytial virus, influenza virus, and human metapneumovirus **(Nishig *et al.*, 2020).**

A number of hematological abnormalities have been observed in the laboratory features of COVID-19 **(Memar *et al.*, 2020**). In study concerning changes of lymphocyte subsets and their correlation with the severity and outcome of the disease have been reported in adults **(Rezaei *et al.*, 2020).**

Hemoglobin (Hb) is the protein contained in red blood cells that is responsible for delivery of oxygen to the tissues. To ensure adequate tissue oxygenation, a sufficient hemoglobin level must be maintained. The amount of hemoglobin in whole blood is expressed in grams per deciliter (g/dl). The normal Hb level for males is 14 to 18 g/dl; that for females is 12 to 16 g/dl. When the hemoglobin level is low, the patient has anemia. An erythrocytosis is the consequence of too many red cells; this results in hemoglobin levels above normal **(Adamson *et al.*, 2020).**

An inflammatory anemia can occur in a situation of acute immune activation; this protective mechanism involves a low circulating iron to prevent the virus from invading the organs, while increasing the effectiveness of cellular immunity **(Brittenham et al., 2020).** The

pathophysiology of this anemia related to decreased proliferation of erythropoietic progenitor cells, reduced stimulation of erythropoietin and a decrease in the half-life of erythrocytes. The imbalance of iron homoeostasis in inflammation is due to increased iron retention within the cells of the reticle-endothelial system. These patients have very high ferritin levels, as an acute phase reactant **(Schroorl *et al.*, 2012).**

The study suggests that hemolysis and erythrocyte structural changes could play the main role in the ferritin elevation and thus the use of iron chelating agents have been proposed **(Liu *et al.,* 2020).**

1.4. Coagulation Dysfunction

The D-dimer is a product of the blood clotting and break-down process that can be measured via analysis of a blood sample. D-dimer is released when a blood clot begins to break down. However, D-dimers have a high sensitivity but low specificity for detecting pulmonary embolism or deep vein thrombosis in low-risk populations **(Ryu *et al.,* 2020).**

COVID-19 related mortality is largely associated with hypercoagulability and increased risk of venous thromboembolism (VTE) events, leading to thrombo-inflammation in severe conditions. Therefore, coagulation biomarkers may indicate disease severity and mortality, and help determine patient triage, therapeutic strategies and prognosis supervision. D-dimer is the product of fibrin degradation, and plays a mechanistic role in thrombo-inflammation in COVID-19**(Bikdeli *et al.*,2020).** Patients with D-dimer >1000 ng/ml present a 20-fold higher mortality risk compared to those with lower D-dimer values **(Zhou *et al.*,2020).** Therefore, D-dimer is a potential screening tool for VTE in COVID-19 patients, and based on D-dimer elevation, adjusting therapeutic anticoagulant doses is more beneficial to the patients

compared to prophylactic doses. Thus, D-dimer levels should be monitored in COVID-19 patients early after admission **(Llitjos *et al.*,2020).**

Ferritin is a protein that stores iron, releasing it when your body needs it. Ferritin usually lives in your body's cells, with very little actually circulating in your blood. Ferritin is stored in the body's cells until it's time to make more red blood cells. The body will signal the cells to release ferritin. The ferritin then binds to another substance called transferrin. Transferrin is a protein that combines with ferritin to transport it to where new red blood cells are made. In one study from China with COVID-19 cases, it was found that individuals with severe diseases often present with increased serum ferritin levels, with a statistically significant difference between severe and mild categories. Whereas another study conducted using records from a large multi-hospital New York City health system demonstrated poor performance of serum ferritin for the prediction of mortality **(Jonathan *et al.*, 2020).**

1.5. Liver enzyme and COVID-19

The pathogen of COVID-19 pneumonia is severe acute respiratory syndrome coronavirus, which mainly causes respiratory, intestinal, liver and nervous system diseases. A study that more than 50% of patients with COVID-19 have different degrees of liver injury. It has reported the clinical characteristics of patients with coronavirus disease 2019 (COVID-19), including some factors that may lead to COVID-19-related liver damage and the relationship between liver function damage and disease prognosis. In these studies, different degrees of elevated levels of alanine aminotransferase (ALT) and aspartate amino trans-

erase (AST) were reported. However, the effect of COVID-19 on liver injury has not been fully presented **(Huang *et al.*, 2020).**

Liver injury is related to the severity and mortality of COVID- **(Praktiknjo *et al.*,2020)** systematically described the clinical characteristics of COVID-19 patients with liver injury and revealed that liver was related to disease severity. In addition, a study reported that liver injury was related to death in patients with COVID-19, and mortality was related to an increase in liver enzyme levels. However, mechanical ventilation, which is the main auxiliary treatment for critical patients and an important clinical outcome of COVID-19, was not involved. On the other hand, dynamic changes in liver functions may indicate a certain relationship between liver injury and mortality. There were few studies on the dynamic changes of liver functions in COVID-19-related liver injury **(Lei *et al.*,2020).**

1.6. Vascular cell adhesion molecule

The adhesion molecules that are expressed on the surface of leukocytes and their respective counter-receptors on Extra cellar Cells (ECs).Lectin-like adhesion glycoproteins, called the selectins, mediate leukocyte rolling, while the firm adhesion and subsequent transendothelial migration of leukocytes are mediated by the interaction of integrins (CD11/CD18, VLA-4) on leukocytes with immunoglobulin-like adhesion molecules on ECs (e.g., ICM-1, VCM-1). The expression of P-selectin, E-selectin ICAM-1, and VCM-l on venular EC are temporally coordinated to ensure that the processes of leukocyte rolling and firm adhesion/emigration can occur for several hours after the initiation of an inflammatory response. The importance of these endothelial cell adhesion molecules and their counter-receptors on leukocyte recruitment in different animal models of inflammation has been demonstrated using either adhesion molecule-specific blocking monoclonal antibodies

(mAbs) or mice that are genetically deficient in one or more adhesion molecules**(Petri & Kubes.,2008).**

1.6.1 Vascular Adhesion molecules (VCM-1)

Vascular Cell Adhesion molecules (VCM-1) are a 90-kDa glycoprotein that is inducible and predominantly expressed in endothelial cells. In1989, VCAM-1was first identified endothelial cell surface glycol protein. VCAM-1 expression is activated by pro-inflammatory cytokines, including (Tumor necrosis factor) TNFα,and also by Reactive Oxygen Species (ROS), oxidized low density lipoprotein, high glucose concentration, toll-like receptor agonists, and shear stress **(Coperchini *et al.*,2020).**

Under high levels of inflammation and chronic conditions in some diseases, VCM-1 also is expressed on the surface of other cells, including tissue macrophages, dendritic cells, bone marrow fibroblasts, myoblasts, oocytes, Kupffer cells, Sterol cells, and cancer cells. Structurally, human VCAM-1 contains an extracellular domain with six or seven immunoglobulin (Ig)-like domains, a trans-membrane domain, and a cytoplasmic domain, whereas the mouse VCM-1 has a three or seven Ig-like domain form, **(Rice *et al.*, 1989).**

The Ig-like domains of the extracellular domain contain both the disulfide-linked loops and the N-glycosylation site that binds to galectin-3 on eosinophil. In addition to galectin-3, Ig-like domain 1 and/or 4 of VCAM-1 is involved in ligand binding, including α4β1 integrin and α4β7 integrin. A4β1 integrin plays a major role in the VCM-1–mediated rolling and firm adhesion of leukocytes to the endothelium, as well as leukocyte transmigration, **(Cook-Mill *et al.*, 2011)**.

1.6.2. Role of VCM-1 in the Inflammation

Inflammation is a protective biological response that recruits immune cells, blood vessels, and molecular mediators to eliminate harmful stimuli, including bacteria, viruses, or damaged cells. In inflammation, leukocyte trafficking is regulated by the complicated and coordinated actions of many molecular mediators, including chemokine, selectins, and cell adhesion molecules, **(Sharma *et al.*, 2017).** Generally, inflammation is initiated by the release of TNFα from immune cells, such as macrophages, T lymphocytes, and natural killer cells, (**Schlesinger& Benda2015).** In turn, TNFα triggers a series of various cell adhesion molecules, such as selectins, ICM-1, and VCM-1, to recruit a subset of leukocytes at inflamed sites through leukocyte adhesion, as shown in figure(1.2) **(Ge *et al.*, 2010).**

Among these adhesion molecules, VCM-1 is a major regulator of leukocyte adhesion and trans-endothelial migration through interaction with α4β1 integrin. A4β1 integrin expressed on leukocytes adheres to VCM-1 on the surface of endothelial cells, and activates signaling pathways within the activated endothelial cells that allow the trans-endothelial migration of leukocytes, **(Beghi *et al*., 2020).**

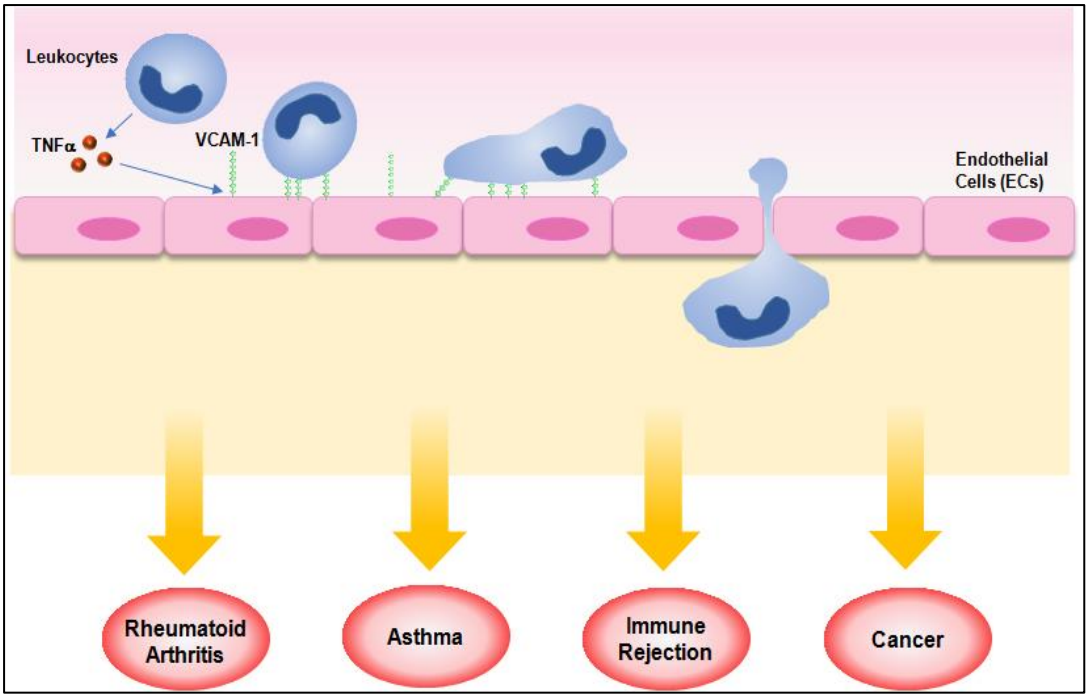

Figure (1.1) Role of Vascular Cell Adhesion molecule-1(VCM-1) inflammation (Cook-Mills *et al.*, 2011)

1.6.3. Vascular adhesion protein (VAP-1)

Vascular adhesion protein-1 (VAP-1) is a homodimeric sialylated glycoprotein originally discovered in inflamed synovial vessels by Salmi and Jalkanen in (1997). VAP-1 is a multifunctional molecule that possesses enzymatic activity known as semicarbazide-sensitive amine oxidase (SSAO) and is involved in the leukocyte recruitment cascade. The VAP-1 molecule consists of an extracellular part, which harbors the catalytic site, a transmembrane segment, and a short intracellular N-terminal tail. On the plasma membrane, VAP-1 normally forms a homodimer of two 90 kDa glycoproteins. The extracellular part of each monomer consists of three domains (D2–D4). VAP-1 has a relatively narrow substrate channel formed by domains D4 and D3, and a key leucine (469 in human) guards the entry of substrates. The large D4 domains, from each subunit, form the dimer interface and each also

contains a catalytic site, buried at the base of a deep cleft, shown as figure (1.2) **(Ernberg *et al.*, 2010).**

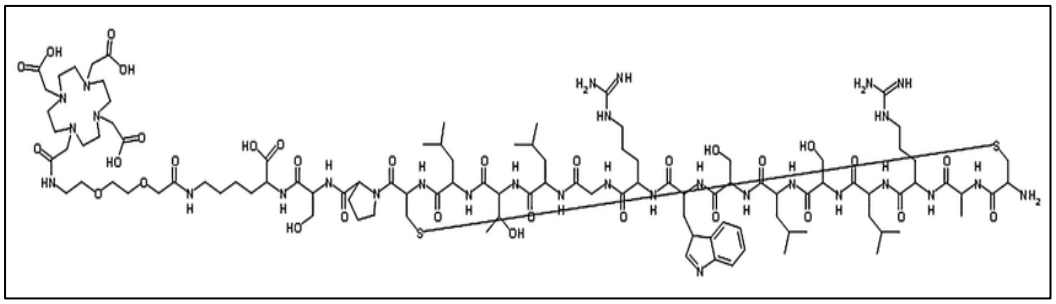

Figure (1.2) the structure of vascular adhesion protein-1 (Finney et al 2014).

Vascular adhesion protein-1 (VAP-1) is one of the endothelial cell adhesion molecules that mediates lymphocyte binding to endothelium under shear stress. The expression of VAP-1 under normal conditions is most prominent in endothelium in lymph nodes and hepatic endothelia; although in the setting of chronic inflammation it is induced in the vessels of several tissues, such as tonsil, gut, skin and synovia. VAP-1 appears to have a particular function in the liver because it can mediate shear-dependent adhesion to hepatic sinusoids, **(Bai *et al.*, 2020).**

1.6.4. Role of vascular adhesion protein-1(VAP-1)

vascular adhesion protein-1**(**VAP-1) is mainly absent from the endothelial cell surface and is stored within intracellular granules in normal conditions, while on inflammation, it is rapidly translocated to the endothelial cell surface and facilitates the recruitment of leukocytes into the inflamed tissues together with other leukocyte adhesion molecules (Figure 1.4). In fact, the VAP-1 is involved in the molecular mechanisms of acute inflammation and leukocytosis under diabetic conditions **(Noda *et al.*, 2009).** Indeed, VAP-1 inhibition may be a novel and potent therapeutic strategy in the treatment of inflammatory diseases. Notably, SSAO/VAP-1 contributes to inflammation not only through its role as an adhesion molecule but also through its function as an enzyme by causing

the formation of cytotoxic molecules such as hydrogen peroxide, aldehyde, and ammonia. These molecules are involved in the pathophysiology of inflammation **(Izuta et al., 2010),** and their inhibition, for instance, through antioxidants, recovers the integrity of the blood-aqueous barrier in endotoxin-induced uveitis (EIU) animals **(Duyndam *et al.*, 2001).**

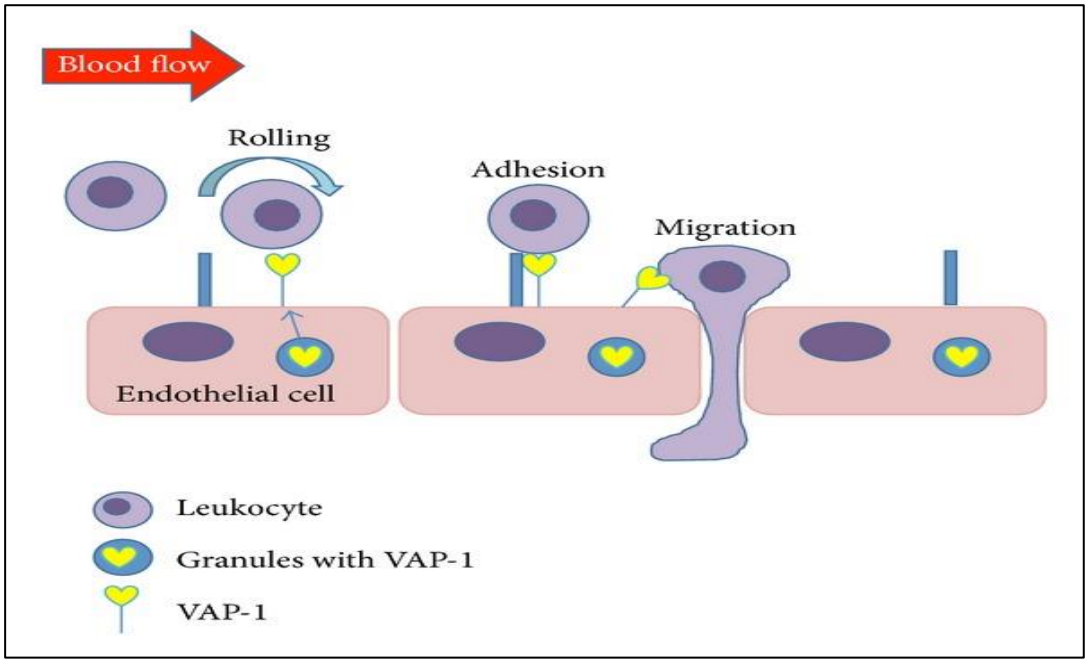

Figure (1.3) Role of vascular adhesion protein-1(vap-1) (Salmi *et al.*, 1997)

1.11. The aims of this study are to:

1- Investigate circulating cell adhesion molecule (VCAM-1) concentrations in patients with COVID 19.

2- Observe characteristics of serum VACM-1 and its relationship with the severity of COVID 19 patients

3- Assess the relationship between VACM-1, diabetes, hypertension and smoking in patients with COVID19

4- Find out the role of VAP-1 in the pathogenesis of COVID 19 by assess its levels in patients' sera and compare it according to the different clinical cases (diabetes, hypertension and smoking)

5- Investigate the correlation among vascular endothelial cell adhesion molecules (VCAM-1), d-dimer, ferritin, and WBC, lymphocyte, Heamgoblin and liver enzymes in patients with COVID 19 patients.

Chapter Two

Materials and Methods

2. Materials and Methods

2.1 Study Design:

The present work included a cross sectional study for (88) patient samples. The study was conducted from October 2020toApril 2021. Patients with Covid-19 were selected from the Al-Hayat unit, Al Hussein Teaching Medical City.

2.2 Inclusion and Exclusion criteria

2.2.1 Inclusion Criteria

All patients were subjected to the clinical history, clinical examination, and relevant laboratory investigations. The diagnosis of the Covid-19 clinical conditions was established according to the latest clinical practice guidelines by the WHO (Handier & Bittner 2020).

2.2.2 Exclusion criteria

All the patients included

2.3. Study variables

2.3.1. Dependent Variable

Serum Total Vap-1and VCAM-1, D-dimer, ferritin, WBC, lymphocyte, Hemoglobin, AST and ALT

2.3.2. Independent Variable

Age, DM, Blood pressure and smoking

2.4. Approval of the Ethical Committee

The protocol of the study was approved by Ethical Committee of Kerbela Medical College, and committee of Al-Hayat unit in Al Hussein Teaching Medical City. Samples from serum were obtained after consent from patients or the patients' relatives.

2.5. Measurement and Data collection

2.5.1. Data Collection

A structured questionnaire was specifically design to obtained information which helps to select individuals according to the selection criteria of the study appendix A.

2.5.2 Blood Collection and Storage

Blood samples were collected from Al-Hayat unit of Al Hussein Teaching Hospital. 5 mls of blood samples were drown by venipuncture using 5 ml disposable syringes, blood was left for (15 min) at room temperature in gel tube, EDTA tubs and Sodium citrate tubs . Serums were separated by centrifuging for 10 minutes at approximately 4000 rpm. Serum samples were aliquot into two eppendrof and store at -20°C to avoiding multiple freezing-thawing cycles.

2.6. Instruments:

Materials, instruments and tools were described and listed in Table.

Table 2.1: The instruments that used in the study

NO.	Instruments	Suppliers
1.	Centrifuge	HETTICH/ Germany
2.	Deep freezer	COOLTECH/ China
3.	ELISA system	UNO/HUMAN/ Germany
4.	Eppendrof Tubes	China
5.	MAGLUMI	
6.	Mini-vads	France
7.	Cobass	Germany
8.	Swelab	USA
	Materials	
1	Vascular adhesion molecules Kit. Vap-1	Elabscience USA
2	Vascular adhesion molecules (VCAM-1)Elisa kit	Elabscience USA
3	Gel –tubes	China
4	Pipette(100-1000µl)	DRAGON MED/ United State

5	Micropipette(10-100 µl)	DRAGON LAB/ United State

2.7. Methods

2.7.1. Determination Vascular adhesion molecule VCM-1.

2.7.1.1. Test principle

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 VCM-1/CD106. 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 VCM-1/CD106 and Vidin-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 VCM-1/CD106, biotinylated detection antibody and Vidin-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 spectrophotometric at a wavelength of 450 nm. The OD value is proportional to the concentration of Human VCM-1/CD10. The concentration of Human VCM-1/CD106 in the samples can be calculated by comparing the OD of the samples to the standard

2.7.1.2. Reagent preparation

1. All reagent were brought at room temperature (18~25℃) before use, then the Micro-plate reader manual for set-up and preheat it for 15 min before OD measurement.

2. Wash Buffer: Wash Buffer was Diluted 30 mL of Concentrated with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer.

3. Standard working solution was centrifuged at 10,000×g for 1 min, and added 1.0 mL of Reference Standard &Sample Diluent, it stand for 10 min and inverted it gently several times. After it was dissolves fully, it mixed thoroughly with a pipette. This reconstitution produces a working solution of 100ng/mL. Then serial dilutions were made as needed. The recommended dilution gradient is as follows: 100, 50, 25, 12.5, 6.25, 3.13, 1.56, and 0 ng/mL.

Dilution method: EP tubes was Taken, then 500uL of Reference Standard & Sample Diluent was added to each tube. Solution working was added of the 100 ng/mL to the first tube and it was mixed up to produce a 50 ng/mL working solution. The solution from the former tube into the latter one according to these steps

4. Biotinylated Detection Ab working solution was calculated the required amount before the experiment (100 μL/well). And Centrifuge gating made to the stock tube before used, the 100× Concentrated Biotinylated Detection Ab diluted to 1×working solution with Biotinylated Detection Ab Diluent.

5. Concentrated HRP Conjugate working solution: The required amount before the experiment (100 µL/well). The 100× Concentrated HRP Conjugate gate diluted to 1× working solution with Concentrated HRP Conjugate Diluent.

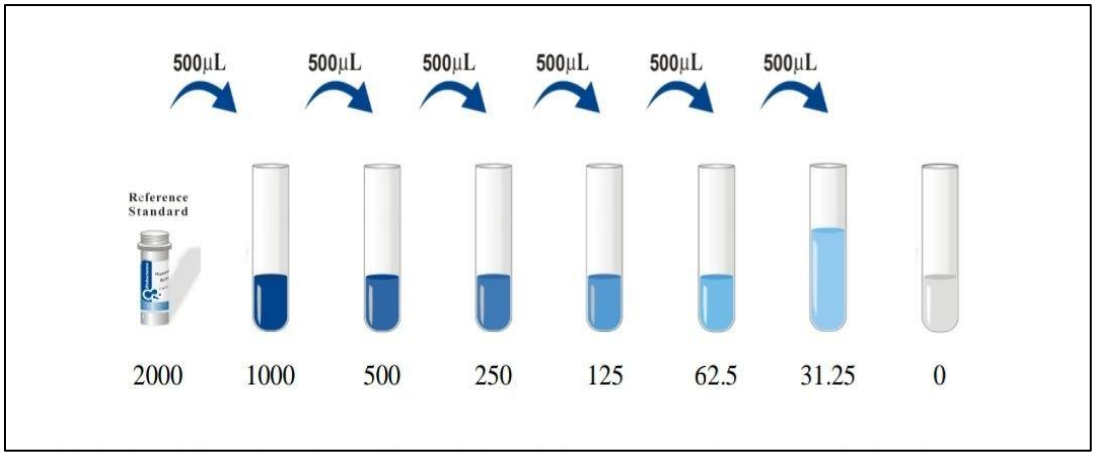

Figure (2.1) Conjugate Diluent of vascular cell adhesion molecule (VCM-1)

2.7.1.3. Assay procedure

1. The Standard working solution was taken the first two columns, and each concentration of the solution was added in duplicate, to one well each, side by side (100 uL for each well). The samples was added to the other wells (100 uL for each well), then cover the plate with the sealer provided in the kit. Incubated for 90 min at 37℃.
2. The liquid was removed out of wells, 100 µL of Biotinylated Detection Ab working solution taken to each well, the Plate sealer was covered the wells with mix up, then Incubation for 1 hour at 37C°.
3. The solution aspirate from each well, the wash buffer350 uL was joined to each well. Soak for 1~2 min and aspirate or decant the solution from each well and it put dry by clean absorbent paper. This repeated wash step 3 times.

4. HRP Conjugate working solution of100 µL was added to each well.
5. The solution Aspirate from each well, the wash process repeated for five times as conducted in step 3.
6. Substrate Reagent was add 90 µL of to each well, and Covered with a new plate sealer Incubation for about 15 min at 37C°.
7. The optical density (OD value) was determined of each well at once with a micro-plate reader set to 450 nm.

2.7.1.4. Calculation of results

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four-parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis. The concentration calculated from the standard curve must be multiplied by the dilution factor, when the samples have been diluted. The OD of the sample surpasses the upper limit of the standard curve; it should re-test it with an appropriate dilution.

2.7.1.4. Typical data

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard data is provided below (2.2).

Concentration(ng/mL)	100	50	25	12.5	6.25	3.13	1.56	0
OD	2.458	1.636	0.918	0.427	0.212	0.153	0.106	0.055
Corrected OD	2.403	1.581	0.863	0.372	0.157	0.098	0.051	-

Table (2.2) Standard data of vascular cell adhesion molecule (vcam-1)

2.7.2. Determination of vascular adhesion protein (VAP-1)

2.7.2.1. Test principle

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 VAP-1. 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 VAP-1 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 VAP-1, 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 spectrophotometric at a wavelength of 450 nm. The OD value is proportional to the concentration of Human VAP-1.The concentration of Human VAP-1 in the samples can be by comparing the OD of the samples to the standard curve.

2.7.2.2. Reagent preparation

1. All reagents were brought to room temperature (18~25℃) before use. Follow the Micro plate reader manual for set-up and preheat it for 15 min before OD measurement.

2. Wash Buffer was Diluted 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer.

3.Standard working solution was Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Reference Standard &Sample Diluent, it stand for 10 min and inverted it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 2000pg/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 2000 、1000、 500、250、125、62.5、31.25、0 pg/mL.

Dilution method: 7 tubes were added 500uL of Reference Standard & Sample Diluent to each tube. Pipette 500uL of the 2000pg/mL working solution to the first tube and mix up to produce a 1000pg/mL working solution. Pipette 500uL of the solution from the former tube into the latter one according to this step. The figure (2.2) is illustration dilution.

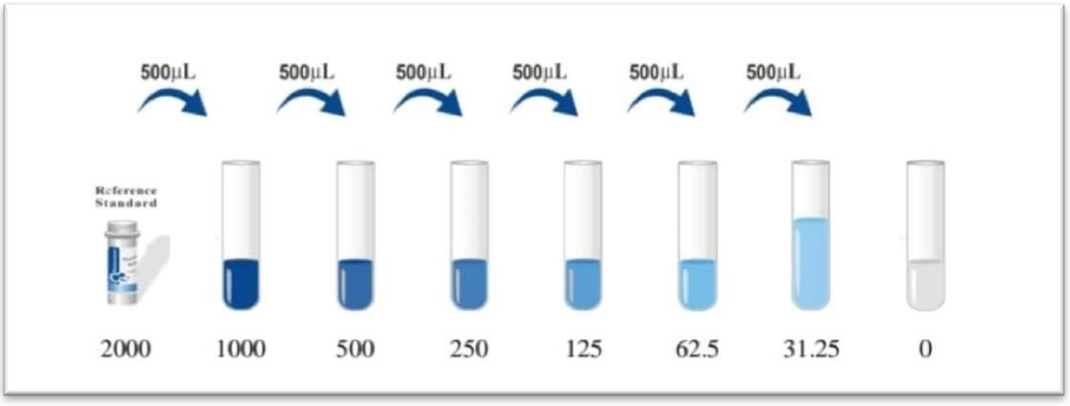

Figure (2.2) Conjugate Diluent of vascular adhesion protein (VAP-1)

4. Biotinylated Detection Ab working solution was calculated the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. The Centrifuge gating made the stock tube before used; dilute the 100× Concentrated Biotinylated Detection Ab diluted to 1×working solution with Biotinylated Detection Ab Diluent.

5. Concentrated HRP Conjugate working solution was calculated the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. It was Diluted the 100× Concentrated HRP Conjugate to 1× working solution with Concentrated HRP Conjugate Diluent

2.7.2.3. Assay procedure

1. The Standard working solution was taken in the first two columns, each concentration of the solution is added in duplicate, to one well each, side by side (100 uL for each well). The samples were added to the other wells (100 uL for each well). Cover the plate with the sealer provided in the kit. Incubation for 90 min at 37℃

2. The liquid was removed out of each well. The 100 µL of Biotinylated Detection taken to each well. The Plate sealer was covered. Incubation for 1 hour at 37°C

3. The solution aspirated as from each well. The 350 uL of wash buffer was added to each well. Soak for 1~2 min and aspirate the solution from each well, and it put dry against clean absorbent paper. This was repeated washing step 3 times.

4. HRP Conjugate working solution was added100 µL to each well, and it covered with the Plate sealer. Incubation for 30 min at 37°C

5. The solution was decanted from each well, the wash process was repeated for five times as conducted in step 3.

6. The Substrate Reagent of 90 μL was added to each well, and Covered with a new plate sealer. Incubation for about 15 min at 37°C

7. The Stop Solution was added 50 μL to each well

8. The optical density (OD value) was determined of each well at once with a micro-plate reader set to 450 nm

2.7.2.4. Calculation of results

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four-parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis. The concentration calculated from the standard curve must be multiplied by the dilution factor, when the samples have been diluted. The OD of the sample surpasses the upper limit of the standard curve; it should re-test it with an appropriate dilution.

2.7.2.5. Typical data

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard data is provided below.

Concentration(pg/mL)	2000	1000	500	250	125	62.5	31.25	0
OD	2.443	1.678	0.936	0.503	0.257	0.174	0.125	0.074
Corrected OD	2.369	1.604	0.862	0.429	0.183	0.1	0.051	-

Table (2.3) the standard data of vascular adhesion protein-1 (VAP-1)

2.7.3. Measurement of D-dimer

2.7.3.1. Test Principle

The D-Dimer assay is a sandwich chemiluminescence immunoassay.

The sample (or calibrator/control, if applicable), ABEI labeled with anti-D-Dimer monoclonal antibody, buffer and magnetic micro-beads coated with another anti-D-Dimer monoclonal antibody are mixed thoroughly and incubated to form a sandwich; after precipitation in a magnetic field, decant the supernatant, and perform a wash cycle. Subsequently, the Starter 1+2 are added to initiate a flash chemiluminescent reaction. The light signal measured by a photomultiplier as relative eight units (RLUs), which is proportional to the concentration of D-Dimer present in the sample (or calibrator/control, if applicable

2.7.3.2. Test Procedure

Preparation of the Reagent

- The Re-suspension of the magnetic micro-beads takes place automatically when the kit is a loaded successfully, ensuring the magnetic micro-beads are totally re-suspended homogenous prior to use.

- The strictly was adhered to operating instruction of MAGLUMI series fully –auto chemiluminescence immunoassay analysers. Each test parameter is identified via a RFID CHIP on Reagent kit.

2.7.3.3. Dilution

Sample dilution by analyser is not available in this reagent kit. Samples with concentrations above the measuring range can be diluted manually.

2.7.3.4. Calculation of Result

The calculate analyzer automatically the D-dimer concentration in each sample by mean of a calibration curve which is generated by a 2-point calibration master curve procedure. The result expressed in µg FEU/ml.

2.7.4. Determination of ferritin:

2.7.4.1. Principle

The assay principle combines a one-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and pre- dispensed in the sealed reagent strips. All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times. The During the final detection step, the substrate (4-Methyl- umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate umbelliferone) the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of antigens present in the sample. At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

2.7.4.2. Procedure

1. The required reagents removed from the refrigerator and allow them to come to room temperature for at least 30 minutes.

2. Ferritin strip use one time and one FER SPR for each sample, control or calibrator to be tested. The storage should have been resealed after the required SPRS have been removed.

3. The calibrator must be identified by "S1", and tested in duplicate. It should be identified by "C1, when the control needs to be tested.

4. The calibrator was mixed with control and samples using a Vortex- type mixer.

5. The calibrator, sample or control was added 100 μL into the sample well.

6. The SPRS and strips inserted into the instrument. .

7. The assay as directed in the Operator's Manual. All the assay steps are performed automatically by the instrument. The assay will be completed within approximately 30 minutes,

8. After the assay is completed, the SPRS and strips were removed from the instrument.

2.7.5. Determination of alanine aminotransferase (ALT):

2.7.5.1. Principle

Alanine aminotransferase (ALT) catalyzes the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD+. The rate of the NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance.

2.7.5.2. Calculation

The Cobas 6000 system automatically calculates the ALT concentration of each sample.

2.7.6. Determination of Aspartate aminotransferase (AST):

2.7.6.1. Principle

Aspartate Aminotransferase AST in the sample catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH), to form NAD+. The rate of the NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance.

2.7.6.2. Calculation

The Cobas 6000 system automatically calculates the AST activity of each sample

2.7.7. Complete Blood Count

The blood specimen in EDTA tube was shaken up then was examined as soon as possible in swelab alfa automated hematology analyzer to account white blood cell, lymphocyte and hemoglobin.

2.8. Biostatistics analysis

In this study the data were calculated by Microsoft Excel 10 with the Statistical Package for the Social Sciences (SPSS) version 24. Descriptive statistic was expressed as mean + standard deviation (SD) for different among four group's patients. Spearman correlation coefficient was used to evaluate the correlations between all variables. Significant differences were considered as $p< 0.05$ and more was non-significant.

Chapter Three

Results and Discussion

3. Results and Discussion

3.1. Demographic characteristics of Patients with covid-19

The present study enrolled 88 patients with covid-19. The demographic characteristics of patients are shown in table (3-1).

Table (3-1): Demographic characteristics of patients

Characteristic	N	(%)
Age (years)		
Mean ± SD	48.55 ± 12.72	
Range	19.00- 75.00	
< 30,	9	10.2 %
30-39,	16	18.2 %
40-59,	48	54.6 %
≥ 60,	15	17.0 %
Gender		
Male,	49	55.7 %
Female,	39	44.3 %
Male: female ratio	1.26:1	
BMI		
Mean ± SD	31.085 ± 4.25	
Range	19.96-39.96	
Smoker		
Yes	48	54.6 %
No	40	45.4 %

N: number of cases; **SD**: standard deviation; †: independent samples t-test; **NS**: not significant at ***P* > 0.05**

The mean age of patients was (48.55 ± 12.72). The frequency distribution of patients according to age was also shown in table (3-1). Although COVID-19 can affect individuals of any age, the present study show the high rate of infection was between 40-59 years 48 (54.6 %), followed by the age group of 30-39 years 16 (18.2 %), suggesting that young adults may be driving resurging epidemics, show in figure (3-1) .

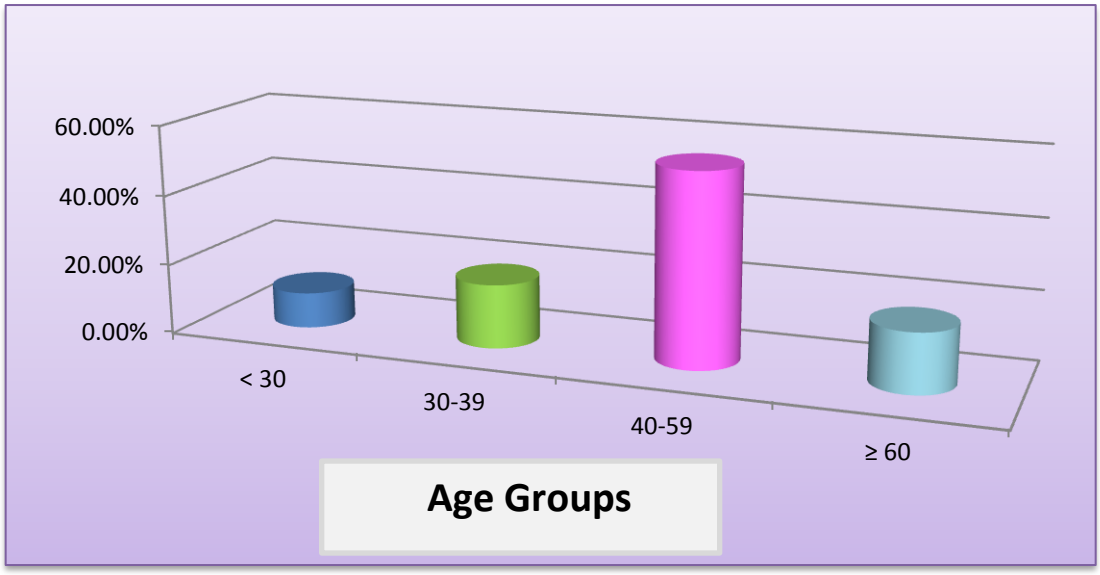

Figure (3-1): Distribution of covid-19 patients according to Age groups.

So, the occupational and behavioral factors might put adults at higher risk for exposure toCOVID-19. Younger adults make up a large proportion of workers in frontline occupations (e.g., retail stores, public transit, child care, and social services) and highly exposed industries (e.g., restaurants, entertainment, and personal services) **(Rho *et al.*, 2020),** Where consistent implementation of prevention strategies might be difficult or not possible. In addition, younger adults might also be less likely to follow community mitigation strategies, Such as social distancing and avoiding group gatherings, **(Kidder *et al.*, 2020).** Younger adults are more likely to have mild or no symptoms, Can contribute to pre-symptomatic

or asymptomatic transmission to others, **(Furuse *et al.*, 2020),** including to persons at higher risk for severe illness. COVID-19 infection is not benign in younger adults, especially among those with underlying medical conditions, who are at risk for hospitalization, severe illness, and death, **(Cunningham *et al.*, 2020).**

According to gender, Covid-19 was found to be more prevalent among males 49 (55.7 %) than females 39 (44.3 %) with male to female ratio of 1.26:1, table (3-1) and figure (3-2), indicate that male patients were more effected by Covid-19,The virus responsible for enters the body through the angiotensin-converting enzyme 2(ACE2),**(Hoffmann *et al.*, 2020).** Differences in the expression of ACE2 caused by sex hormones may help in explaining the sex disparities in COVID-19 infection, severity, and fatality, **(Gheblawi et al. 2020).** The men had more severe disease than the women. Respiratory rate, oxygen requirement and lung infiltrates were the clinical parameter to stratify the severity of disease, **(Jin *et al.*, 2020).**

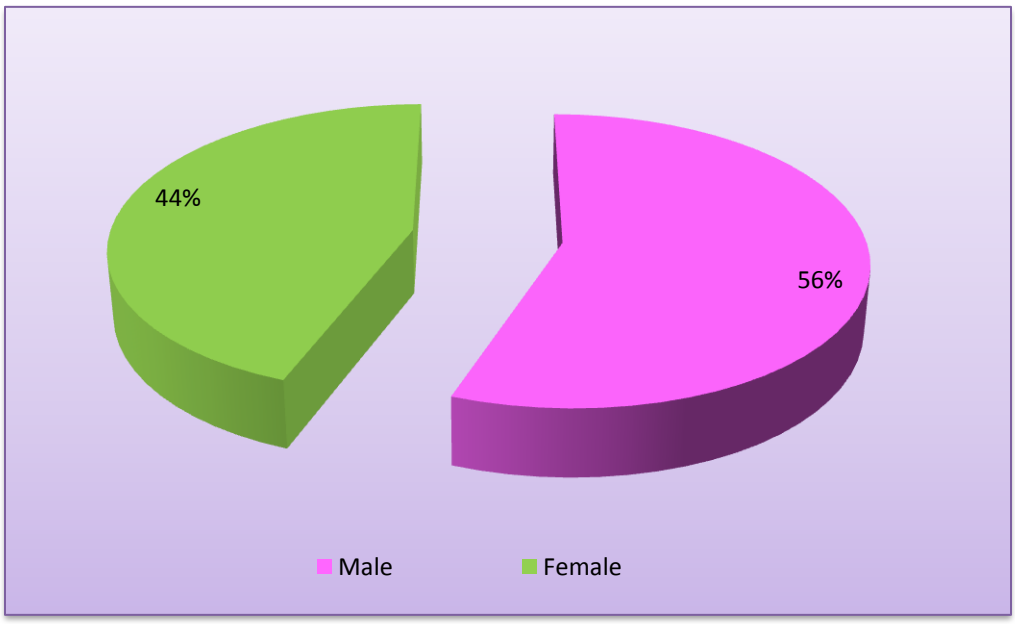

Figure (3-2): Distribution of covid-19 patients according to Gender

AS regard he mean of Body mass indices (BMI) was (31.085 ± 4.25), some study were stimulated by observations in hospital intensive care showed that body mass indices (BMI) above 35 or 40 were associated with poorer prognosis. However, a body mass index (BMI) is linearly associated with COVID-19**, (Simon *et al.*, 2020).**

The number of smoker was 48(54.6%), while the number of non-smoker was 40(45.4 %). Previous study have shown that smokers are twice more likely than non-smokers to contract influenza and have more severe symptoms, while smokers were also noted to have higher mortality in the previous COVID-19 outbreak, **(Park *et al* .,2017).**

3.2. Chronic illnesses patients with COVID-19

The rates of chronic illnesses including diabetes mellitus, systemic Hypertension, heart disease and kidney disease, are shown in table (3-2)

Table (3-2): Chronic illnesses in patients with covid-19

Chronic illnesses	**Patients**	
	N	**%**
Diabetes mellitus		
Yes	33	37.5 %
No	55	62.5 %
Systemic hypertension		
Yes	49	55.7 %
No	39	44.3 %
Heart disease		
Yes	16	18.2 %
No	72	81.8 %

***N*:** number of cases.

The frequency distribution of patients according to chronic illnesses show 33 (37.5 %) of patients with covid-19 have diabetes mellitus. In the present study, 49

(55.7 %) of patients have systemic hypertension. The current findings show only 19 (18.2 %) of patient with covid-19 have heart disease, and only 7 (7.9 %) have kidney disease, Since some researchers found diabetes, Hypertension, Heart disease and Any other coexisting diseases were related with severity of COVID-19. In the USA, Patients with diabetes mellitus have a higher possibility of hospitalization and greater illness severity, **(Gregory *et al.*, 2021**). Some research suggested that diabetic patients' cytokine responses may be related to the severity of COVID-19, **(Pal *et al.*, 2020).**

3.3 The Severity ofCOVID-19.

Table (3-3): The distribution COVID-19 patient according to the severity

Characteristic	Patients	
	N	%
Mild, *n* (%)	**69**	**78.4**
Severe, *n* (%)	**19**	**21.6**
Total	**88**	**100.00**

N: number of cases.

Patients with Covid-19 were classified according to the severity, based on pneumonia in the CT scan, multi-organ failure and ICU admission. The evaluation was completed by a specialist physician. Table (3-3) appear a sixty nine patients (78.4%) had mild disease and 19 (21.6%) of patients were admitted to critical care with severe disease. The difference in severity of disease could be explained by immunological factors; young children are more adept at fighting off novel diseases, whereas the older population is more accustomed to having immune memory responses acquired over a lifetime **(Brodin., 2021).** Differences in the immune system could also explain why severe COVID-19 is much more common in men than in women, **(Casanova *et al.*, 2020).** In consideration of symptom

factors, cough, (**Azab *et al.*, 2020),** dyspnea**, (Hu *et al.*, 2020),** fatigue**, (Tang *et al.*, 2020),** and fever,**(Qiu *et al.*, 2020)** were the main symptoms of severe COVID-19. Unsurprisingly, the existence of these symptoms could lead to severe illness. Cough and fever were globally regarded as main factors in previous study, **(Driessche *et al.*, 2020).** The symptoms could be found in children, adolescents, and adults, **(Viner *et al.*, 2021)** in research on Europe,**(Lechien *et al.*, 2020),** The UK, **(Docherty *et al.*,2020),** And other countries. Considering dyspnea, infection with COVID-19 could result in severe outcomes and death from pneumonia with severe symptoms, **(Azabou *et al.*, 2020).** Fatigue could even be found in the post-COVID-19 period,**(Wostyn, 2020).**

3.4-The comparison between covid-19 patients with diabetic and covid-19 patients without diabetic

Table (3-4): Frequency distribution of covid-19 patients with diabetic and covid-19 patients without diabetic according to some variables.

	Cases of covid-19		
	with diabetic **N=33**	***without diabetic*** **N=55**	***P***
White blood cells g\l			
Mean± SD	**15.73** ± 3.25	**15.7**± 3.95	**0.962 † NS**
Range	10.80 – 23.60	10.44 – 26.20	
SE	**0.55**	**0.528**	
Lymphocytes			
Mean± SD	**5.64** ± 3.31	**6.19** ± 3.66	**0.469 † NS**
Range	1.10- 13.20	1.10 - 17.20	
SE	**0.0.56**	**0.49**	
Hemoglobin g\dl			
Mean± SD	**12.49** ± 1.63	**12.14** ± 1.85	**0.373 † NS**
Range	7.70 - 16.00	8.10 - 17.70	
SE	**0.276**	**0.247**	
AST U\L			
Mean± SD	**35.69** ± 12.46	**37.785** ± 12.64	**0.441**

Range	15.00 - 55.00	13.00 - 59.00	† **NS**
SE	**2.106**	**1.68**	
ALT U\L			
Mean± SD	**35.68** ± 13.99	**36.46** ± 13.577	**0.794** † **NS**
Range	12.00 - 54.00	10.00 - 58.00	
SE	**2.36**	**1.81**	
Ferritin ng\ml			
Mean± SD	**498.92** ± 135.24	**483.81** ± 95.208	**0.534** † **NS**
Range	213.40 - 829.80	140.40- 751.00	
SE	**22.86**	**12.72**	
D-dimer ng\ml			
Mean± SD	**1183.32** ± 695.766	**677.40** ± 413.26	**0.039** † **S**
Range	160.00 - 7622.00	140.40- 3483.00	
SE	**286.63**	**68.58**	

N: number of cases; **SE**: standard Error ; †: independent samples t-test; **HS**: Highly significant at **P** ≤ 0.001; NS: not significant at **P** ≤ 0.05.

The comparison of white blood cell between covid-19 patients with diabetic and covid-19 patients without diabetic has been carried out and the results were demonstrated in table (3-4), Mean levels of WBC in covid-19 patients with diabetic were higher than mean WBC of covid-19 patients without diabetic, (15.73 ± 3.25)versus(15.7± 3.95),The difference was non-significant (P =0.962). Much similar study among diabetics mean leukocyte was higher when compared to their non-diabetic counterpart, **(Yang *et al.*, 2020).**

In the same hand, the difference of hemoglobin, lymphocyte, AST, ALT and ferritin were non-significant, except the mean of D-dimer was significant. The mean of hemoglobin in patient's covid-19withe diabetic was greater than mean of non-diabetic (12.49 ± 1.63) versus (12.14 ± 1.85).

Thus, the mean of hemoglobin in covid-19 patients with diabetic (12.49 ± 1.63) was more than without diabetic (12.14 ± 1.85) Several hypothesis to RBCs are rather unique body cells, since they have lost all organelles when mature and they only conserve a few metabolic pathways for obtaining energy and reducing the power consumption for the key functions they need to fulfill **(Barasa &**

Slijper2014). This makes RBCs highly sensitive to any disorder. In addition, RBCs are involved in the transport and delivery of nutrients such as amino acids. It has been shown that the metabolism of RBCs is altered in DM, including the glycosylation of the heme group by an excess of glucose **(Contreras-Zentella et al., 2019).** This heme glycosylation tends to shift the oxygen dissociation curve to left, leading to an increase in hemoglobin–oxygen affinity and reduced oxygen delivery to tissues. Furthermore, metabolite transport and basal metabolism of RBCs can be compromised in DM, thus impairing the delivery of certain compounds to several tissues **(Gay *et al.*, 2017).**

The mean lymphocyte of patients of covid-19 without diabetic was higher than (6.19 ± 3.66) versus (5.64 ± 3.31). the epidemiological study have determined that DM is associated with chronic inflammation, which may contribute to the acceleration of diabetic micro-antipathy, and the development of macro-antipathy, insulin resistance (IR) is a characterized of DM and the exact molecular action leading to IR is not yet understood. The study, it have associated IR with inflammation experimental study have demonstrated a link between chronic inflammation and insulin resistance through mechanisms involving obesity, **(Pitsavos *et al.*, 2007).**

While, the means of liver enzyme patient of covid-19 without diabetes were (37.785 ± 12.64 AST), (ALT 36.46 ± 13.577) greater than with diabetic (AST35.69±12.46),(35.68±13.99ALT)(p=0.373,0.794,respactivaly).Since,The liver is a vital organ in metabolism that plays an important role in the regulation of glucose homeostasis **(Wang *et al.*,2016)** . In another study, it significance of abnormal liver biochemistries remains uncertain **(Schaefer *et al.*, 2020).**

Then, the mean of ferritin of diabetic in covid-19 patients more than it is mean without diabetic (498.92 ± 135.24) versus (483.81 ± 95.208).Most of study,

there were found a strong positive correlation between ferritin and the hepatic enzymes, ALT. It is plausible that iron overload in the liver damages hepatocytes, which in turn would result in elevated transaminases and GGT, **(Choi *et al.*, 2005).**

Finally, the mean of D-dimer with diabetic in covid-19 was increased (1183.32 ± 695.7669) Than patients without diabetic in covid- (677.40 ± 413.26), But the difference was non-significant (P=0.039), During hyperglycemia-hyper-insulinemia, which is a characteristic of diabetes, elevation of plasma coagulation factors may constitute a potential for enhanced thrombin generation and thrombosis when triggered by exposure of transcription factor TF, Such as during arterial plaque rupture, **(Vaidyula *et al.*, 2006).**

Table (3-5): Frequency distribution of covid-19 patients with diabetic and covid-19 patients without diabetic according to level of Serum VCM-1.

	Cases of COVID-19		
VCM-1 (pg/ml)	***with diabetic*** **N=33**	***without diabetic*** **N=55**	***P***
Range	112.36 – 562.40	34.66 – 456.00	**0. 302 † NS**
Median (IQR)	199.00 (113.20)	195.75 (114.34)	

***N*:** number of cases; **IQR:** inter-quartile range; **†:** Mann Whitney U test; **HS:** Highly significant at $P \leq 0.001$

The comparison of serum VCM-1 level between covid-19 patients with diabetic and covid-19 patients without diabetic has been carried out and the results were demonstrated in table (3-5) and figure (3-3), Median levels of serum VCM-1 in covid-19 patients with diabetic were higher than in comparison with it is median levels of covid-19 patients without diabetic, 199.00 (113.20) pg/ml versus 195.75 (114.34) pg/ml, the difference was non-significant (P = 0.302). This simulative effect of high glucose concentration on the expression of adhesion molecules by

endothelial cells has been previously reported in various study performed both in vivo and in vitro. However, the results concerning the rate of induction of various types of adhesion molecules have not been consistent depending on the experimental system used. The Stimulation of ICM-1was express by high glucose concentration, **(Esposito et al., 2001).**

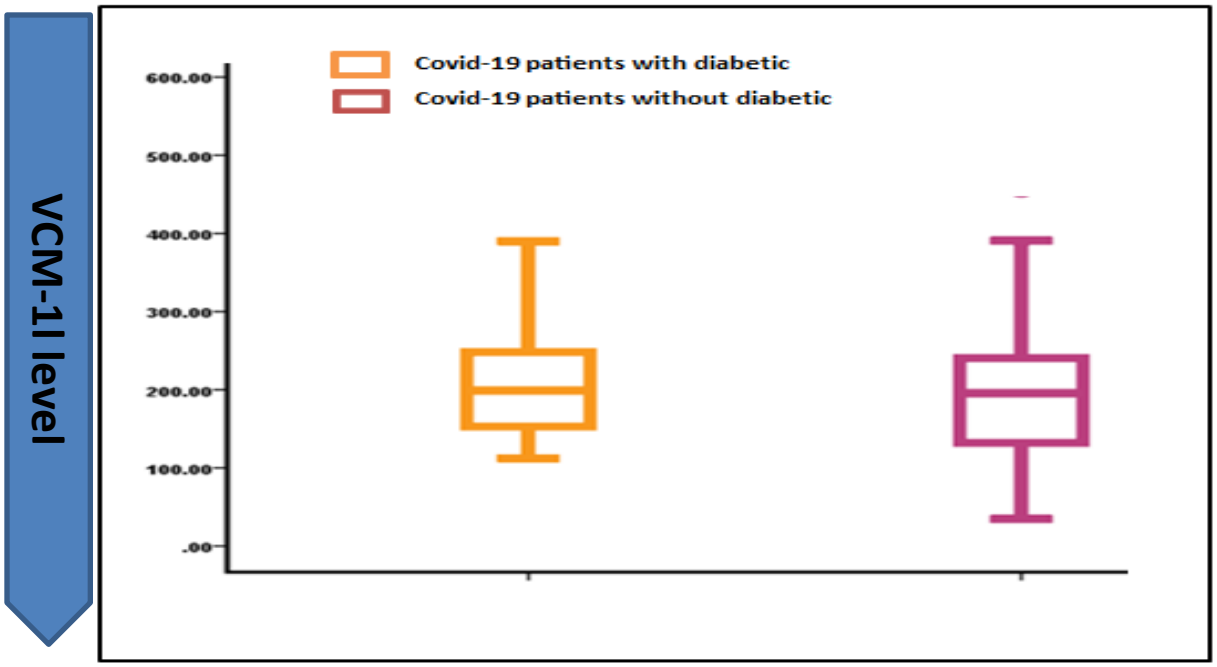

Figure (3-3): Box plot OF comparison of median serum VCM-1 level among covid-19 patients with diabetic and covid-19 patients without diabetic.

The comparison of serumVap-1level between covid-19 patients with diabetic and covid-19 patients without diabetic has been carried out and the results were demonstrated in table (3-6) and figure (3- 4).

Table (3-6): Frequency distribution of covid-19 patients with diabetic and covid-19 patients without diabetic according to level of Serum VAP-1.

	Cases with COVID-19		
VAP-1 (pg/ml)	***with diabetic*** **N=33**	***Non- diabetic*** **N=55**	***P***
Range	2089.00– 8743.00	2347.00– 9130.00	**< 0.001 † HS**
Median (IQR)	3690.00 (1230.00)	3678.50 (1673.25)	

***N*: number of cases; IQR:** inter-quartile range; †: Mann Whitney U test; **HS:** Highly significant at $P \leq 0.001$

Median levels of serum VAP-1in covid-19 patients with diabetic were increased than in comparison with its median levels in covid-19 patients without diabetic, 3690.00 (1230.00)pg/ml versus(3678.50) (1673.25) pg/ml, the difference was highly significant (P =< 0.001). Previous study there was found a correlation between insulin resistance and the presence of adhesion protein in diabetes. Depending on the adhesion protein, the corresponding soluble form can be released into the circulation via different mechanisms: Shedding from the membrane-Bound form, or alternatively splicing of the gene encoding the integral protein **(Leinonen *et al.*, 2009).**

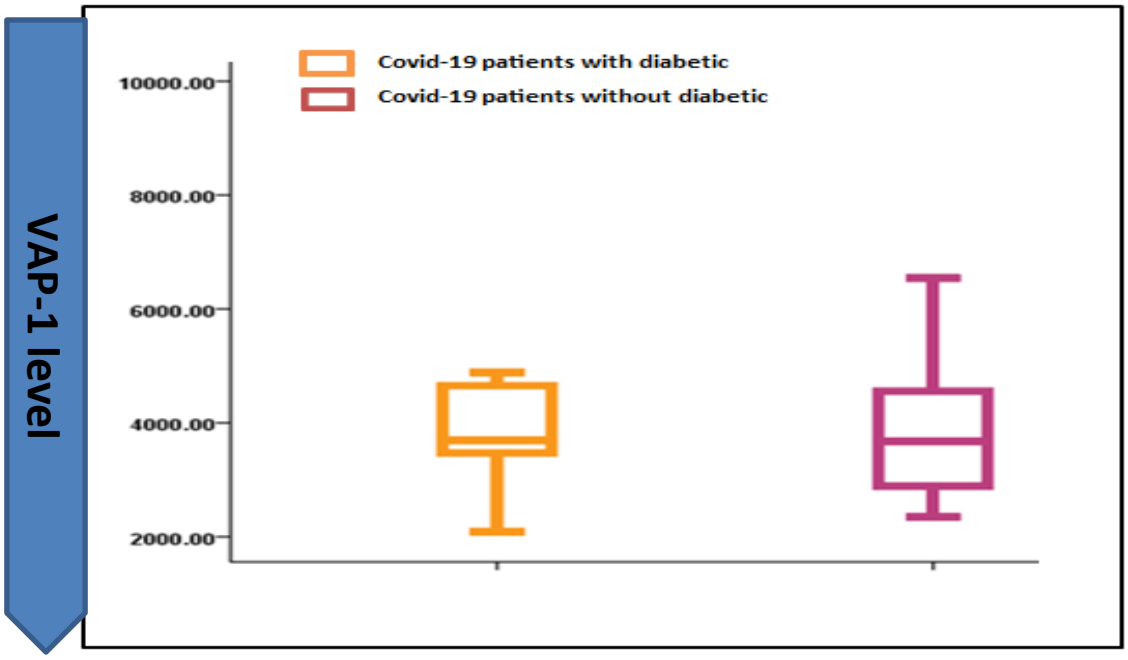

Figure (3-4): Box plot of comparison of median serum VAP-1 level among covid-19 patients with diabetic and covid-19 patients without diabetic

3.5 The comparison between covid-19 patients with and without systemic hypertension

There was non-significant differences between patient's covid-19 with hypertension and without hypotension were such as white blood cell, lymphocyte, heamgoblin and D-dimer, In contrast the differences in liver enzyme (AST, ALT), Ferritin were significant, table (3-7).

Table (3-7): Frequency distribution of covid-19 patients with systemic hypertension and covid-19 patients without systemic hypertension according to some variables.

	Case with COVID-19		
	with systemic hypertension N=49	*without systemic hypertension* N=39	*P*
White blood cells g\l			
Mean± SD	**15.45** ± 3.34	**16.76** ± 4.48	**0.122 † NS**
Range	10.44 – 24.80	10.80 – 26.20	
SE	**0.46**	**0.75**	
Lymphocyte g\l			
Mean± SD	**5.91** ± 3.66	**5.31** ± 3.16	**0.431 † NS**
Range	1.10- 17.20	1.20 - 13.40	
SE	**0.508**	**0.534**	
Hemoglobin g\dl			
Mean± SD	**12.18** ± 1.66	**12.60** ± 1.88	**0.279 † NS**
Range	8.10 - 16.00	7.70 - 17.70	
SE	**0.230**	**0.318**	
AST U\L			
Mean± SD	**35.85** ± 12.18	**41.42** ± 11.71	**0.037 † S**
Range	13.00 - 55.00	15.00 - 59.00	
SE	**1.68**	**1.97**	
ALT U\L			
Mean± SD	**34.74** ± 13.15	**40.83** ± 13.33	**0.038 † S**
Range	10.00 - 54.00	12.00 - 58.00	
SE	**1.82**	**2.25**	
Ferritin ng\ml			
Mean± SD	**471.75** ± 87.77	**525.92** ± 136.99	**0027 † S**
Range	213.40- 751.00	279.80- 844.10	
SE	**12.17**	**23.15**	
D-dimer ng\ml			
Mean± SD	**1005.51** ± 439.28	**754.02** ± 514.30	**0.325 †**
Range	101.00 - 7622.00	311.41- 3483.00	

SE	**199.59**	**86.93**	**NS**

***N*:** number of cases**; SD:** standard deviation**; †:** independent samples t-test; **HS:** Highly significant at **P ≤ 0.001; NS:** not significant at **P ≤ 0.05.**

The comparison of white blood cell between covid-19 patients with hypertension and covid-19 patients without hypertension has been carried out, Mean levels of WBC in covid-19 patients with hypotension were higher than mean WBC in covid-19 patients without hypertension, (16.76 ± 4.48) versus, (15.45 ± 3.34) the difference was non-significant (P =0.122).That identified evidence in support of a causal relationship between circulating lymphocytes and risk of Chronic heart disease (CHD), Although the genetic effect of lymphocyte count on CHD reported that Driven by variants in the major histocompatibility complex locus, **(Astle *et al.*, 2012).** The study provides a plausible mechanism by which lymphocytes might cause CHD, through increases in blood pressure(BP)parameters The relationship between total lymphocyte count and both Systolic Blood Pressure (SBP) and Diastolic blood pressure (DBP) that was independent of the major histocompatibility complex region. This is of importance because SBP and DBP are recognized causal risk factors for cardiovascular disease, **(Flint *et al.*, 2019).** The mechanism by which white blood cells, At the effects of lymphocytes might be mediated by modulating vascular function, Sympathetic outflow, And hypertension as well as renal sodium reabsorption and salt handling by antigen presenting cells, **(Van *et al.*,2019).**

Thus, the mean of hemoglobin in covid-19 patients with hypertension (12.18 ± 1.66) was lower than in patients without hypertension (12.60 ± 1.880). In pervious study, the Person was evaluated Hemoglobin level in arterial blood pressure in persons, **(Göbel *et al.*, 1991).** The mechanism for blood pressure increase with increased Hb levels would be increased blood viscosity. That elevation of hematocrit and Hb levels increases blood viscosity and that increased

viscosity partly through an effect on blood pressure may worsen cardiovascular function. However, other research is inconclusive about the role of blood viscosity in high blood pressure and hypertension. Studies in hypertensive patients do support the role of increased blood viscosity in raising blood pressure but not in healthy individuals, **(Vázquez *et al.*, 2012).**

The mean lymphocyte in patients of covid-19 hypertension was (5.91 ± 3.66) higher than non-hypertension (5.31 ± 3.16). In the epidemiological study, the lymphocyte were elevated in BP. **(Demir., 2013).** The pathologic and molecular mechanisms by which BP variability leads to vascular disease are controversial. It has been suggested that BP variability may promote endothelial expression of cytokines and stimulate inflammation. The total white blood cell (WBC) count and its subtypes, such as neutrophil, lymphocyte and neutrophil/lymphocyte ratio (NLR) can be used as an indicator of systemic inflammation, **(Chae *et al.*, 2001).**

While, the means of liver enzyme patient of covid-19 without hypertension were (41.42 ± 11.71AST), (40.83 ± 13.33ALT) greater than with hypertension (AST 35.85 ± 12.18), (34.74 ± 13.15ALT).since, that the content of hypertensinogen was reduced in human plasma in patients with hepatic insufficiency. Renin is generally considered to be destroyed in the liver since hepatectomy will delay its removal from the blood. Likewise hypertensinase, the hypertension splitting enzyme, is also said to be present in liver as well as in many other parts of the organism; however, its concentration in the blood is said not to be changed in hepatic insufficiency **(Preetha S., 2016).**

Then, the mean of ferritin of hypertension in covid-19 patients was increased of mean without hypertension (471.75 ± 87.77) versus (525.92 ± 136.99), the difference was significant (P =0.027). The ferritin levels were associated with insulin resistance and the development of diabetes, (**Bonnet *et al.*, 2017).** This

association can be mediated by the presence of fatty liver disease. Then, it associated with a progressive increase in BP over time and with incident hypertension. Iron overload might play a role in the pathogenesis of fatty liver disease by increasing insulin resistance and oxidative stress (**Kim *et al.*, 2012).**

Finally, the mean of D-dimer with hypertension raised 1005.51 ± 439.28 then hypotensien754.02 ± 514. Similar finding, it observed higher D-dimer levels among the hypertensive patients, when compared with the normal healthy controls. This difference may probably be related to a difference in blood pressure values of patients included in these study or interference of antihypertensive treatments, **(Zhang *et al.*,2003).**

Table (3-8): Frequency distribution of covid-19 patients with systemic hypertension and covid-19 patients without systemic hypertension according to level of VCM-1.

	Cases with COVID-19		
VCM-1 (pg/ml)	***covid-19 patients with systemic hypertension* N=49**	***covid-19 patients without systemic hypertension* N=39**	***P***
Range	34.66– 562.40	114.00 – 456.00	**0.681 † NS**
Median (IQR)	195.75 (108.26)	199.00 (114.34)	

***N*:** number of cases; **IQR**: inter-quartile range; **†:** Mann Whitney U test; **HS:** Highly significant at ***P* ≤ 0.001**

The comparison of serum VCM-1 level between covid-19 patients with systemic hypertension and covid-19 patients without systemic hypertension has been carried out and the results were demonstrated in table (3-8) and figure (3-5), Median levels of serum VCM-1 in covid-19 patients with systemic hypertension were lower than in comparison the median levels of covid-19 patients without systemic hypertension, 199.00 (113.20) pg/ml versus 195.75 (114.34) pg/ml, The difference was non-significant (P = 0.681). Previous report, it have shown that

increased levels of soluble VCM-1 were found in patients with complicated hypertension **(Ferri *et al.*, 1999).** With increased BP, sheer stress may induce an inflammatory condition and endothelial cells may be activated by inflammatory cytokines, whereas ICM-1 and VCM-1 mediate strong adhesion of leukocytes to the endothelium. In addition, it was reported that endothelial dysfunction reflects the effects of chronic hypertension and it is more likely in hypertensive patients with cardiac hypertrophy than in patients without cardiac hypertrophy, **(Huo *et al.*, 2000).**

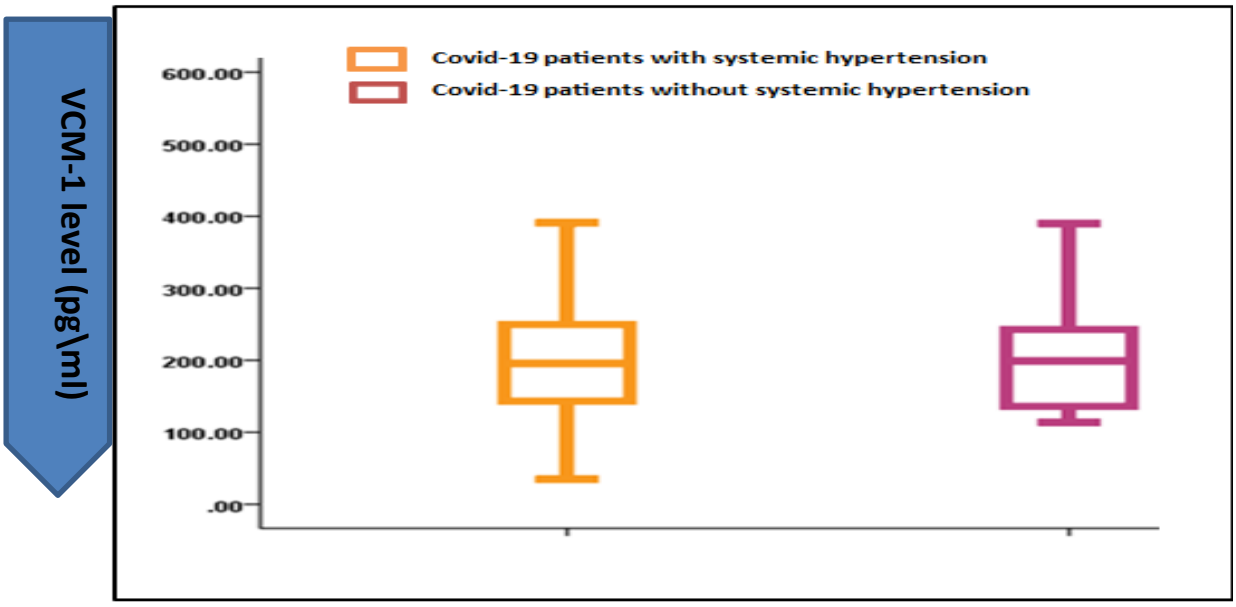

Figure (3-5): Box plot comparison of median serum VCM-1 level among covid-19 patients with systemic hypertension and covid-19 patients without systemic hypertension

Table (3-9): Frequency distribution of covid-19 patients with systemic hypertension and covid-19 patients without systemic hypertension according to level of Vap-1.

	Cases with COVID-19		
VAP-1 (pg/ml)	***with systemic hypertension*** **N=49**	***without systemic hypertension*** **n=39**	***P***
Range	2459.00– 9130.00	2089.00– 8140.00	**0.014 † S**
Median	3719.50 (1108.75)	3600.50 (1029.75)	

(IQR)			

***N*:** number of cases; **IQR:** inter-quartile range**; †:** Mann Whitney U test; **HS:** Highly significant **at *P* ≤ 0.001**

The comparison of serum VAP-1 level between covid-19 patients with systemic hypertension and covid-19 patients without systemic hypertension has been carried out and the results were demonstrated in table (3-9) and figure (3-6), Median levels of serum Vap-1 in covid-19 patients with systemic hypertension were higher than the median levels of covid-19 patients without systemic hypertension,3719.50 (1108.75)pg/ml versus3600.50 (1029.75) pg/ml, the difference was significant (P =0.014).Since in study, VAP-1 was significantly higher in hypertensive patients when compared to their non- hypertensive. It was possible relations between VAP-1 and cardiovascular complications, **(Aronow *et al.*, 2011).** Endothelial VAP-1 can participate in inflammation by binding granulocytes, Lymphocytes, and monocytes, with the aid of SSAO activity. Fibrinogen is an acute phase reactant and is also associated with progression of atherosclerosis in patients with chronic kidney disease, **(Hiramoto *et al* ., 2012).**

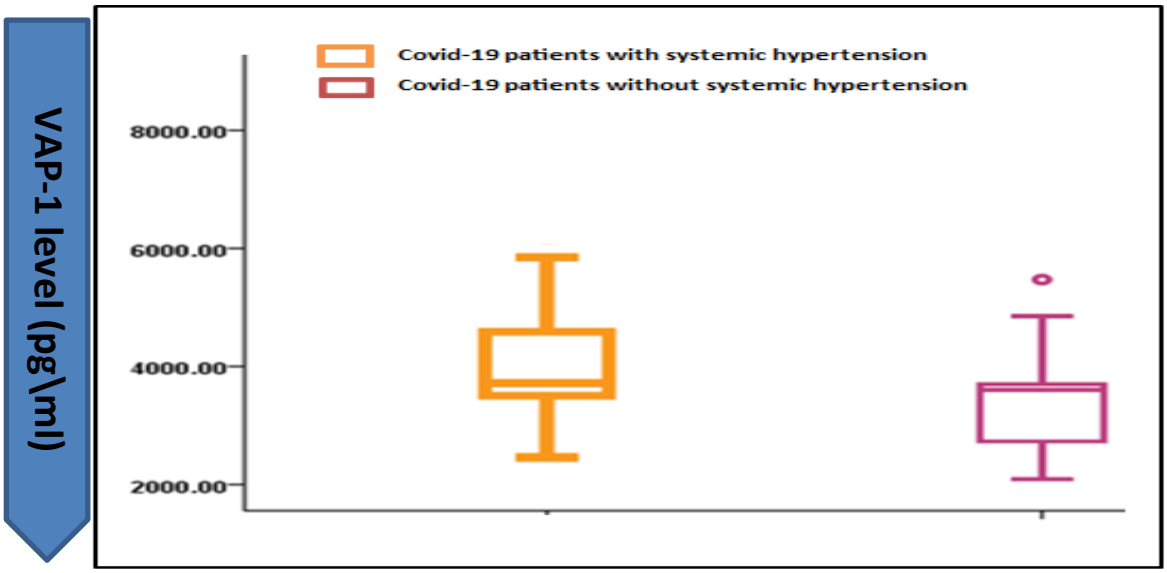

Figure (3-6): Box plot comparison of median serum VAP-1 level among covid-19 patients with systemic hypertension and covid-19 patients without systemic hypertension.

3.6 The comparison between covid-19 patient's smoker and covid-19 patient's non-smoker.

The comparison of white blood cell between covid-19 patients smoker and covid-19 patients non-smoker has been carried out and the results were demonstrated in table (3- 6).

Table (3-10): Frequency distribution of covid-19 patient's smoker and covid-19 patient's non-smoker according to some variables.

	Cases of COVID -19		
	***patients smoker* N=48**	***non-smoker* N=40**	***P***
White blood cells g\l			
Mean± SD	**15.66** ± 3.73	**16.36** ± 3.90	**0.407 † NS**
Range	10.44 – 26.20	10.80 – 24.80	
SE	**0.53**	**0.65**	
Lymphocyte g\dl			
Mean± SD	**6.30** ± 3.62	**4.83** ± 2.91	**0.049 † S**
Range	1.60- 17.20	1.20 - 11.20	
SE	**0.51**	**0.49**	
Hemoglobin g\dl			
Mean± SD	**12.53** ± 1.69	**12.08** ± 1.86	**0.258 † NS**
Range	8.10 - 17.70	7.70 – 16.60	
SE	**0.242**	**0.315**	
AST U\L			
Mean± SD	**36.463** ± 12.91	**40.211** ± 11.44	**0.173 † NS**
Range	13.00 - 59.00	14.00 - 59.00	
SE	**1.64**	**1.93**	
ALT U\L			
Mean± SD	**35.62** ± 14.199	**39.57** ± 12.86	**0.196 † NS**
Range	10.00 - 57.00	12.00 - 58.00	
SE	**2.028**	**2.17**	
Ferritin ng\ml			
Mean± SD	**521.45** ± 141.47	**447.47** ± 66.46	**0.005 † S**
Range	140.40- 844.10	340.20 - 623.10	
SE	**20.21**	**11.23**	
D-dimer ng\ml			
Mean± SD	**978.42** ± 418.44	**775.48** ± 360.65	**0.443 † NS**
Range	101.00 - 7622.00	213.20- 5011.00	
SE	**202.63**	**128.57**	

***N*:** number of cases; **SD**: standard deviation; †: independent samples t-test; **HS**: Highly significant at **P ≤ 0.001**; **NS**: not significant at **P ≤ 0.05**.

Mean levels of WBC in covid-19 patients non- smoker were higher than mean WBC of covid-19 patients smoker, (15.66 ± 3.73) versus **(**16.36± 3.90), the difference was non-significant (P =0**.**407**)**. In previous study, elevation of inflammatory markers, such as the WBC count, CRP, IL-6, and IL-8, had been reported in COPD patients, these associations were more significant in the non-current smoker group than in the current smoker group, **(Singh *et al.*, 2010).**

While, the mean of hemoglobin in covid-19 patient's smoker was12.53 ± 1.69 Increase in comparison with non-smoker 12.08 ± 1.86.But, the difference was non-significant (P = 0.258). The hypothesis to explain, it confirmed that hemoglobin levels were higher for smokers than non-smokers, **(Gumus *et al.*, 2010).** Other study, smoking is associated with increase hemoglobin levels in total blood, **(Mahsud *et al.*, 2010).**

The mean lymphocyte of patients of covid-19 smoker was higher than non-smoker (6.30 ± 3.62) versus (4.83 ± 2.91). While, the difference was significant (P = 0.049).the epidemiological study, the lymphocyte have been used as an indicator of inflammation and have been associated with chronic obstructive pulmonary disease (COPD), systemic inflammatory response in rheumatic disease; they can be derived from routine blood investigations without any additional cost. Thus, that lymphocyte increased in the smokers, **(Gumus *et al.*, 2018).**

While, the means of liver enzyme patient of covid-19 non-smoker were (40.211±11.44AST), (39.57±12.86ALT) greater than smoker (AST36.463 ± 12.91), (35.62 ± 14.199ALT).Since, the difference was non- significant (P =0.173, 0.196, respectively). The study, that is on the association between smoking and liver enzymes, **(Breitling *et al.*, 2009).** The particularly hazardous effect of heavy drinking and smoking on liver enzyme, Which is a risk factor for liver cancer and

cirrhosis, it may contribute to the synergistic effects of smoking and alcohol on liver cancer and alcoholic cirrhosis reported in study, **(Altamirano *et al.*,2010).**

Then, the mean of ferritin of smoker in covid-19 patients increased of mean non-smoker 521.45 ± 141.47versus447.47 ± 66.46.thus, the difference was significant (P =0.005)the lung epithelial cells can rapidly increase the expression of ferritin protein. Ferritin restricts the capacity of iron to generate reactive oxygen species and free radicals via iron sequestration and, thus, serves as an antioxidant protein, **(Ghi *et al.*, 2009).**

Finally, the mean of D-dimer smoker increased in comparison with (978.42 ± 418.44) than non-smoker (775.48 ± 360.65), But the difference was non-significant (P =0.443). In the study, had numerically higher levels of D-dimer than non-smokers, this difference were in healthy HIV-infected (human immunodeficiency virus) adults **(Caponnetto *et al.*, 2011).**

Table (3-11): Frequency distribution of covid-19 patient's smoker and covid-19 patient's non-smoker according to level of Serum VCM-1

	Cases of COVID-19		
VCM-1 (pg/ml)	***covid-19 patients smoker*** **N=48**	***covid-19 patients non-smoker*** **N=40**	***P***
Range	34.66– 562.40	98.20– 219.80	**0.001 † S**
Median (IQR)	236.00 (167.42)	180.30 (56.66)	

***N*:** number of cases; **IQR:** inter-quartile range; **†:** Mann Whitney U test; **HS:** Highly significant at $P \leq 0.001$

The comparison of serum VCM-1 level between covid-19 patients smoker and covid-19 patients non-smoker has been carried out and the results were demonstrated in table (3-11) and figure (3-7), Median levels of serum VCM-1 in covid-19 patients smoker were higher than in comparison the median levels of covid-19 patients non-smoker , 236.00 (167.42)pg/ml versus 180.30 (56.66)

pg/ml, The difference was highly significant (P = 0.001). A previous study, the patients diagnosed with atherosclerosis observed that VCM-1 values higher concentration, these molecules were strong and independent predictors of mortality in patients with atherosclerosis. The primary origin of CAD (Coronary artery disease) is atherosclerosis, which is a chronic inflammatory process that induces the expression of adhesion molecules at the endothelial membrane. VCM-1, ICAM-1 and E-selectin promote the connection of leukocytes and their migration into the intima, whereas a metalloproteinase plays a special role in the instability of atherosclerotic plaques. These molecules can also be found in the serum in a soluble form due to the protolithic rupture of cellular membranes in which adhesion molecules are expressed. Therefore, their measurement can be evaluated in several clinical situations in which inflammation is a causal contributor, making these molecules easily accessible serum markers, **(Hoke *et al.*, 2015).**

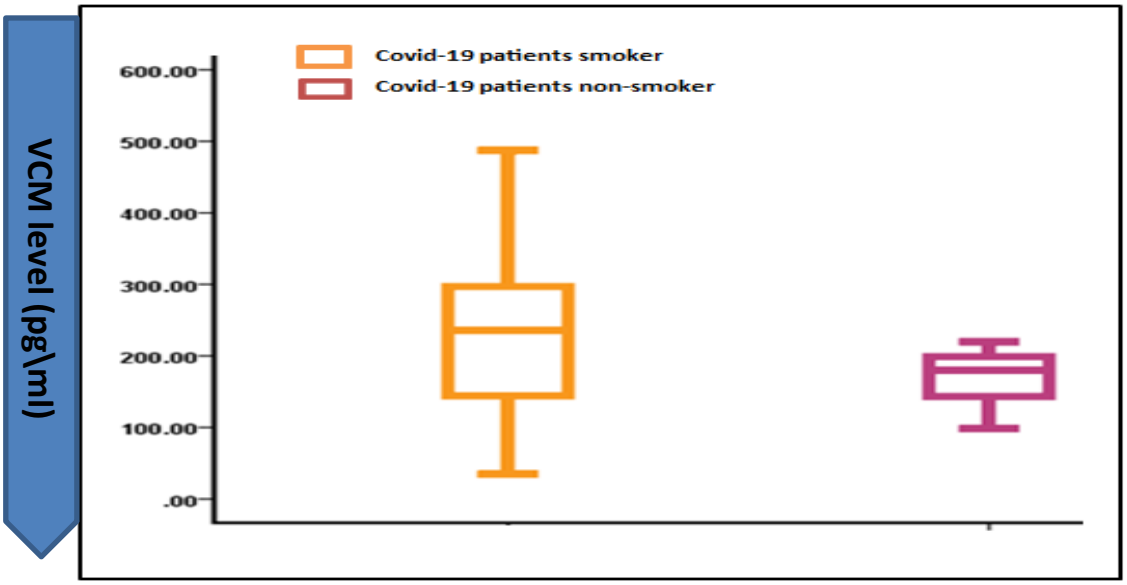

Figure (3-7): Box plot of comparison of median serum VCM-1 level among covid-19 patient's smoker and covid-19 patient's non-smoker

Table (3-12): Frequency distribution of covid-19 patient's smoker and non-smoker according to level of Serum VAP-1

VAP-1 (pg/ml)	*Cases COVID-19*		
	covid-19 patients smoker **N=48**	***covid-19 patients non-smoker*** **N=40**	***P***
Range	2456.00– 9130.00	2089.00– 4889.00	**0.009** † **S**
Median (IQR)	3698.00 (1571.50)	3550.00 (1516.00)	

N: number of cases; **IQR**: inter-quartile range; †: Mann Whitney U test; **HS**: Highly significant at $P \leq 0.001$

The comparison of serum Vap-1 level between covid-19 patients smoker and covid-19 patients non-smoker has been carried out and the results were demonstrated in table (3-12) and figure (3-8), Median levels of serum Vap-1 in covid-19 patients smoker were higher than in comparison the median levels of covid-19 patients non-smoker, 3698.00 (1571.50)pg/ml versus3550.00 (1516.00) pg/ml, the difference was highly significant (P = 0.009).since, the serum VAP-1 levels in those with a smoking history were higher than in those without a smoking history, although, **(Wan *et al.*, 2013).** The enzymatic activity of VAP-1 converts methylamine to hydrogen peroxide, ammonium, and aldehydes, all of which promote inflammation by up-regulating the expression and release of selectins, which are classic adhesion molecules, onto endothelium, **(Jalkanen *et al.*, 2007).**

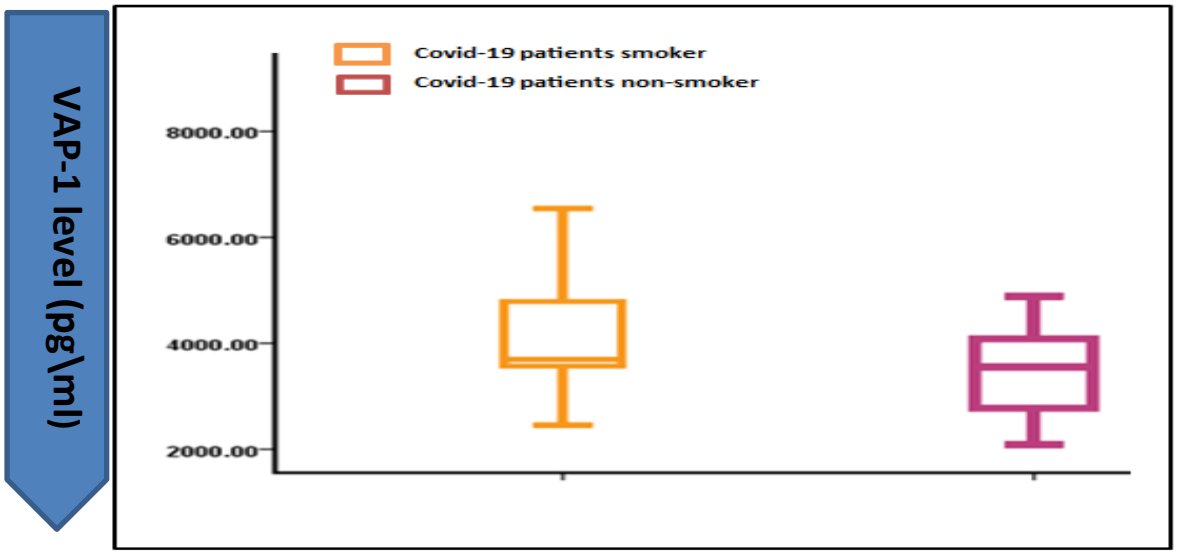

Figure (3-8): Box plot showing comparison of median serum VAP-1 level among covid-19 patient's smoker and covid-19 patient's non-smoker.

3.7 -The comparison between mild covid-19 patients and severe covid-19 patients

The comparison of white blood cell between sever covid-19 patients and mild covid-19 patients has been carried out and the results were demonstrated in table (3- 13).

Table (3-13): Frequency distribution of mild covid-19 patients and severe covid-19 patients according to some variables

	Cases of COVID-19		
	mild covid-19 patients **N=69**	***severe covid-19 patients*** **N=19**	***P***
White blood cells g\l			
Mean± SD	**14.89** ± 3.09	**19.076** ± 3.87	**0.001 † HS**
Range	10.44 – 26.20	12.60 – 24.80	
SE	**0.37**	**0.94**	
Lymphocyte g\dl			
Mean± SD	**6.279** ± 3.45	**4.27** ± 3.01	**0.031 † S**
Range	1.60- 17.20	1.20 - 11.20	
SE	**0.41**	**0.73**	
Hemoglobin g\dl			
Mean± SD	**12.25** ± 1.69	**12.61** ± 2.161	**0.468 †**
Range	7.70 - 16.00	9.60 – 17.70	

SE	**0.203**	**0.524**	**NS**
AST U\L			
Mean± SD	**32.91** ± 12.15	**42.66** ± 11.28	**0.039 † S**
Range	13.00 - 59.00	15.00 - 53.00	
SE	**2.43**	**2.73**	
ALT U\L			
Mean± SD	**35.06** ± 13.54	**42.49** ± 11.73	**0.041 † S**
Range	10.00 - 57.00	12.00 - 58.00	
SE	**1.63**	**2.84**	
Ferritin ng\ml			
Mean± SD	**485.40** ± 109.97	**513.98** ± 156.02	**0.382 † NS**
Range	140.40 - 829.80	213.40 - 844.10	
SE	**13.23**	**37.84**	
D-dimer ng\ml			
Mean± SD	**913.317** ± 262.15	**815.124** ± 209.51	**0.759 † NS**
Range	101.00 - 7622.00	367.70- 3483.00	
SE	**151.94**	**172.08**	

***N*:** number of cases; **SD**: standard deviation; **†:** independent samples t-test; **HS:** Highly significant at **P** ≤ 0.001; **NS**: not significant at **P** ≤ 0.05

Mean levels of WBC in sever covid-19 patients were higher than mean WBC of mild covid-19 patients, 19.076 ± 3.87 versus14.89 ± 3.09 , The difference was highly significant (P =0.001). In study, it found elevated neutrophil useful predictor for severity and mortality of SARS-CoV-2 infection, **(Liao *et al.*, 2020).**

Thus, the mean of hemoglobin in sever covid-19 patient's was Increase 12.61 ± 2.161Than mild12.25 ± 1.69. But, the difference was non-significant (P = 0.468). Where autoimmune hemolytic anemia occurred during the worsening of symptoms of Covid-19 infection. Hb level with the associated reduction of oxygen carrying capacity of the blood together with CT diagnosed lung parenchymal pathology explains the significant dyspnea associated with increased disease severity, **(Lopez *et al.*, 2020).**

The mean lymphocyte of sever patients of covid-19 was (4.27 ± 3.01) Dropped than (6.279 ± 3.45) mild. While, the difference was significant (P =0.031). The epidemiological study, that Lymphopenia was correlates with several poor patient outcomes, including mortality, Acute Respiratory Distress Syndrome, ICU care, and severe diseases. COVID-19 disease severity has also been linked to the lymphocyte-to-neutrophil cell count ratio, **(Lagunas *et al.*, 2020).** It has been suggested that the reduction of immune cell counts in the peripheral blood during viral infection may be caused by the mobilization of immune cells to sites of infection, such as the lungs, and potentially by virus-induced destruction of T cells, Future prospective studies designed to investigate the utility of lymphocyte subset measurements as prognostic biomarkers of disease severity, mortality, and response to treatment in patients infected with covid-19 are strongly encouraged, **(Merad *et al.*, 2020).**

While, the means of liver enzyme sever patient of covid-19 were (42.66 ± 11.24AST), (42.49 ± 11.73ALT) greater than mild, (AST32.91 ± 12.15), (35.06 ± 13.54ALT).since, the difference was significant (P = 0.039, 0.041, respectively). The epidemiological study, In terms of laboratory finding AST, ALT were significantly higher in severe cases than mild cases, which indicate a degree of cardiac, liver coagulation function abnormality and infection, it should be paid for these patients to avoid delayed treatment, **(Wang *et al.*, 2019).** In another study, that the incidence of hepatic abnormalities was significantly increased after infection with COVID-19**,(Zhang *et al* 2020).**

Then, the mean of ferritin in sever in covid-19 patients increased mean (513.98 ± 156.02) versus (485.40 ± 109.97).thus, the difference was non-significant (P =0.759). The value of serum ferritin was significantly increased in severe and critical patients as compared to mild and moderate patient. In study from (**Wuhan *et al.*, 2020).**

Finally, the mean of D-dimer sever patients raised(815.124 ± 209.51) than mild (913.317 ± 262.15)But the difference was non-significant (P =0.759), further research to investigate the cause-effect relationship between serum D-dimer concentrations, COVID-19 disease severity, The onset of pulmonary complications and clinical outcomes, the identification of D-dimer as a biomarker of COVID-19 severity is potentially clinically relevant. Its relatively simple and inexpensive determination might assist, particularly with serial assessments, with the rapid identification of those patients developing pulmonary compromise, or at risk of venous thromboembolism that requires aggressive care and intensive monitoring, (**Lopez *et al.*, 2020).**

The comparison of serum VCM-1 level between severs covid-19 patients and mildcovid-19 has been carried out and the results were demonstrated in table (3-14).

Table (3-14): Frequency distribution of mild covid-19 patients and severe covid-19 patients according to level of Serum VCM-1.

	Cases of COVID-19		
VCM-1 (pg/ml)	***mild covid-19 patients*** **N=69**	***severe covid-19 patients*** **N=19**	***P***
Range	34.66– 562.40	98.20– 467.65	**0.004 † S**
Median (IQR)	198.30 (105.05)	271.31 (255.95)	

***N*:** number of cases; **IQR**: inter-quartile range; **†:** Mann Whitney U test; **HS**: Highly significant at $P \leq 0.001$

Median levels of serum Vcm-1 in sever covid-19 patients were higher than in comparison the median levels of mild covid-19 patients, 271.31 (255.95)pg/ml versus198.30 (105.05) pg/ml, the difference was highly significant (P =0.004) figure (3-9). Thus, Various infections, including those caused by the severe acute respiratory syndrome (SARS) family of viruses, cause endothelial dysfunction,

which is characterized by a diminished ability to produce nitric oxide and the release of inflammatory markers, such as VCM-1 The unique marker d affinity of coronaviruses to the host a angiotensin converting enzyme 2 receptor, which is expressed in endothelial cells of blood vessels, means a direct effect of covid-19 on the vascular endothelium is distinctly possible. Endothelial dysfunction manifested by reduced nitric oxide bio availability is thought to be an early event in hypertension, diabetes, CHD, and even kidney dysfunction, which were shown here to be significantly associated with patients COVID -19, **(Varga *et al.*, 2020).**

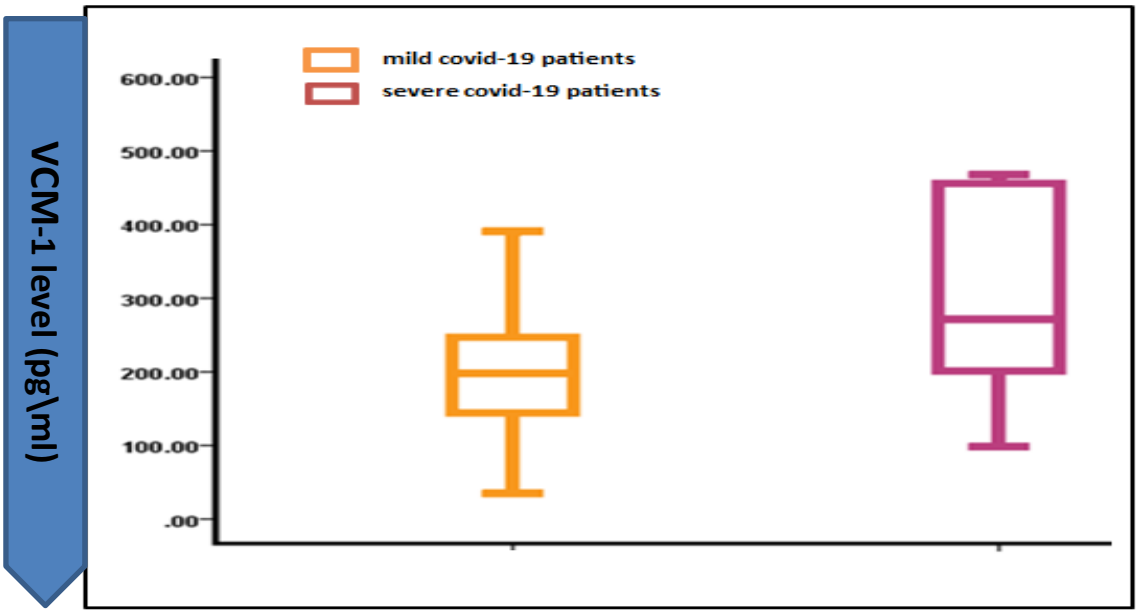

Figure (3-9): Box plot of comparison of median serum VCM-1 level among mild covid-19 patients and severe covid-19 patients.

The comparison of serum Vap-1 level between sever covid-19 patients and mildcovid-19 has been carried out and the results were demonstrated in table (3-15)

Table (3-15): Frequency distribution of mild covid-19 patients and severe covid-19 patients according to level of Serum Vap-1.

	Cases of COVID-19		
VAP-1 (pg/ml)	***mild covid-19 patients* N=69**	***severe covid-19 patients* N=19**	***P***
Range	2089.00– 9130.00	2347.00– 4756.00	**0.009 † S**
Median (IQR)	3687.00 (1654.50)	3664.00 (1055.50)	

***N*: number of cases; IQR:** inter-quartile range**; †:** Mann Whitney U test**; HS:** Highly significant at $P \leq 0.001$

Median levels of serum Vap-1 in mild covid-19 patients were higher than in comparison the median levels of sever covid-19 patients, 3687.00 (1654.50) pg/ml versus3664.00 (1055.50) pg/ml, the difference was highly significant (P =0.009) figure (3-10). According to previous studies, the chronic HCV infection patients, the VAP-1 concentration was significantly elevated in patients with moderate or severe fibrosis especially in the presence of cirrhosis. Fibrosis stages were determined from the liver stiffness. VAP-1 supports leukocyte transmigration in the inflamed tissue and the deleterious effects of its SSAO activity lead to enhanced production of extracellular matrix proteins and increased oxidative stress. This process contributes to progressive fibrosis that ultimately leads to cirrhosis**, (Van der et al., 1992).**

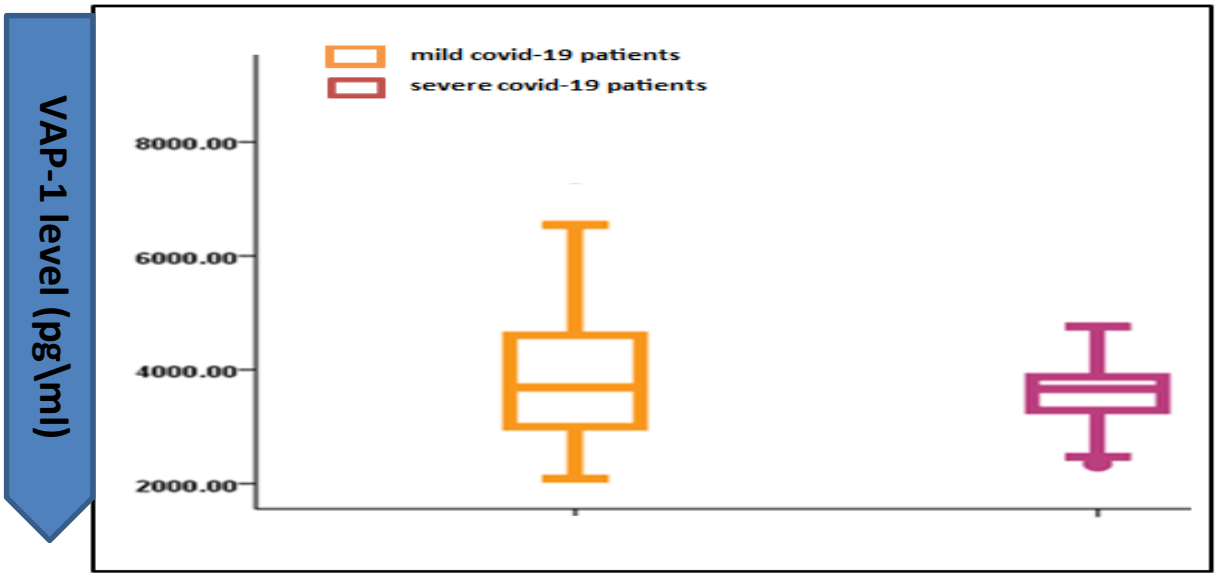

Figure (3-10): Box plot of comparison of median serum VAP-1 level among mild covid-19 patients and severe covid-19 patients.

3.8. Correlations of Serum VCM-1 level and Serum VAP-1 level according to some variable.

Table (3-16): Correlations of Serum VCM-1 level and Serum VAP-1 level according to some variable.

Characteristics	VCM-1		Vap-1	
	R	P	R	P
White blood cells	0.106	0.327 NS	-0.129	0.229 NS
Lymphocyte	-0.003	0.978 NS	0.052	0.630 NS
Hemoglobin	0.322	0.002 S	-0.115	0.288 NS
AST	0.018	0.871 NS	-0.290	0.006 S
ALT	0.059	0.585 NS	-0.228	0.033 S
Ferritin	0.168	0.117 NS	0.254	0.017 S
D-dimer	- 0.150	0.164 NS	- 0.114	0.291 NS

***R*: Spearman correlation coefficient; NS:** not significant **at *P* > 0.05**

As shown in table (3.16) the correlation between white blood cell and vascular adhesion molecule were positive, but it was non-significant (p=0.372, 0.769, respectively). This notion is also supported by the parallelism observed over time between enhanced leukocyte adhesion and VCM-1 expression. The study, although intravital microscopy does not allow discerning which type of leukocytes is being recruited, the predominant role of VCM-1 suggests that most adherent cells correspond to mononuclear leukocytes. However, recent evidence indicates that activated neutrophils can also express the VCM-1 receptor $\alpha_4\beta_1$ and can adhere via this integrin under flow conditions. **(Reinhardt *et al.*, 1997).** Previous investigator, a role for VAP-1 in mediating leukocyte migration and propagation of inflammation has been proposed to be facilitated by both its enzymatic activity, **(Koskinen *et al.*,2004).**

Then, the correlation between hemoglobin and VCM-1 was positive and significant (p=0.327, 0.229, respectively), But the correlation between hemoglobin and vap-1 was negative and non-significant. So, the relation between hemoglobin and vascular adhesion molecules were non- significant, **(Wick *et al.*, 1987).**

The relationship between liver enzyme (AST, ALT) and VCM-1 were positive and non-significant. But, the relation VAP-1and liver enzyme was negative and significant. Since, all relation between vascular adhesion molecule and all liver enzymes were non- significant. Vascular adhesion molecule may be a useful biomarker in patients with chronic liver disease, both for the diagnosis of liver fibrosis as well as its associated complications. Moreover, evidence has mounted for a role for endothelial adhesion molecules in fibrotic diseases in general, as serum VCM-1 levels were elevated in patients with idiopathic pulmonary fibrosis and were predictive of overall and post-transplant survival, **(Haller *et al.*, 1995).**

The correlation between ferritin and VCM-1 were positive and non-significant. But, the relationship between ferritin and VAP-1 were negative and significant (p=0.117, 0.017, respectively). The correlation vascular adhesion molecule and ferritin were significant; ferritin induction by aspirin might explain earlier observations showing that aspirin is capable of directly protecting endothelial cells from oxidant injury. Aspirin-dependent endothelial protection reported was most pronounced when toxicity was induced by exogenous ferrous iron, the main Fenton catalyst of oxygen radical formation in living cells. From the results of the present investigation, **(D*owney, et al* 2017).**

Finally, the relation between d-dimer and vascular adhesion molecule were negative and non-significant (p=0.164, 0.291, respectively). Thus, the correlation between d-dimer and vascular adhesion molecule were non-significant, **(Escher *et al., 2020*).**

3.9. VCM-1 levels in severe covid-19 patients and mild covid-19 patients

To evaluate the VCM-1 cutoff value as well as to predict covid-19 severity as diagnostic tests or adjuvant diagnostic tests, receiver operator characteristic (ROC) curve analysis was carried out and the results are shown in (3.17)

Table (3.17): Sensitivity and specificity of VCM-1 level (< 180.80-fold) in covid-19 severity

VCM-1 level (fold)	Severe Patients N = 19	Mild Patients N = 69
> 180.80	15 (%)	33 (%)
≤ 180.80	4 (%)	36 (%)
Sensitivity %	78.9 %	
Specificity %	52.2 %	
PPV %	31.3 %	
NPV %	90.0 %	

AUC (95% CI)	0.729 (0.591- 0.867)

CI: Confidence interval, **AUC:** Area under curve**, NPV:** Negative predictive value, **PPV:** Positive predictive value.

The VCM-1 cutoff value was > 180.80 with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Area under curve of 78.9 %, 52.2 %, 32.3 %, 90.0 % and 0.729 (0.591- 0.867), figure (3.11).

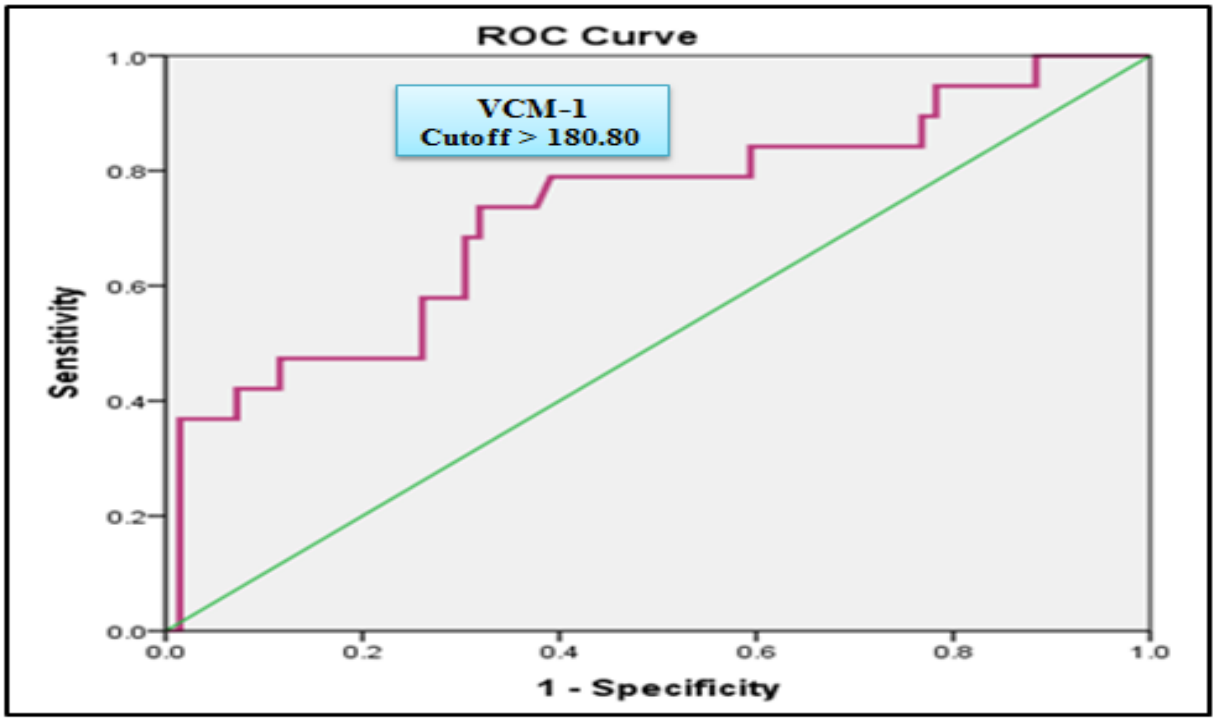

Figure (3.11): Receiver operator characteristic curve analysis for the calculation of VCM-1 possible diagnostic cutoff value

3.10. VAP-1 levels in in severe covid-19 patients and mild covid-19 patients

To evaluate the Vap-1 cutoff value as well as to predict covid-19 severity as diagnostic tests or adjuvant diagnostic tests, receiver operator characteristic (ROC) curve analysis was carried out and the results are shown in (3.18)

Table (3.18): Sensitivity and specificity of Vap-1 level (< 3612.0-fold) in covid-19 severity

VAP-1 level (fold)	Severe Patients N = 19	Mild Patients N = 69
> 3612.0	14 (%)	35 (%)
≤ 3612.0	5 (%)	34 (%)
Sensitivity %	73.7 %	
Specificity %	49.3 %	
PPV %	28.6%	
NPV %	87.2 %	
AUC (95% CI)	0.524 (0.368- 0.679)	

CI: Confidence interval, **AUC:** Area under curve, **NPV:** Negative predictive value**, PPV:** Positive predictive value.

The VAP-1 cutoff value was > 3612.0 with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Area under curve of 73.7 %, 49.3 %, 28.6 %, 87.2 % and 0.524 (0.368- 0.679), figure (3.12).

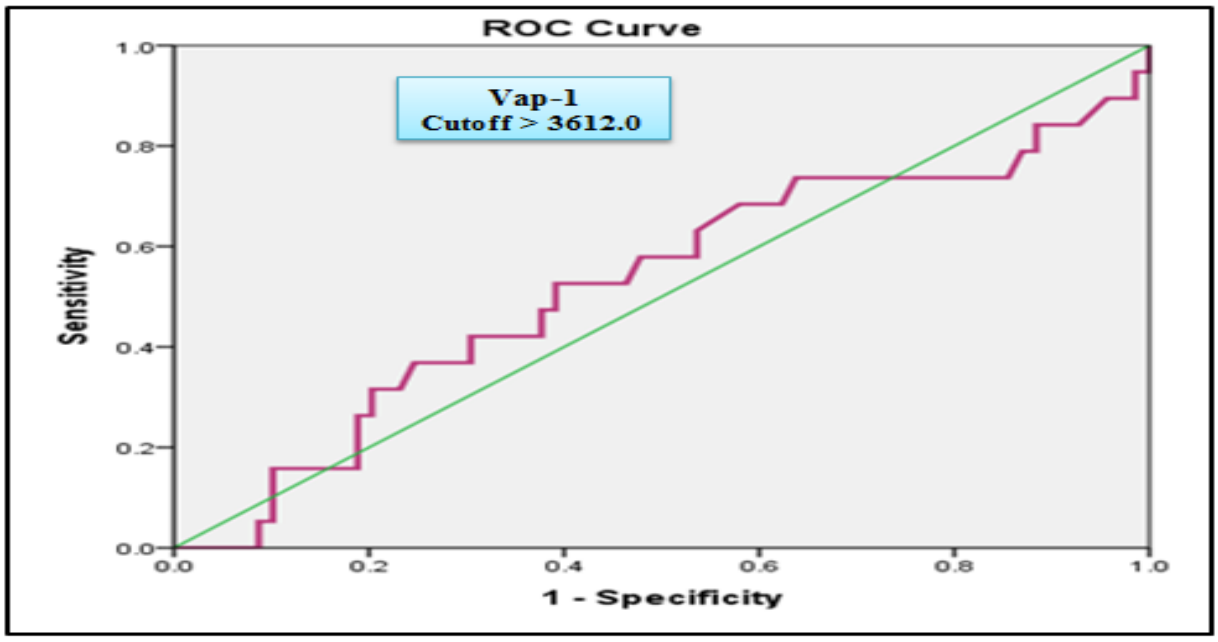

Figure (3.12): Receiver operator characteristic curve analysis for the calculation of VAP-1 possible diagnostic cutoff value.

Chapter Four

Conclusion &Future work

4. Conclusion and future work

According to the results of this study we concluded that

- Serum levels of VAP-1 and VCM-1 associated with the severity of covid-19.
- Serum levels of VAP-1 associated with the risk factors (diabetes, hypertension and smoking) in patients with covid-19.
- The serum levels of Vcam-1 associated just with smoking status in patients with covid-19.
- Lymphocyte and liver enzymes associated with the severity of covid-19.
- Liver enzymes associated with the severity of covid-19 as well as with the hypertension in patients with covid-19.
- Vascular endothelial cell adhesion molecules (VAP-1 and VCM-1) are not predictors for the severity of COVID-19.

4.2. Future work

1. Further studies should be conducted to confirm the significant association of hemoglobin and AST with vascular adhesion molecule
2. Another study for use immunological markers is performed to take the association and effects of this marker with COVID-19infection.
3. Conduct massive studies on covid-19 to found treatments for population

.

Reference

References:

- **Adamson JW, Finch CA(2012)**. Hemoglobin function, oxygen affinity and erythropoietin. Annu Rev Physiol.;37:351.
- **Aronow W. S., Fleg J. L., Pepine C. J., Artinian N. T., Bakris G., Brown A. S., *et al.* (2011).** ACCF/AHA expert consensus document on hypertension in the elderly: a report of the American College of Cardiology Foundation Task Force on clinical expert consensus documents developed in collaboration with the American Academy of Neurology, American Geriatrics Society, American Society for Preventive Cardiology, American Society of Hypertension, American Society of Nephrology, Association of Black Cardiologists, and European Society of Hypertension. *Journal of the American College of Cardiology*, *57*(20), 2037-2114.
- **Bai, Y., Yao, L., Wei, T., Tian, F., Jin, D. Y., Chen, L., Wang, M. (2020).** Presumed asymptomatic carrier transmission of COVID-19. *Jama*, *323*(14), 1406-1407.
- **Barasa, B., Slijper, M. (2014).** Challenges for red blood cell biomarker discovery through proteomics. *Biochimic et Biophysic Acta (BBA)-Proteins and Proteomics*, *1844*(5), 1003-1010.
- **Beghi, E., Feigin, V., Caso, V., Santalucia, P., & Logroscino, G. (2020).** COVID-19 infection and neurological complications: present findings and future predictions. *Neuroepidemiology*, *54*(4), 1-6.
- **Bikdeli, B., Madhavan, M. V., Jimenez, D., Chuich, T., Dreyfus, I., Driggin, E., *et al.* (2020).** COVID-19 and thrombotic or thromboembolic disease: implications for prevention, antithrombotic therapy, and follow-up: JACC state-of-the-art review. *Journal of the American college of cardiology*, *75*(23), 2950-2973.
- **Brittenham GM, Weiss G, Brissot P, Lainé F, Guillygomarch A,*et al.* (2000)** .Clinical consequences of new insights in the pathophysiology of disorders of iron and heme metabolism. *Hematology Am Soc Hematol Educ Program* 2000: 39-50.
- **Brodin, P. (2021)**. Immune determinants of COVID-19 disease presentation and severity. *Nature Medicine*, *27*(1), 28-33.
- **Chan, J. F. W., Yuan, S., Kok, K. H., To K. K. W., Chu, H., Yang, J.,**

***et.al*. (2020).** A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *The lancet*, *395*(10223), 514-523.

- **Chan, *J. F*., Lau, S. K., To, K. K., Cheng, V. C., Woo, P. C., & Yuen, K. Y. (2015).** Middle East respiratory syndrome coronavirus: another zoonotic beta coronavirus causing SARS-like disease. *Clinical microbiology reviews*, *28*(2), 465-522.
- **Choi, K. M., Lee, K. W., Kim, H. Y., Seo, J. A., Kim, S. G., Kim, N. H., *et al.* (2005).** Association among serum ferritin, alanine aminotransferase levels, and metabolic syndrome in Korean postmenopausal women. *Metabolism*, *54*(11), 1510-1514.
- **Contreras-Zentella M. L., Sánchez-Sevilla L., Suárez-Cuenca J. A., Olguín-Martínez M., Alatriste-Contreras M. G., García-García N. *et al.* (2019).** The role of oxidant stress and gender in the erythrocyte arginine metabolism and ammonia management in patients with type 2 diabetes. *PloS one*, *14*(7), e0219481.
- **Cook-Mills J. M., Marchese M. E., Abdala-Valencia, H. (2011).** Vascular cell adhesion molecule-1 expression and signaling during disease: regulation by reactive oxygen species and antioxidants. *Antioxidants & redox signaling*, *15*(6), 1607-1638.
- **Coperchini F., Chiovato L., Croce L., Magri F., Rotondi M. (2020).** The cytokine storm in COVID-19: An overview of the involvement of the chemokine/chemokine-receptor system. *Cytokine & growth factor reviews*, 53:25-32.
- **Cunningham JW, Vaduganathan M, Claggett BL, *et al* (2020).** Clinical outcomes in young US adults hospitalized with COVID-19. *JAMA Intern Med* . 10.1001/jamainternmed.2020.5313.
- **Demir M. (2013).** The relationship between neutrophil lymphocyte ratio and non-dipper hypertension. *Clinical and Experimental Hypertension*, *35*(8), 570-573.
- **Downey R. M., Liao P., Millson E. C., Quyyumi A. A., Sher S., Park J. (2017)**. Endothelial dysfunction correlates with exaggerated exercise pressor response during whole body maximal exercise in chronic kidney

disease. *American Journal of Physiology-Renal Physiology*, *312*(5), F917-F924.

- **Driessche K. V., Nestele J., Grouwels J., Duval E. L. (2020)**. Exposure to cough aerosols and development of pulmonary COVID-19. *Journal of Breath Research*, *14*(4), 041003.
- **Duyndam M. C., Hulscher T. M., Fontijn D., Pinedo H. M., Boven E. (2001).** Induction of vascular endothelial growth factor expression and hypoxia-inducible factor 1α protein by the oxidative stressor arsenite. *Journal of Biological Chemistry*, 276(51), 48066-48076.
- **Ernberg K., McGrath A. P., Peat T. S., Adams T. E., Xiao X., Pham T., *et al.* (2010).** A new crystal form of human vascular adhesion protein 1. *Acta Crystallographica Section F: Structural Biology and Crystallization Communications*, *66*(12), 1572-1578.
- **Escher R., Breakey N., Lämmle B. (2020).** Severe COVID-19 infection associated with endothelial activation. *Thrombosis research*, *190*, 62.
- **Esposito C., Fasoli G., Plati A., Bellotti N., Conte M. M., Cornacchia F., *et al.* (2001).** Long-term exposure to high glucose up-regulates VCAM-induced endothelial cell adhesiveness to PBMC. *Kidney international*, *59*(5), 1842-1849.
- **Feld J, Tremblay D, Thibaud S, Kessler A, Naymagon L. (2020)**Ferritin levels in patients with COVID-19: A poor predictor of mortality and hemophagocytic lymphohistiocytosis. Int J Lab Hematol. 42:773–779
- **Flint A. C., Conell C., Ren X., Banki N. M., Chan S. L., Rao V. A., *et al.* (2019).** Effect of systolic and diastolic blood pressure on cardiovascular outcomes. *New England Journal of Medicine*, *381*(3), 243-251.
- **Furuse Y., Sando E., Tsuchiya N., Miyahara R., Yuda I., Ko Y. K., *et al.* (2020).** Clusters of coronavirus disease in communities, Japan. *Emerging infectious diseases*, *26*(9), 2176.
- **Gay F.; Aguera K.; Sénéchal K.; Tainturier A.; Berlier W.; Maucort-Boulch D. *et al.* (2017)**: Methionine tumor starvation by erythrocyte-encapsulated methionine gamma-lyase activity controlled with per os vitamin B6. Cancer Med. 6, 1437–1452.
- **Ge X. N., Bahaie N. S., Kang B. N., Hosseinkhani, M. R., Ha, S. G.,**

Frenzel, E. M., *et al.* (2010). Allergen-induced airway remodeling is impaired in galectin-3–deficient mice. *The Journal of Immunology*, *185*(2), 1205-1214.

- **Gheblawi, M., Wang, K., Viveiros, A., Nguyen, Q., Zhong, J. C., Turner, A. J., *et al.* (2020).** Angiotensin-converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system: celebrating the 20th anniversary of the discovery of ACE2. *Circulation research*, *126*(10), 1456-1474.
- **Ghiasvand F., Miandoab S. Z., Harandi H., Golestan F. S., Alinaghi S. A. (2020).** A patient with COVID-19 disease in a Referral Hospital in Iran: a typical case. *Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders)*, *20*(4), 559-562.
- **Ghio A. J. (2009).** Disruption of iron homeostasis and lung disease. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1790*(7), 731-739.
- **Göbel B. O., Schulte-Göbel A., Weisser B., Glönzer K., Vetter H., Düsing R. (1991).** Arterial blood pressure: Correlation with erythrocyte count, hematocrit, and hemoglobin concentration. *American journal of hypertension*, *4*(1), 14-19.
- **Gregory, J. M., Slaughter, J. C., Duffus, S. H., Smith, T. J., LeStourgeon, L. M., Jaser, S. S., ... & Moore, D. J. (2021).** COVID-19 severity is tripled in the diabetes community: a prospective analysis of the pandemic's impact in type 1 and type 2 diabetes. *Diabetes Care*, *44*(2), 526-532.
- **Guan, C. S., Lv, Z. B., Yan, S., Du, Y. N., Chen, H., Wei, L. G., ... & Chen, B. D. (2020).** Imaging features of coronavirus disease 2019 (COVID-19): evaluation on thin-section CT. *Academic radiology*, *27*(5), 609-613.
- **Guan, C. S., Lv, Z. B., Yan, S., Du, Y. N., Chen, H., Wei, L. G., *et al.* (2020).** Imaging features of coronavirus disease 2019 (COVID-19)
- **Gumus, F., Solak, I., & Eryilmaz, M. A. (2018)**. The effects of smoking on neutrophil/lymphocyte, platelet//lymphocyte ratios. *Bratislavske lekarske listy*, *119*(2), 116-119.
- **Haller, G. W., Langrehr, J. M., Blumhardt, G., Platz, K. P., Müller, A.,**

Reference

Bechstein, W. O., ... & Knoop, M. (1995, February). Factors relevant to the development of primary dysfunction in liver allografts. In *Transplantation proceedings* (Vol. 27, No. 1, p. 1192).

- **Heneka M. T., Golenbock D., Latz E., Morgan D., Brown, R. (2020)**. Immediate and long-term consequences of COVID-19 infections for the development of neurological disease. *Alzheimer's research & therapy*, *12*(1), 1-3.
- **Heneka M. T., Golenbock D., Latz E., Morgan D., Brown, R.(2020)** 2020). Immediate and long-term consequences of COVID-19 infections for the development of neurological disease. *Alzheimer's research & therapy*, *12*(1), 1-3.
- **Henry BM, de Oliveira MHS, Benoit S, Plebani M, Lippi G. (2020)**Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. *Clin Chem Lab Med.*58:1021–8. doi: 10.1515/cclm-2020-0369.
- **Henry, B. M., De Oliveira, M. H. S., Benoit, S., Plebani, M., & Lippi, G. (2020**). Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. *Clinical Chemistry and Laboratory Medicine (CCLM)*, *58*(7), 1021-1028
- **Hiramoto J. S., Katz R., Peralta C. A., Ix J. H., Fried L., Cushman M., *et al.* (2012).** Inflammation and coagulation markers and kidney function decline: the Multi-Ethnic Study of Atherosclerosis (MESA). *American journal of kidney diseases*, *60*(2), 225-232.
- **Hoke M., Winter M. P., Wagner O., Exner M., Schillinger M., Arnold, Z., *et al.* (2015).** The impact of selectins on mortality in stable carotid atherosclerosis. *Thrombosis and haemostasis*, *114*(09), 632-638.
- **Hu Y., Sun J., Dai Z., Deng H., Li X., Huang Q., *et al.* (2020).** Prevalence and severity of corona virus disease 2019 (COVID-19): A systematic review and meta-analysis. *Journal of Clinical Virology*, *127*, 104371.
- **Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China (2020)**. Lancet.

;395:497-506liver failure. Liver Int. 2020;40:1457-1466.

- **Huo, Y., Hafezi-Moghadam A., Ley, K. (2000).** Role of vascular cell adhesion molecule-1 and fibronectin connecting segment-1 in monocyte rolling and adhesion on early atherosclerotic lesions. *Circulation research*, *87*(2), 153-159.
- **Izuta H., Matsunaga N., Shimazawa M., Sugiyama T., Ikeda T., Hara, H. (2010).** Proliferative diabetic retinopathy and relations among antioxidant activity, oxidative stress, and VEGF in the vitreous body. *Molecular vision*, *16*, 130.
- **Jalkanen S., Karikoski M., Mercier N., Koskinen K., Henttinen T., Elima, K., *et al.(*2007).** The oxidase activity of vascular adhesion protein-1 (VAP-1) induces endothelial E-and P-selectins and leukocyte binding. *Blood, The Journal of the American Society of Hematology*, *110*(6), 1864-1870.
- **Jin J. M., Bai P., He W., Wu F., Liu X. F., Han D. M., *et al.* (2020).** Gender differences in patients with COVID-19: focus on severity and mortality. *Frontiers in public health*, *8*, 152.
- **Jonathan-Moyal, É., Vendrely, V., Motte, L., Balosso, J., Thariat, J. (2020):** Radioresistant tumours From identification to targeting. *Cancer/Radiotherapie*, *24*(6-7), 699-705.
- **Kidder D. P., Czeisler C. A. (2020).** Public attitudes, behaviors, and beliefs related to COVID-19, stay-at-home orders, nonessential business closures, and public health guidance—United States, New York City, and Los Angeles, *Morbidity and Mortality Weekly Report*, *69*(24), 751.
- **Kim, M. K., Baek, K. H., Song, K. H., Kang, M. I., Choi, J. H., Bae, J. C., *et al.* (2012).** Increased serum ferritin predicts the development of hypertension among middle-aged men. *American journal of hypertension*, *25*(4), 492-497.
- **Koskinen K., Vainio P. J., Smith D. J., Pihlavisto M., Ylä-Herttuala, S., Jalkanen S., *et al.* (2004):** Granulocyte transmigration through the endothelium is regulated by the oxidase activity of vascular adhesion protein-1 (VAP-1). *Blood*, *103*(9), 3388-3395.
- **Koskinen, K., Vainio P. J., Smith D. J., Pihlavisto M., Ylä-Herttuala S.,**

Jalkanen, S., *et al.* (2004): Granulocyte transmigration through the endothelium is regulated by the oxidase activity of vascular adhesion protein-1 (VAP-1). *Blood*, *103*(9), 3388-3395.

- **Lagunas-Rangel F. A. (2020):** Neutrophil-to-lymphocyte ratio and lymphocyte-to-C-reactive protein ratio in patients with severe coronavirus disease 2019 (COVID-19): a meta-analysis. *Journal of medical virology*.
- **Lechien J. R., Chiesa-Estomba C. M., Place S., Van Laethem Y., Cabaraux P., Mat Q., *et al.* (2020):** Clinical and epidemiological characteristics of 1420 European patients with mild-to-moderate coronavirus disease 2019. *Journal of internal medicine*, *288*(3), 335-344.
- **Lee G. K., Lee L. C., Chong E., Lee C. H., Teo S. G., Chia B. L., *et al.* (2012):** The long-term predictive value of the neutrophil-to-lymphocyte ratio in Type 2 diabetic patients presenting with acute myocardial infarction. *QJM: An International Journal of Medicine*, *105*(11), 1075-1082.
- **Lei F, Liu YM, Zhou F, Qin JJ, Zhang P, Zhu L, *et al.* *(*2020)**: Longitudinal association between markers of liver injury and mortality in COVID-19 in China. Hepatology 72(2):389-398..
- **Leinonen, E., Hurt-Camejo, E., Wiklund, O., Hultén, L. M., Hiukka, A., Taskinen, M. R. (2003):** Insulin resistance and adiposity correlate with acute-phase reaction and soluble cell adhesion molecules in type 2 diabetes. *Atherosclerosis*, *166*(2), 387-394.
- **Liao D., Zhou F., Luo L., Xu M., Wang H., Xia J., *et al.* (2020).** Haematological characteristics and risk factors in the classification and prognosis evaluation of COVID-19: a retrospective cohort study. *The Lancet Haematology*, *7*(9), e671-e678.
- **Liu W, Li H (2020) COVID-19:** Attacks the 1-Beta Chain of Hemoglobin and Captures the Porphyrin to Inhibit Human Heme Metabolism. ChemRxiv.
- **Llitjos J. F., Leclerc M., Chochois C., Monsallier J. M., Ramakers M., Auvray M., *et al.* (2020).** High incidence of venous thromboembolic events in anticoagulated severe COVID-19 patients. *Journal of Thrombosis and Haemostasis*, *18*(7), 1743-1746.
- **Lopez C., Kim J., Pandey A., Huang T., DeLoughery T. G. (2020).**

Simultaneous onset of COVID-19 and autoimmune haemolytic anaemia. *British journal of haematology*. 190(1):31-32.

- **Mahsud, M. A. J., Khan, A., & Hussain, J. (2010).** Hematological changes in tobacco using type 2 diabetic patients. *Gomal Journal of Medical Sciences*, *8*(1):8-11.
- **Memar EHE, Mamishi S, Ekbatani MS, Alimadadi H, Yaghmaei B, Chegini V, *et al*(2020).** Fulminant hepatic failure: a rare and devastating manifestation of Coronavirus disease 2019 in an 11-year-old boy. *Arch Pédiatr*. 27:502–5. doi: 10.1016/j.arcped.2020.09.009.
- **Merad M., Martin J. C. (2020).** Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. *Nature reviews immunology*, *20*(6), 355-362.
- **Montalvan V., Lee J., Bueso T., De Toledo J., Rivas K. (2020).** Neurological manifestations of COVID-19 and other coronavirus infections: a systematic review. *Clinical neurology and neurosurgery*, *194*, 105921.
- **Ng CT, Snell LM, Brooks DG, Oldstone MB(2013).** Networking at the level of host immunity: immune cell interactions during persistent viral infections. *Cell Host Microbe.* 13:652–64.
- **Nishiga M, Wang DW, Han Y, Lewis DB, Wu JC. COVID-19 and cardiovascular disease (2020):** from basic mechanisms to clinical perspectives. *Nat Rev Cardiol*17:543–58.
- **Noda K., Nakao S., Zandi S., Engelstädter V., Mashima Y., Hafezi-Moghadam, A. (2009).** Vascular adhesion protein-1 regulates leukocyte transmigration rate in the retina during diabetes. *Experimental eye research*, *89*(5), 774-781.
- **Pal R., Bhadada S. K. (2020).** COVID-19 and diabetes mellitus: An unholy interaction of two pandemics. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, *14*(4), 513-517.
- **Park J., Marvar P. J., Liao P., Kankam M. L., Norrholm S. D., Downey R. M., *et al.*(2017).** Baroreflex dysfunction and augmented sympathetic nerve responses during mental stress in veterans with post-traumatic stress disorder. *The Journal of physiology*, *595*(14), 4893-4908.
- **Petri B., Phillipson M., Kubes P. (2008).** The physiology of leukocyte

recruitment: an in vivo perspective. *The Journal of Immunology*, *180*(10), 6439-6446.

- **Pitsavos C., Tampourlou M., Panagiotakos D. B., Skoumas Y., Chrysohoou C., Nomikos T., *et al.* (2007).** Association between low-grade systemic inflammation and type 2 diabetes mellitus among men and women from the ATTICA study. *The review of diabetic studies: RDS*, *4*(2), 98.
- **Praktiknjo M, Monteiro S, Grandt J, *et al.* (2020)**: Cardiodynamic state is associated with systemic inflammation and fatal acute-on-chronic liver failure. Liver Int.;40:1457-1466.
- **Preetha S. (2016).** Estimation of liver function test in hypertension patients. *Journal of Pharmaceutical Sciences and Research*, *8*(8), 869.
- **Reinhardt P. H., Elliott J. F., Kubes P. (1997).** Neutrophils can adhere via α4β1-integrin under flow conditions. *Blood, the Journal of the American Society of Hematology*, *89*(10), 3837-3846.
- **Rezaei M, Marjani M, Mahmoudi S, Mortaz E, Mansouri D(2021).** Dynamic changes of lymphocyte subsets in the course of covid-19. *Int Arch Allergy Immunol*. 182:1–9.
- **Rho H. J., Brown H., Fremstad S. (2020).** A basic demographic profile of workers in frontline industries. *Center for economic and policy research*
- **Rice G. E., Bevilacqua M. P. (1989).** An inducible endothelial cell surface glycoprotein mediates melanoma adhesion. *Science*, *246*(4935), 1303-1306.
- **Ryu SH, Min SW, Kim JH, Jeong HJ, Kim GC, Kim DK, Sim YJ(2020)**. Diagnostic Significance of Fibrin Degradation Products and D-Dimer in Patients With Breast Cancer-Related Lymphedema. Ann Rehabil Med. Feb;43(1):81-86.
- **Salmi M., Rajala P., Jalkanen S. (1997)**. Homing of mucosal leukocytes to joints. Distinct endothelial ligands in synovium mediate leukocyte-subtype specific adhesion. *The Journal of clinical investigation*, *99*(9), 2165-2172.
- **Schlesinger, M., Bendas, G. (2015).** Vascular cell adhesion molecule-1 (VCAM-1)—an increasing insight into its role in tumorigenicity and metastasis. *International journal of cancer*, *136*(11), 2504-2514.
- **Schroorl M, Snijders D, Schroorl M, Boersma WG, Bartels PCM**

(2012). Temporary impairment of reticulocyte haemoglobin content in subjects with community-acquired pneumonia. *Int Jnl Lab Hem* 34: 390-395.

- **SeyedAlinaghi S., Ghadimi M., Hajiabdolbaghi M., Rasoolinejad M., Abbasian L., Nezhad, M. H., *et al.* (2020).** Prevalence of COVID-19-like symptoms among people living with HIV, and using antiretroviral therapy for prevention and treatment. *Current HIV research*, *18*(5), 373-380.
- **Sharma, R., Sharma, R., Khaket, T. P., Dutta, C., Chakraborty, B., & Mukherjee, T. K. (2017).** Breast cancer metastasis: Putative therapeutic role of vascular cell adhesion molecule-1. *Cellular Oncology*, *40*(3), 199-208.
- **Simon P., Ebner P., Eichner D., Reynolds J., Bendavid E., Bhattacharya J. (2020).** Seroprevalence of SARS-CoV-2–specific antibodies among adults in Los Angeles County, California, on April 10-11, 2020. *Jama*, *323*(23), 2425-2427.
- **Singh D., Edwards L., Tal-Singer R., Rennard, S. (2010).** Sputum neutrophils as a biomarker in COPD: findings from the ECLIPSE study. *Respiratory research*, *11*(1), 1-12.
- **Tang X., Du R. H., Wang R., Cao T. Z., Guan L. L., Yang C. Q., *et al.* (2020).** Comparison of hospitalized patients with ARDS caused by COVID-19 and H1N1. *Chest*, *158*(1), 195-205.
- **Tyrrell D. A. J., Bynoe M. L. (1966).** Cultivation of viruses from a high proportion of patients with colds. *Lancet*, 76-7.
- **Vaidyula V. R., Rao A. K., Mozzoli M., Homko C., Cheung P., Boden, G. (2006).** Effects of hyperglycemia and hyperinsulinemia on circulating tissue factor procoagulant activity and platelet CD40 ligand. *Diabetes*, *55*(1), 202-208.
- **Van Beusecum, J. P., Barbaro, N. R., McDowell, Z., Aden, L. A., Xiao, L., Pandey, A. K., *et al.* (2019).** High salt activates CD11c+ antigen-presenting cells via SGK (Serum Glucocorticoid Kinase) 1 to promote renal inflammation and salt-sensitive hypertension. *Hypertension*, *74*(3), 555-563.
- **Van der Wal A. C., Das P. K., Tigges A. J., Becker A. E. (1992).** Adhesion molecules on the endothelium and mononuclear cells in human

atherosclerotic lesions. *The American journal of pathology*, *141*(6), 1427.

- **Varga Z., Flammer A. J., Steiger P., Haberecker M., Andermatt R., Zinkernagel, A. S., *et al.* (2020).** Endothelial cell infection and endotheliitis in COVID-19. *The Lancet*, *395*(10234), 1417-1418.
- **Vázquez, B. Y. S. (2012).** Blood pressure and blood viscosity are not correlated in normal healthy subjects. *Vascular health and risk management*, *8*, 1.
- **Velavan T. P., Meyer C. G. (2020).** The COVID-19 epidemic. *Tropical medicine & international health*, *25*(3), 278..
- **Viner R. M., Ward J. L., Hudson L. D., Ashe M., Patel S. V., Hargreaves D., *et al*.(2021).** Systematic review of reviews of symptoms and signs of COVID-19 in children and adolescents. *Archives of disease in childhood*, *106*(8), 802-807.
- **Wagener F. A., Pickkers P., Peterson S. J., Immenschuh S., Abraham, N. G. (2020).** Targeting the heme-heme oxygenase system to prevent severe complications following COVID-19 infections. *Antioxidants*, *9*(6), 540.
- **Wang D., Hu B., Hu C., Zhu F., Liu X., Zhang J., *el al.* (2020).** Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. *Jama*, *323*(11), 1061-1069.
- **Wang Y. C., Li H. Y., Wei J. N., Lin M. S., Shih S. R., Hua C. H., *et al.* (2013).** Serum vascular adhesion protein-1 level is higher in smokers than non-smokers. *Annals of human biology*, *40*(5), 413-418.
- **Wang Y-L, Koh W-P, Yuan J-M, Pan A(2016).** Association between liver enzymes and incident type 2 diabetes in Singapore Chinese men and women. *BMJ Open Diabetes Res Care*.;4(1):e000296.
- **Wick T. M., Moake J. L., Udden M. M., Eskin S. G., Sears D. A., McIntire L. V. (1987).** Unusually large von Willebrand factor multimers increase adhesion of sickle erythrocytes to human endothelial cells under controlled flow. *The Journal of clinical investigation*, *80*(3), 905-910.
- **Wostyn, P. (2021).** COVID-19 and chronic fatigue syndrome: Is the worst yet to come?*Medical hypotheses*, *146*, 110469.
- **Yang A. P., Liu J. P., Tao W. Q., Li, H. M. (2020).** The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19

patients. *International immunopharmacology*, *84*, 106504.

- **Yazdanpanah F, Hamblin MR, Rezaei N. The immune system and COVID-19: friend or foe? *Life Sci.* (2020).** 256:117900.
- **Youssef M., Hussein M., Attia A. S., M Elshazli R., Omar M., Zora G., Kandil, E. (2020).** COVID-19 and liver dysfunction: a systematic review and meta-analysis of retrospective studies. *Journal of medical virology*, *92*(10), 1825-1833.
- **Zhang C., Shi L., Wang F. S. (2020).** Liver injury in COVID-19: management and challenges. *The lancet Gastroenterology & hepatology*, *5*(5), 428-430.
- **Zhang W. R., Sun M., Luo, J. K. (2003).** Serum nitric oxide and D-dimer before and after administering antihypertensive drugs in essential hypertension. *Hunan yi ke da xue xue bao= Hunan yike daxue xuebao= Bulletin of Hunan Medical University*, *28*(4), 382-384.
- **Zhou P., Yang X. L., Wang X. G., Hu B., Zhang L., Zhang W., *et al.* (2020).** Discovery of a novel coronavirus associated with the recent pneumonia outbreak in humans and its potential bat origin. *BioRxiv*.579(7798) 270-273

APPENDIX

Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Kerbela

College of Medicine/ Department of Chemistry and Biochemistry

Experimental data:

Sample No. …….

Date……

Name …….

Age……..

Address…….

Phone…….

Gender: Male or Female

Smoking: Yes or No

Diabetic mellitus: Yes or No

Medications: Yes or No

Blood pressure: hypotension or hypertension

COVID-19: Yes or No

Weight:

Height:

Date of diagnosis……

Biomarker:

Vascular cell adhesion molecule-1 (VCM-1)

Vascular adhesion protein-1 (VAP-1)

الخلاصة

المقدمة انتشر وباء فيروس كورونا 2019 (COVID-19) في جميع أنحاء العالم. وبالتالي ، هناك حاجة ماسة لإجراء فحوصات سريعة وبسيطة ودقيقة لتشخيص عدوى فيروس كورونا 2 (SARS-CoV-2) المتلازمة التنفسية الحادة الوخيمة.

الهدف من الدراسة

متوسط vcm-1 في مرضى السكري 199.00 وفي غير مرضى السكري 195.75 (NS) ، بينما متوسط vap-1 في مجموعة مرضى السكري 3690.00 وغير مصاب بمرض السكري 3678.50 (HS). في أيدي أخرى ، متوسط 3719.50 vap-1 بوصة مجموعة ارتفاع ضغط الدم ولكن الوسيط في مجموعة انخفاض ضغط الدم 3600.50 (S). في المقابل vcm-1 ، فإن الوسيط في انخفاض ضغط الدم أعلى من ارتفاع ضغط الدم (199.00 ، 195.75 على التوالي). أيضًا ، في مجموعات المدخنين ، يكون متوسط vcm-1in مدخنًا أكثر من غير المدخن (236.00 مقابل 180.30) (S) ، في حين أن متوسط vap-1 في المدخن أكبر منه في غير المدخن (3698.00 ، 3550.00 ، على التوالي). ، وسيط مجموعة vcm-1 المعتدلة 198.30 أقل من مجموعة (S) VV 271.31 ، بينما متوسط vap-1 في معتدل 3678.00 أكثر من (S) 3664.00 S.

.

المواد وطريقة العمل: دراسة مقطعية للأفراد العراقيين المصابين بـ COVID19. هناك مرضى مختلفين مصابين بفيروس كورونا المستجد (كوفيد -19) للحالات الخفيفة والشديدة وسيكون العدد حسب معادلة حجم العينة. يبلغ حجم 5 مل من عينة الدم ، وسيتم جمعها ومعالجتها وسيفصل المصل لقياس المعلمات البيوكيميائية

النتائج: مستوى vcm-1 في مرضى السكري 199.00 وفي غير مرضى السكري 195.75 (NS) ، بينما مستوى vap-1 في مجموعة مرضى السكري 3690.00 وغير مصاب بمرض السكري 3678.50 (HS). في أيدي أخرى ، مستوى 3719.50 vap-1 بوصة مجموعة ارتفاع ضغط الدم ولكن الوسيط في مجموعة انخفاض ضغط الدم 3600.50 (S). في المقابل vcm-1 ، فإن الوسيط في

انخفاض ضغط الدم أعلى من ارتفاع ضغط الدم (199.00 ، 195.75 على التوالي). أيضًا ، في مجموعات المدخنين ، يكون مستوى vcm-1in مدخنًا أكثر من غير المدخن (236.00 مقابل 180.30 (S) ، في حين أن متوسط vap-1 في المدخن أكبر منه في غير المدخن (3698.00 ، 3550.00 ، على التوالي). ، وسيط مجموعة vcm-1 المعتدلة 198.30 أقل من مجموعة (S) 271.31 ، بينما متوسط vap-1 في معتدل 3678.00 أكثر من (S) 3664.00 S.

بسم الله الرحمن الرحيم

فَتعَالى الله المِلكُ الحَق ولا تَعْجَل بِالقُرآنِ مِن قَبل

أن يُقْضى إليكَ وحُيهُ وقُل رَبي زِدنِي عِلماً

صدق الله العظيم

الآية 114 من سورة طه

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء/ كلية الطب

فرع الكيمياء والكيمياء الحياتية

جزيئات التصاق لخلايا البطانية الوعائية كمؤشرات لشدة COVID-19

رسالة مقدمة

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

من قبل

سجى حيدر فاضل

بكالوريوس تحليلات مرضية – جامعة كربلاء – 2017

اشراف

ا. م.د. شيماء زهراوي ندى

ا.م.د .لمياء عبد الكريم درويش

1443 هـ

2021 م