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The association of vitamin D3 and Cathelicidin with the severity of COVID 19 in a sample of Iraqi patients

A Thesis

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

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Dedication

To my Lovely homeland ..... Iraq

To my parents ..

To my husband ..

To my brothers and sisters ..

I dedicate my work with love .....

Muntaha

Supervisors Certification

We certify that this M.Sc. thesis titled:

The association of vitamin D3 and Cathelicidin with the severity of COVID 19 in a sample of Iraqi patients

was prepared under our supervision at the College of Medicine/ University of Kerbala, as a partial requirement for the degree of Master in Clinical chemistry.

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بسم الله الرحمن الرحيم

نَرْفَعُ دَرَجَاتٍ مَّن نَّشَاءُ

وَفَوْقَ كُلِّ ذِي عِلْمٍ

عَلِيمٌ

صدق الله العلي العظيم

الآية ﴿٧٦﴾ من سورة يوسف

Acknowledgement

In the name of God, the most merciful and most gracious.

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List of Contents

List of Abbreviations

µm	Micrometre
1,25(OH)2D3	1,25-dihydroxy vitamin D3
ACE2	Angiotensin converting enzyme 2
AMPs	Antimicrobial peptides
ARDS	Acute respiratory distress syndrome
AUC	Area under the curve
BOV	Bocavirus
CAMP	Cationic antimicrobial peptide
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease 2019
CSS	Cytokine storm syndrome
CT	Computed Tomography
DBP	Vitamin D binding protein
DC	Direct current resistance method
DIC	Disseminated intravascular coagulation
ERGIC	Endoplasmic reticulum-Golgi intermediate compartment
FEU/ml.	Fibrinogen equivalent unit
GCS-F	Granulocyte colony stimulating factor
hCAP	Human cationic antimicrobial protein
HCAp18	Human cationic antimicrobial peptide
MCP	Monocyte chemoattractant protein
MERS-COV	Middle East respiratory syndrome coronavirus
MPV	Metapneumovirus
NK	Natural killer cell
PD	Pe-diluted mode
PIV	Parainfluenza virus
PPE	Personal protective equipment
RAS	Renin-angiotensin system
RBD	Receptor binding domain
RT-PCR	Real time polymerase chain reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
VTE	Venous thromboembolism
WB	Whole blood mode

List of figures

List of Tables

Abstract

Background: Pneumonia is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has emerged in Wuhan City, Hubei Province, China in December 2019. By Feb. 11, 2020, the World Health Organization (WHO) officially named the disease resulting from infection with SARS-CoV-2 as coronavirus disease 2019 (COVID-19). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for COVID-19 infection. Vitamin D3 is a fat-soluble vitamin and has immune function, particularly in modulating the inflammatory response to viral infection. vitaminD3 modulates both the adaptive and innate immune at cellular level. Cathelicidin is Antimicrobial peptide and main components of innate immunity.

Aims: To find the association between vitamin D3 levels and the severity of COVID-19 in studied subjects. Study the mechanisms of Vitamin D3 action in patients with COVID-19 and discus how it will be used in reduction the severity of this disease and To study the association between Cathelicidin and vitamin D3 levels in order to support the use of vit D3 in treatment of Iraqi patients with COVID 19.

Materials and methods: This case–control study consisted of 90 samples, 60 of them have COVID-19. Patient divided into three group (20 mild, 20moderate, 20 sever cases of COVID-19). Classified into three severity levels depending on clinical manifestations: mild, moderate, and severe disease depended on pulmonary imaging, however, respiratory characteristics such as respiratory rate, oxygen saturation, and lesion development are employed as categorization criteria. Different organ

dysfunctions, such as septic shock, heart failure, and disseminated intravascular coagulation, worsen severe cases (DIC).

Control individuals were 30 and age range of individuals (patient and control) was (20-70).

Detection of cathelicidin levels were carried out by Enzyme-Linked Immunosorbent Assay (ELISA). vitamin D3 levels were carried out by fully automated chemiluminescence immunoassay analyser. D-dimer was tested using Cobas e 411 fully automated analyzer and ferritin level was tested using

automated Mini-Vidas system. CBC measurements were done by XP 300™ Automated hematology analyzer device.

Results: Monitoring of the Vitamin D3, D-dimer and ferritin in the studied groups were shown a highly significant in severe patients and related to severity of COVID-19 in studied subjects(p-value <0.05).D-dimer and ferritin levels were significantly increased, while vitamin D3 was decreased in COVID-19 patient The present study showed negative correlation between Vitamin D3 and Cathelicidin in covid19 patients. Vitamin D3, cathelicidin and D-dimer showed high specificity and sensitivity in ROC analysis.

Conclusion; vitamin D3, and D-dimer are factors for severity of COVID-19 in studied subjects and Vitamin D3 deficiency is more susceptible to development of COVID-19 diseases by affecting on innate immunity. Therefor, this study may suggest recommended the monitoring of cathelicidin, vitamin D3 and D-dimer levels in COVID19 good biomarkers for prognosis and treatment.

Chapter One

Introduction & Literature Review

1.Introduction

1.1.Coronavirus disease 2019 (COVID-19)

Pneumonia is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection emerged in Wuhan City, Hubei Province, China in December 2019. By Feb. 11, 2020, the World Health Organization (WHO) officially named the disease resulting from infection with SARS-CoV-2 as coronavirus disease 2019 (COVID-19). The global epidemic of coronavirus disease 2019 (COVID-19) has presented a major threat to public health worldwide. COVID-19 is the result of infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that was first isolated and identified in patients who were exposed at a seafood market in Wuhan City (Shi *et al.*, 2020). COVID-19 is classified into three severity levels depending on clinical manifestations: mild, moderate, and severe disease. Differentiating severe patients from non-severe patients can assist improve the COVID-19 cure rate to some extent. In pulmonary imaging, however, respiratory characteristics such as respiratory rate, oxygen saturation, and lesion development are employed as categorization criteria. Different organ dysfunctions, such as septic shock, heart failure, and disseminated intravascular coagulation, worsen severe cases (DIC). Some thrombotic consequences have been recorded in medical practice, including ischemic limbs, strokes, and venous thromboembolism. In patients with advanced illness, venous thromboembolism is prevalent. Although a low platelet count and a high D-dimer have been linked to severe COVID-19 and a high mortality rate. hematological and coagulation parameters in COVID-19 individuals with mild, moderate, and severe cases Finding effective hematology and coagulation measures for risk

classification and prognosis prediction is therefore a primary aim (Liao et al., 2020) .

In China alone, 80,409 cases had been confirmed as of March 4, 2020, with over 3,000 deaths reported (National Health Commission of the People's Republic of China, 2020). In other nations, there were a total of 12,668 confirmed cases and 214 deaths, respectively (World Health Organization, 2020a). COVID-19 has been declared a public health emergency by the World Health Organization (WHO) (J. Zhang *et al.*, 2020). Coronaviruses are enveloped, positive single stranded huge RNA viruses that can infect humans as well as a variety of other animals. Tyrell and Bynoe, who grew the viruses from patients with common colds, initially characterized coronaviruses in 1966. They were named coronaviruses (Latin: corona = crown) because of their shape as spherical virions with a core shell and surface projections resembling a solar corona. Coronaviruses are divided into four subfamilies: alpha, beta, gamma, and delta. While alpha and beta coronaviruses are thought to have originated in mammals, particularly bats, gamma and delta viruses are thought to have originated in pigs and birds. The genome size ranges from 26 to 32 kb. Beta coronaviruses, one of seven coronavirus subtypes that can infect humans, can cause serious disease and death, whereas alphacoronaviruses induce asymptomatic or slightly symptomatic infections.

(SARS Cove) is a beta coronavirus that belongs to the B lineage and is closely related to the SARS virus.

The nucleocapsid protein (N), spike protein (S), small membrane protein (SM), and membrane glycoprotein (M) are the four major structural genes, with an extra membrane glycoprotein (HE) found in the beta coronaviruses as shown in figure(1.1). SARSCoV2 is 96 % identical to a bat coronavirus at the complete genome level (Velavan and Meyer, 2020). SARS-CoV-2 is the pathogen that causes COVID-19 in human. Severe acute respiratory

syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), both of which have a significant fatality rate, are two more coronaviruses known to cause human disease. Deep sequencing demonstrated that the coronavirus identified from lower respiratory tract samples of COVID-19 patients belonged to the -CoV family (Zhou, Zhang, and Qu, 2020).

The CoV is a non-profit organization that provides assistance to individuals. A "spike protein" is found in the CoV family. The spike (S) protein, which has 1300 amino acids, interacts with host cells such as pulmonary and parabronchial epithelial cells and promotes the coronavirus in entering through the epithelial cell membrane. Furthermore, the virus's target, angiotensin-converting enzyme 2 (ACE2), is abundantly expressed in alveolar epithelial cells. The recognition of ACE2 by the virus's S protein allows the coronavirus to enter the human circulatory system (Hendaus, 2020). Spike protein is important for determining host tropism and transmission capability because it plays an important function in binding to receptors. It is functionally separated into two domains: S1 domain for receptor binding and S2 domain for cell membrane fusion. The -CoV receptor binding domain (RBD) is typically found in S1's C-terminal domain. The SARS-CoV-2 spike protein's cryogenic electron microscopy (Cryo-EM) structure revealed that it had a 10 to 20-fold higher binding affinity to human angiotensin-converting enzyme 2 (ACE2) than SARS-CoV. SARS-CoV-2 has a closer sequence similarity to SARS-CoV genomes than MERS-CoV genomes, according to phylogenetic analyses of the evolution history (Zhou, Zhang and Qu, 2020).

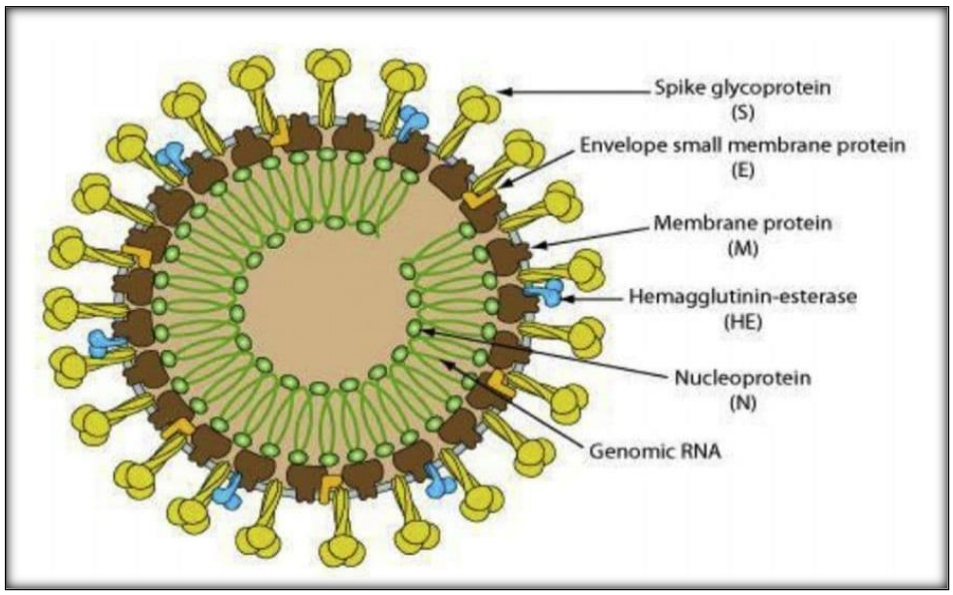


Figure (1.1) Coronavirus structure (Mousavizadeh and Ghasemi, 2020)

1.1.1. Symptoms

The symptoms of COVID-19 infection appear after an incubation period of approximately 5 days. The period from the onset of COVID-19 symptoms to death ranged from 6 to 41 days with a median of 14 days. This period is dependent on the age of the patient and status of the patient's immune system .when comparing patients above the age of 70 to those under the age of 70, it was found to be shorter (Rothan and Byrareddy, 2020). Patients are affected by symptoms. Cough, sore throat, fever, diarrhoea, headache, muscle or joint discomfort, exhaustion, and loss of smell and taste are common symptoms of mild COVID19 (mild symptoms). Breathlessness, lack of appetite, confusion, pain or pressure in the chest, and a high temperature (over 38 °C) are all indications of COVID19 pneumonia (severe symptoms). Lung sounds, blood pressure, and heart rate are among the signs examined by clinical examination (Emperador et al., 2020). Due to significant alveolar destruction, elderly men with comorbidities are more likely to develop respiratory failure. In severe situations, disease onset can lead to organ dysfunction (e.g., shock, acute respiratory distress syndrome, acute heart injury, and acute kidney

injury), as well as death. Patients may experience normal or low white blood cell counts, lymphopenia, or thrombocytopenia in the meantime (Shrestha and Shrestha, 2020).

COVID-19 can simultaneously present with other infections such as influenza, and it can be hard to distinguish the symptoms of the two conditions from each other. However, there are differences and these are summarized in table 1.1(**Balla *et al.*, 2020**)

Table 1.1: differences between covid19, flu and common cold symptoms (Awadasseid et al., 2020)

Common cold	Viral pneumonia
Breathing - without difficulty or shortness of breath	Breathing is accelerated or even difficult
Cough - occurs later	Spray the symptom is serious, give priority to with dry coughSpray Accompanied by phlegm sound, wheeze, influence Morpheus
Fever normal 48 to 72 hours later, fever medicine has a good effect	Fever-a high fever lasts over 72 hours
There is no significant difference in body and mind, or appetite and sleep	General mental disorder, or poor appetite
Incubation period:1-3 days	Incubation period: 2-14 days, 7 days on average

Respiratory infections can be transmitted through droplets of various sizes: respiratory droplets are larger than 5-10 m in diameter, while droplet nuclei are less than 5 m in diameter. COVID-19 virus is largely spread between persons by respiratory droplets and contact routes, according to existing findings. Airborne transmission was not recorded in a study of 75,465 COVID-19 cases in China. When a person comes into close contact (within 1 m) with someone who has respiratory symptoms (e.g., coughing or sneezing), his or her mucosae are at risk of being infected (mouth and nose) or Exposure of the conjunctiva (eyes) to possibly infectious

respiratory droplets. Transmission can also happen through fomites in the sick person's immediate environment. As a result, COVID-19 virus transmission can occur through direct touch with infected people as well as indirect contact with surfaces in the nearby area or things used on the infected person (e.g., stethoscope or thermometer). Airborne transmission differs from droplet transmission in that it relates to the existence of germs within droplet nuclei, which are typically 5m in diameter and can float in the air for long periods of time and be transmitted to others over large distances (van Doremalen, 2020).

1.1.2. Transmission

Although droplet and touch are thought to be the primary modes of transmission for SARS-CoV-2, other coronaviruses have been demonstrated to survive for days on filthy surfaces. In addition, SARS-CoV-2 RNA was found in a stool sample from a person who had symptoms, despite the serum sample being negative. Chinese researchers recently recovered SARS-Cov-2 from a swab sample of a confirmed patient's feces, indicating the possibility of fecal-oral transmission. Even in the context of isolation efforts in medical settings, studies have revealed successful person-to-person transmission of 2019-nCoV. A case study of nine infected pregnant women found no evidence of third trimester complications After a caesarian incision , vertical transmission occurs (Jiang et al., 2020). While the SARS-CoV-II virus is only viable for three hours in aerosols, it can survive for three days on polypropylene plastic, a couple of days on stainless steel, twenty-four hours on cardboard, and four hours on copper. COVID-19 transmission can be reduced to some extent by increasing temperature and humidity. Surface disinfectants such as 62–71 percent ethanol, 0.5 percent hydrogen peroxide, or 0.1 percent sodium

hypochlorite can inactivate SARS-CoV-II in less than a minute (Gasmi *et al.*, 2020).

1.1.3. Risk factor of COVID-19

The COVID-19 pandemic is a major healthcare problem around the world, with significantly higher morbidity and mortality in older people, comorbidities (including hypertension, diabetes, cardio-vascular disease, chronic lung disease, and cancer (Jothimani et al., 2020), and (Phua et al., 2020). Obesity exacerbates the severity of respiratory disorder, however it is unclear whether obese people are also more likely to have greater COVID-19 sickness severity. In individuals with laboratory-confirmed SARS-CoV-2 infection, there is association between obesity and COVID-19 severity of illness. Obesity was found in 75 of the participants (Gao *et al.*, 2020). Adipose tissue may play a key role in the evolution of COVID-19, and obesity may be a significant risk factor.

Obese patients have higher levels of leptin and lower levels of adiponectin, as well as higher concentrations of pro-inflammatory cytokines like tumor necrosis factor (TNF)-alpha, monocyte chemoattractant protein (MCP)-1, and interleukin (IL)-6, which are produced primarily by adipose tissue and may contribute to a weakened immune response. Inflammatory and immunological responses may be affected by several factors. Another factor could be a sedentary lifestyle, which, alone or in combination with insulin resistance, affects the immune response to microbial agents by impairing macrophage differentiation and modulating proinflammatory cytokine levels, allowing infectious pathogens to invade the body. In some diseases, such as community-acquired pneumonia, the obesity survival paradox has been discovered, where, despite an increased risk of getting pneumonia, an inverse relationship between obesity and mortality has been observed Individuals with a weakened immune system, such as those with various underlying or

persistent infections, and maybe pregnant women (Földi et al., 2020). Women are more susceptible to respiratory illnesses during pregnancy (Phoswa and Khaliq, 2020).

1.1.4. Pathogenesis

1.1.4.1. virus entry and replication

SARS-CoV-2 is mostly transferred through respiratory, contact, and fecal-oral transmission.

Primary viral replication is thought to occur in the upper respiratory tract's mucosal epithelium (nasal cavity and pharynx), followed by multiplication in the lower respiratory tract and gastrointestinal mucosa, resulting in a mild viremia. At this stage, only a few infections are under control and are symptom-free. Non-respiratory symptoms such as severe liver and heart injury, kidney failure, and diarrhea have also been reported in some cases, indicating that many organs are involved. The nasal mucosa, bronchus, lung, heart, esophagus, kidney, stomach, bladder, and ileum all have high levels of ACE2.SARS-CoV-2 is able to infect all of these human organs as shown in figure(1.2).

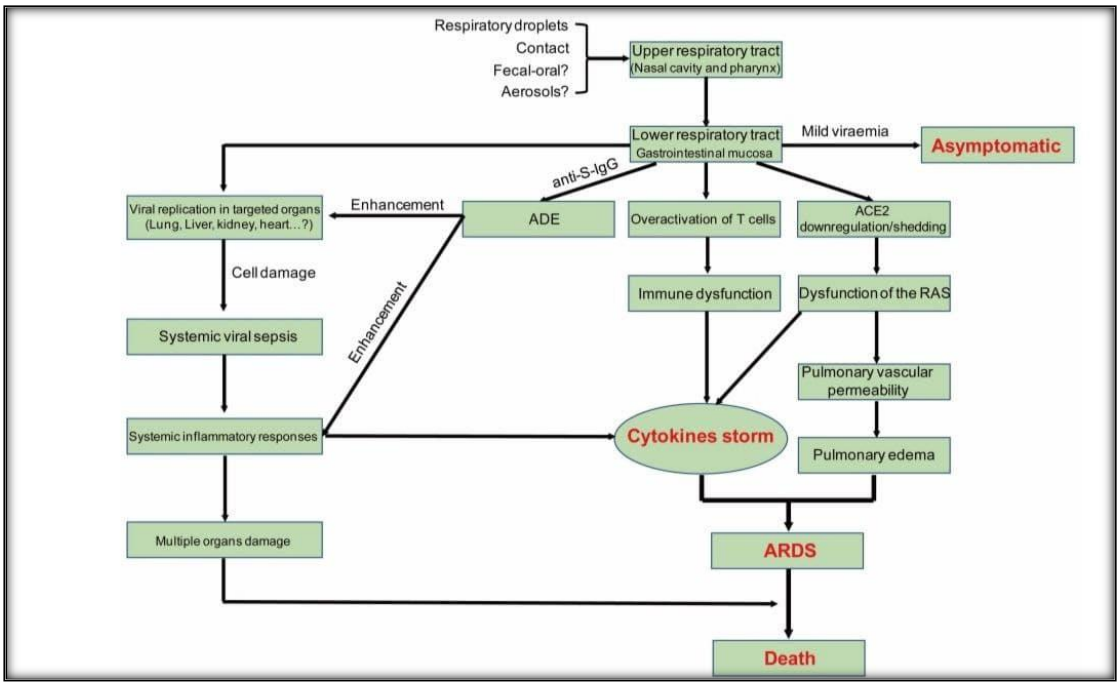


Figure (1.2) pathogenesis of SARS-CoV-2 (Duan, 2020)

Clinicians have recently suggested that SARS-CoV-2 may be harmful to testicular tissues, raising concerns about fertility in young patients (Duan, 2020) . For SARS-CoV and SARS-CoV-2, the envelope spike glycoprotein interacts to its cellular receptor, ACE2. SARS-CoV infection was first thought to be conducted via direct membrane fusion between the virus and the plasma membrane. The membrane fusion and viral infectivity were mediated by a proteolytic cleavage event at position (S2′) of the SARS-CoV S protein. SARS-CoV entrance was mediated by clathrin dependent and -independent endocytosis, in addition to membrane fusion. The viral RNA genome is released into the cytoplasm after the virus penetrates the cells and is translated into two polyproteins and structural proteins, following which the viral genome begins to replicate (Li et al., 2020). As part of the innate immunity to combat the infection, once the virus has access to lung tissue, it will activate an inflammatory cascade. This inflammatory activation, on the other hand, causes severe pulmonary damage. Among the many pro-inflammatory mediators that get triggered

with COVID-19, are distinct cytokines and chemokines of the innate immunity (Abdin *et al.*, 2020) .

1.1.4.2. Cytokine Strom and acute respiratory distress syndrome

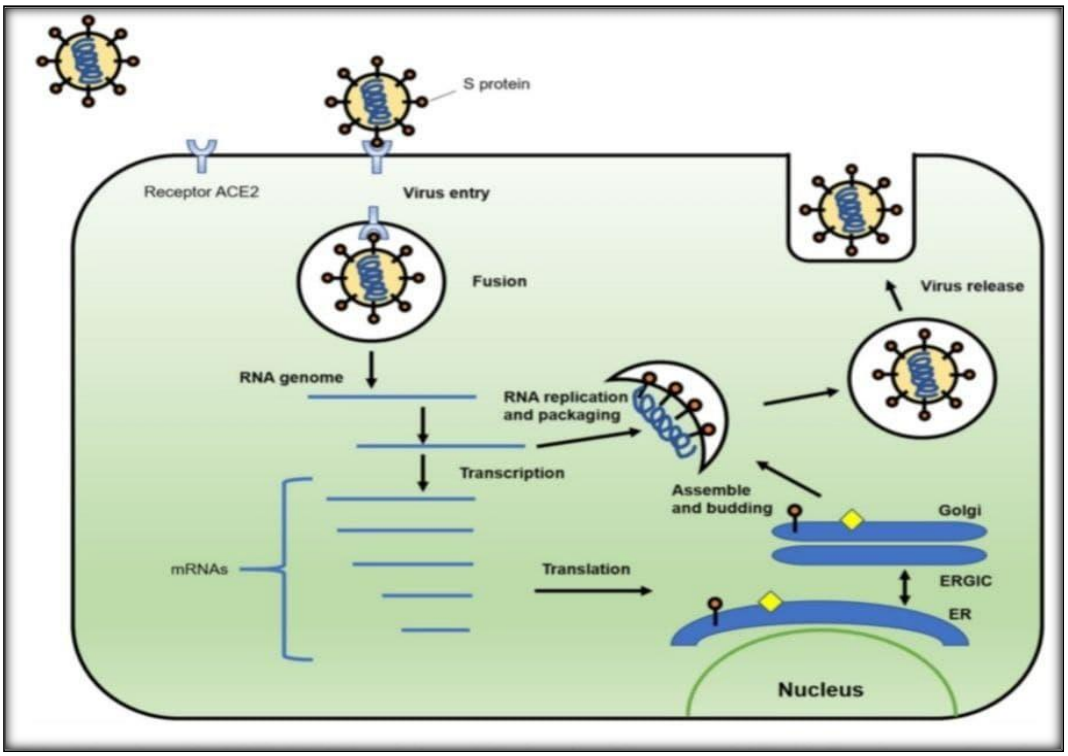
A systemic cytokine barrage disrupts host immunological responses, causing acute respiratory distress syndrome (ARDS) to develop. This is especially important in the elderly, who are more susceptible to cytokine storm. COVID-19 patients had a high level of interleukin (IL)-6, a major inflammatory mediator involved in respiratory failure, shock, and multi-organ dysfunction, and the SARS and MERS viruses, which are related, are known to cause cytotoxic T cell hyper-activation. Similarly, patients with severe COVID-19 symptoms and pneumonia were found to have the highest levels of circulating pro-inflammatory cytokines such as IL-2, IL-7, granulocyte colony stimulating factor (G-CSF), and tumor necrosis factor (TNF) (Shakoor et al., 2021). Recruited monocytes produce pro-inflammatory cytokines, inducing pneumocyte apoptosis; B - Recruited macrophages release other cytokines, C - Neutrophils migrate into the interstitial/alveolar space and degranulate, leading in irreversible damage to pneumocytes and endothelial cells, resulting in alveolar-capillary barrier disruption; D - Interstitial and alveolar edema due to blood protein transmigration. Regardless of which mechanism occurs, the end result is the transmigration of blood proteins, Interstitial and alveolar edema are the end results (Batah and Fabro, 2021). The majority of COVID-19-related morbidity and mortality occurs during the inflammatory phase, which is marked by a dysregulated immune response and life-threatening complications, such as ARDS. ARDS is acute hypoxemic respiratory failure that manifests as bilateral pulmonary infiltrates on lung imaging in the absence of a purely cardinal cause. ARDS is a clinical condition with a

wide range of symptoms. The virus has now reached the lung's gas exchange units and infected alveolar type II cells. In comparison to type I cells, SARS-CoV and influenza preferentially infect type II cells. The infected alveolar units are usually found on the periphery and in the subpleural space. SARS-CoV replicates in type II cells, releasing a huge number of virus particles before the cells die of apoptosis (Mason, 2020).

1.1.4.3. Immune dysfunction

Patients with COVID-19 had a dysregulated immunological response (decreased T, B, and NK cells and increased inflammatory cytokines). In extreme cases, they also had higher amounts of white blood cells, neutrophil count, and D-dimer. In a severe case, peripheral CD4 and CD8 T cells revealed a decrease. There were also high quantities of proinflammatory CD4 T cells and cytotoxic granular CD8 T cells, implying antiviral immune responses and T cell overactivation (Duan, 2020).

As shown in figure (1.3), S protein binds to the cellular receptor ACE2 to facilitate the entry of the virus. After the fusion of viral and plasma membranes, virus RNA undergoes replication and transcription. The proteins are synthesized. Viral proteins and new RNA genome are subsequently assembled in the ER(endoplasmic reticulum) and Golgi, followed by budding into the lumen of the ERGIC(endoplasmic reticulum-Golgi intermediate compartment), New virions are released through vesicles (He, Deng and Li, 2020).



Figure(1.3) Schematic model of SARS-CoV-2 life cycle(He, Deng and Li, 2020).

1.2. Vitamin D3

7-dehydrocholesterol reacts with UVB sunlight in the skin to produce vitamin D3 (cholecalciferol) as shown in figure (1.4), a fat-soluble vitamin (King, 2020). The vitamin D binding protein (DBP), a particular binding protein for vitamin D and its metabolites in serum transports vitamin D to the liver through the bloodstream. Vitamin D is hydroxylated in the liver, resulting in 25-hydroxyvitamin D3 (25(OH)D3) production via 25-hydroxylase Christakos et al., 2010). The DBP transports 25(OH)D3, the most common circulating form of vitamin D, to the kidney. 1-alpha-hydroxylase hydroxylates -25(OH)D3 in the kidney, resulting in the hormonally active form of vitamin D, 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), which is responsible for the majority, if not all, of vitamin D's physiologic effects (Kamen and Tangpricha, 2010).

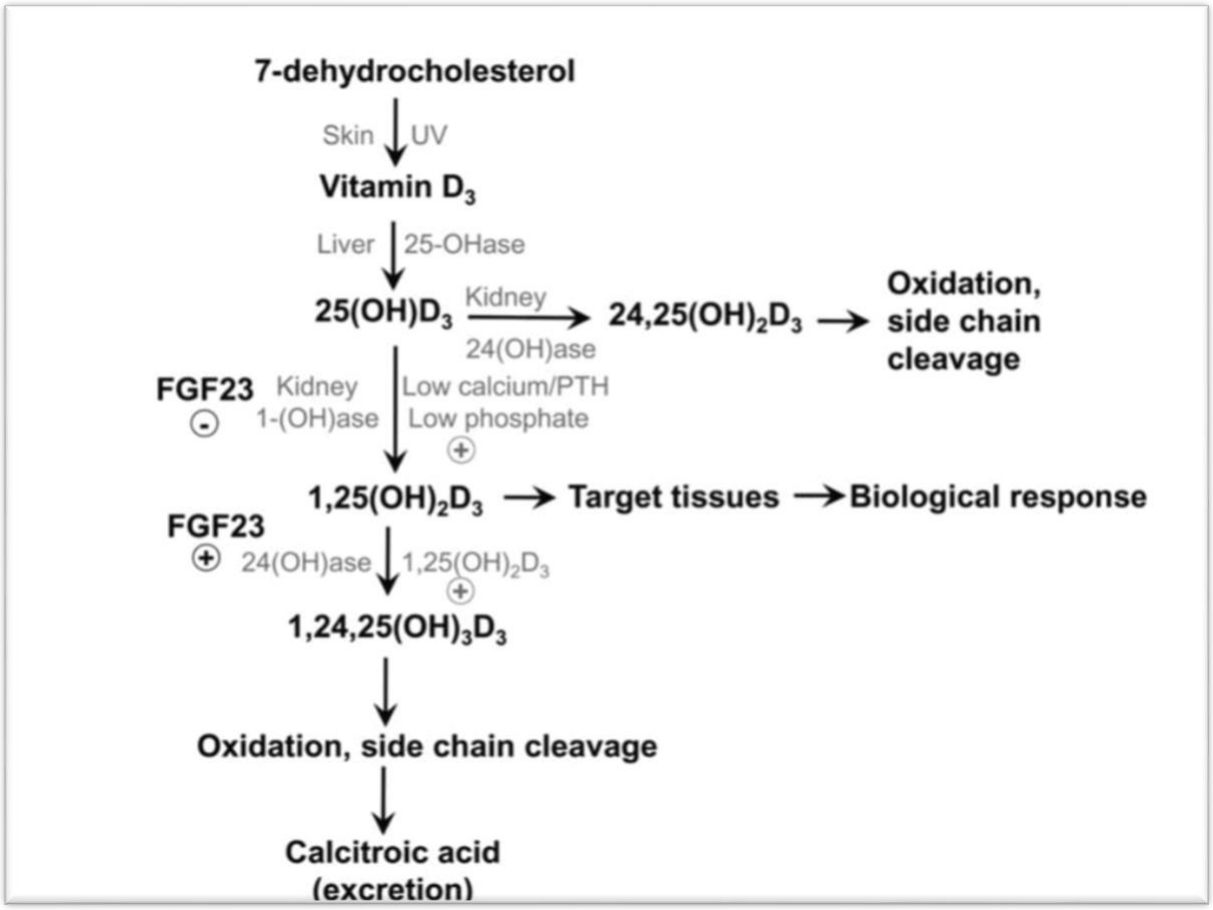


Figure (1.4) metabolic pathway of vitamin D (Christakos *et al.*, 2010)

1.2.1. Function of vitamin D3

It helps to maintain calcium and bone homeostasis (Saponaro, Saba, and Zucchi, 2020). Vitamin D3 influences gene expression in both genomic and non-genomic ways. It has a crucial role in the barrier function of mucosal membranes in the respiratory system (Biesalski, 2020). Vitamin D plays an important role in immune cell function, particularly in regulating the inflammatory response to viral infection. Vitamin D3 regulates cytokines and cell signaling pathways at the cellular level, modulating both the adaptive and innate immune systems. Vitamin D3 regulates the proliferation, inhibition, and differentiation of T and B immunological cells through the vitamin D receptor (VDR). Vitamin D suppresses the synthesis of pro-inflammatory cytokines while increasing

the production of anti-inflammatory cytokines (Zapata, 2020) and (Biesalski, 2020).

1.2.2. Vitamin D deficiency

A 25-hydroxyvitamin D (25(OH)D) concentration of less than 30nmol/L Obesity, older age (the skin of older people is less able to absorb vitamin D from sunshine), and other factors all contribute to vitamin D3 insufficiency(Zapata, 2020). Persons who live at higher latitudes or have darker skin color (Black Asian Minority ethnics - BAME in the UK) are more likely to have vitamin D3 deficiency(Cowbrough, 2015). Vitamin D3 production is lower in those with darker skin. Low levels of 25(OH)D in the blood are linked to cardiovascular disease (CVD) (Kubiak *et al.*, 2018). diabetes and cigarette smokers (Meltzer *et al.*, 2020),(Montano-loza, 2020). A recent research of volunteers at the Bahrain Defense Force hospital's blood bank facility discovered vitamin D insufficiency in 169 (67.6%) of females and 78 (31.2%) of males(Al-Mahroos *et al.*, 2013).

1.3. Cathelicidin

Antimicrobial peptides (AMPs) play an essential role in innate immunity in species all over the world (Sitaram and Nagaraj, 2005). AMPs were once thought to be endogenous antibiotics because of their ability to destroy microorganisms by disrupting their membranes. They have wide antibacterial action, which means they can kill both gram-positive and gram-negative bacteria, viruses, and fungi (Reinholz, Ruzicka and Schauber, 2012). Antimicrobial peptides are secreted by epithelial surfaces from both barrier epithelia and glandular structures. In the process of phagocytosis, granules fuse to phagocytic vacuoles containing ingested microorganisms, exposing the microbes to extremely high concentrations of microbicidal and digesting enzymes. Antimicrobial peptides are found

in the fluid portion of blood (hemo- lymph) and the granules of phagocytic cells (heo- monocytes) in invertebrates (Ganz, 2003). There are about 2,000 of these peptides in the AMP database. They are classified into seven categories: (I) linear peptides; (II) cyclic peptides; (III) glycopeptides; (IV) lipoglycopeptides; (V) lipopeptides and (VII) thiopeptides and chromopeptides. AMPs are small proteins with fewer than 100 amino acids that are found in a variety of cell types. Homologous peptides can be found in vertebrates, invertebrates, and plants, and are generally cationic and amphipathic. Defensin and cathelicidin are two families of AMPs found in mammals (Kuroda et al., 2015).

Antimicrobial peptides from the cathelicidin family can be discovered in a variety of mammalian species, including bovine, mouse, rabbit, and humans. They share a highly conserved signal sequence (proregion) and a cathelin-like proregion (cathelin = cathepsin L inhibitor), but the C-terminal domain, which encodes the mature peptide, is highly heterogeneous. In humans, rabbits, and mice, the microbicidal C-terminal domain can be 12 to 80 amino acids long, with -helical structures in human, rabbit, and mouse, and -sheet structures in pigs. The sole human cathelicidin, LL-37/hCAP-18, was first isolated from bone marrow (Herr, Shaykhiev and Bals, 2007).

In humans, just one cathelicidin gene (CAMP) has been discovered. CAMP codes for the 37-aa peptide LL-37, which has a molecular weight of 18 kDa and starts with two leucine residues at its N-terminus. Human cationic antimicrobial peptide is also known as hCAP-18, FALL-39, or CAMP. LL-37 is found in circulating neutrophils and myeloid bone marrow, It is found in epithelial cells of the skin, as well as the gastrointestinal system, the epididymis, and the lungs, (Strzałkowska and Jo, 2012).

1.4. Vitamin D and innate immunity

Toll like receptors (TLRs) are activated in polymorphonuclear cells, monocytes, and macrophages, as well as a variety of epithelial cells, including those in the epidermis, gingiva, gut, vagina, bladder, and lungs, as part of the innate immune response. A lot of these TLRs use CD14 as a coreceptor. When TLRs are activated, antimicrobial peptides and reactive oxygen species are produced, killing the organism (Bikle, 2009). With the discovery of the vitamin D receptor (VDR) and major vitamin D metabolizing enzymes expressed by immune cells over the last decade, the role of vitamin D on the control of immune cells has grown in recognition.In reaction to endogenously produced 1,25(OH)2D, macrophages produce the antimicrobial peptide two Leucine peptide(LL-37), which enhances innate immunity, according to this research (Kamen and Tangpricha, 2010).

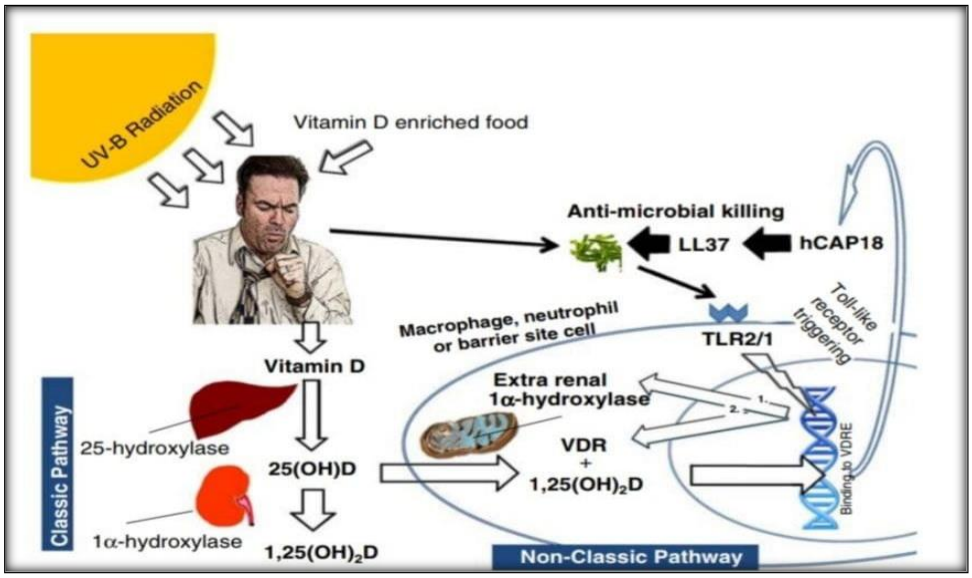


Figure (1.5) Proposed mechanism for vitamin D's action on the innate immune system (Kamen and Tangpricha, 2010)

Monocytes and macrophages are important elements of the innate immune system, and they serve as a first line of defense against microbial

intruders. As shown in figure (1.5) ,1,25(OH)2D3 has been identified as an important modulator of innate immune responses in this context, Immune cells like monocytes and macrophages have antibacterial properties that can be enhanced. In addition, 1,25(OH)2D3 improves macrophage chemotactic and phagocytic abilities. The antibacterial effects of 1,25(OH)2D3 have been shown to be mediated via the VDR and linked to the overexpression of the cathelicidin hCAP-18 gen (Baeke *et al.*, 2010). Vitamin D also inhibits the pro-inflammatory cytokines IL17 and interferon gamma, while increasing the production of the anti-inflammatory cytokine interleukin10 by CD4+ T cells, with effects that are considerably stronger in female T cells than in male T cells. Similarly, men's anti-CD3 and anti-CD28-stimulated peripheral blood mononuclear cells produced significantly less regulatory CD4+CD25+ FoxP3+ T lymphocytes in response to vitamin D than women's cells, but this gender difference vanished when oestradiol was added. This could explain why men have been observed to have a higher severity of COVID-19 (Griffin et al., 2020). The amount of vitamin D required in the blood to promote continuous AMP synthesis against pathogenic microorganisms is unknown at this time, however it is thought to be in the 10,000 (250 mcg) IU/day to 25,000 (625 mcg) IU/day range. The photosynthetic response to sunlight produces 10,000 (250 mcg) IU/day to 25,000 (625 mcg) IU/day of vitamin D, which efficiently treats TB infections, rickets, and psoriasis plaques without producing hypercalcemia. As a result, vitamin D supplementation in this range be considered right in order to protect patients from the coronavirus. (Mccullough *et al.*, 2020).

1.5. Association between vitamin D and COVID-19

Vitamin D has been identified as a key cofactor in a variety of physiological processes related to bone and calcium metabolism, as well as non-skeletal consequences such as autoimmune disorders, cardiovascular diseases, type 2 diabetes, obesity, cognitive decline, and infections. The impact of vitamin D metabolites on the immune system response and the development of COVID-19 infection by the new SARS-CoV-2 virus has been described in specific (Merzon et al., 2020). Other activities of this vitamin have recently been proposed, including immune response modulation in viral and autoimmune illnesses. and has a significant impact on immunological functioning, It inhibits the expression of inflammatory cytokines [e.g., IL-1, IL-1, tumor necrosis factor-] and its deficiency has been linked to T1 cytokine overexpression (Jain et al., 2020). Vitamin D deficiency has been linked to viral respiratory tract infections in epidemiological research (Montano-loza, 2020). Vitamin D [1,25(OH)2D] affects the innate and acquired immune systems in response to bacterial and viral pathogen invasion by interacting with its receptor (VDR) in immune cells. It also serves as a down-regulator of ACE-2 and modulates the renin-angiotensin system. As a result, vitamin D may aid in the treatment of COVID-19 by reducing the cytokine storm and, as a result, ARDS, which is a common cause of death (Maghbooli, 2020). Angiotensin-converting enzyme 2 is the receptor for SARS-CoV-2, as it is for SARS-CoV. (ACE2). ACE2 may play multiple roles that are mutually exclusive. Given that it is the SARS-CoV-2 receptor, it is logical to expect that increased expression of ACE2 would be detrimental to the human host. However, several investigations have revealed that ACE2 plays a critical role in guarding against acute lung injury and ARDS in experimental animals since its discovery twenty years ago (Rhodes et al., 2021). Following deregulation of the renin-angiotensin system, increased

cytokine production may develop, potentially leading to catastrophic ARDS .Vitamin D3 was found to have activity in lung tissue in both mice models and human cell lines, as well as protective benefits against experimental interstitial pneumonitis Several in vitro investigations have shown that vitamin D3 plays an important role in local "respiratory homeostasis," either by increasing the production of antimicrobial peptides or by directly interfering with respiratory virus proliferation. As a result, vitamin D3 deficiency may have a role in ARDS and heart failure, which are symptoms seen in COVID-19 patients who are critically unwell. As a result, vitamin D insufficiency stimulates the renin-angiotensin system (RAS), which can lead to CVD and deterioration of lung function. In COVID-19, those with certain comorbidities account for a larger percentage of serious illness case (Ali, 2020) .

1.6.Diagnosis

1.6.1 RT_PCR

Real-time fluorescence (RT-PCR) was used to detect SARS-CoV-2 positive nucleic acid in sputum, throat swabs, and lower respiratory tract secretions (Adhikari et al., 2020). However, the sensitivity of testing varies depending on when it is performed in relation to exposure. Sensitivity was estimated to be 33% 4 days after exposure, 62 percent on the day of symptom start, and 80 percent 3 days following symptom onset in one modeling study. 61-63 The appropriateness of the specimen collecting technique, time from exposure, and specimen source are all factors that contribute to false-negative test findings. Bronchoalveolar lavage fluid and other lower respiratory samples are more sensitive than upper respiratory samples (Wiersinga , 2020).

1.6.2. Diagnosis using radiology images

With the speedy and precise diagnosis of COVID-19, lives could be saved, disease spread could be limited, and massive data could be gathered from AI models. Scientists working on applications have demonstrated that it can provide radiologists more time and help them make a diagnosis faster and less expensively than traditional coronavirus tests. X-rays and CT scans, or computed tomography, can be used for this purpose. Tests for the Coronavirus are in poor supply and expensive; nevertheless, all emergency clinics have X-Ray or CT (Kumar, Gupta and Srivastava, 2020).

1.6.3. Laboratory test results

Leucopenia (9–25%) or leucocytosis (24–30%), lymphopenia (63%) and high levels of alanine aminotransferase and aspartate aminotransferase (37%) were the most prevalent laboratory abnormalities reported on admission among hospitalized patients with pneumonia. A total of 83 percent of the 1099 COVID-19 patients exhibited lymphocytopenia, with 36 percent having thrombocytopenia and 34 percent having leucopenia. There have also been reports of moderate thrombocytopenia and a rise in lactate dehydrogenase. Clinical severity is linked to elevated inflammation indices, which typically include higher C-reactive protein (CRP) values. Individuals with normal percentage oxygen saturation ($SatO_2$) had a CRP level of 1.1 mg/dL, while hypoxemic patients had a CRP level of 6.6 mg/dL, Furthermore they discovered a link between CRP and mortality risk. Troponin levels were also found to be elevated in 7% of patients who died as a result of fulminant myocarditis. Troponin appears to be a reliable predictor of mortality. Finally, it was discovered that D-dimer and ferritin levels in hospitalized individuals were typically high (Pascarella, 2020),(Kermali *et al.*, 2020).

1.6.4. Serological tests

Anti-SARS-CoV-2 immunoglobulins are detected using this method. Serological assays have sparked considerable attention as a potential alternative or complement to RT-PCR in the diagnosis of acute infection, as some may be less expensive and easier to use at the point of care (Lisboa Bastos et al., 2020). To capture prior infections, serological platforms were created and tested for validation of SARS-CoV-2-specific antibody responses. Highlighting the shortcomings of RT-PCR as a solitary diagnostic tool in surveillance due to its inability to detect past infection, and the added usefulness of serological testing, which can detect both ongoing and past infections if obtained within the appropriate interval after disease onset (Winter and Hegde, 2020).

1.7. Treatment

1. Treatment in general COVID 19 confirmed patients require complete bed rest and supportive care, as well as enough calorie and water intake to avoid dehydration. Homeostasis and electrolyte balance in water If the fever rises above 38.5 °C, antipyretic medication should be taken. As a prophylactic strategy to reduce the temperature, a warm water bath and antipyretic patches are preferred (Hafeez *et al.*, 2020).
2. Antiviral therapy, antivirals (e.g., remdesivir, favipiravir), antibodies (e.g., convalescent plasma, hyperimmune immunoglobulins), anti-inflammatory drugs, targeted immunomodulatory therapies, anticoagulants (e.g., heparin), and antifibrotics are all examples of antiviral therapies (Wiersinga *et al.*, 2020) ,(Zhang J.*et al.*, 2020).
3. Patients are treated with supportive treatment in addition to antiviral therapy, such as oxygen therapy, dialysis, noninvasive ventilator support, ventilator support, and extracorporeal membrane oxygenation therapy when they develop respiratory failure (Hung 2020). Current

research suggests that corticosteroids did not improve survival in SARS and MERS patients, but rather slowed virus clearance. As a result, unless otherwise indicated, corticosteroids should not be used on a regular basis. Because of its direct antiviral impact on SARS-CoV in cell culture, arbidol is utilized experimentally in China(He, Deng and Li, 2020).

4. The macro- and micronutrient state of the host as COVID-19 prevention measures Diet and nutrition have a strong influence on immune system competency and infection risk and severity. Diet, nutrition, infection, and immunity all have bidirectional interactions. These micro- and phytonutrients, which include beta-carotene, vitamin C, vitamin E, and polyphenolic substances, provide antioxidants and anti-inflammatory nutrients that control immunological activities. The use of anti-inflammatory foods, nutrients, or pharmaceuticals as part of a COVID-19 treatment approach is a potential alternative. Dietary supplementation of micronutrients with known roles in immune function can improve immune response regulation and reduce infection risk. Zinc and vitamins C and D are the micronutrients with the most evidence of immunomodulating activity (Gasmi *et al.*, 2020) ,(Name *et al.*, 2020),(Alexander *et al.*, 2020).
5. The best hope for halting the pandemic is a vaccine to prevent COVID-19 infection (Malik et al., 2020). COVID-19 vaccination from Russia On August 11, 2020, Russia became the first country in the world to approve a vaccine against the coronavirus that causes severe acute respiratory syndrome (SARS- CoV-2). The Gamaleya National Center of Epidemiology and Microbiology created the vaccine, which is based on two adenovirus vectors (Burki, 2020).

1.8. Prevention

Because there are no approved treatments for this infection at this time, prevention is essential. Non-specific features of the disease, infectivity even before onset of symptoms in the incubation period, transmission from asymptomatic people, long incubation period, tropism for mucosal surfaces such as the conjunctiva, prolonged duration of the illness, and transmission even after clinical recovery are all factors that make prevention difficult (Singhal,2020) .Suspected cases with symptoms of respiratory infections (e.g., runny nose, fever, and cough) who present to healthcare institutions must wear a face mask to confine the virus and follow the triage method to the letter. They should not be allowed to wait in the same facilities as other patients seeking medical attention. They should be kept in a separate, well-ventilated room that is at least 2 meters away from other patients and has easy access to respiratory hygiene products. If a confirmed COVID-19 case requires hospitalization, they must be put in a single patient room with negative air pressure and at least six air changes per hour. Personal protective equipment (PPE) such as gloves, gowns, disposable N95, and eye protection should be used by anybody entering the room, including medical personnel. The room should be decontaminated or cleaned once the cases have been recovered and discharged, and personnel entering the room should wear PPE such as a facemask, gown, and eye protection (Harapan et al., 2020).

1.9. Research Aims and Objectives

Our objectives were to:

1. Find the association between vitamin D3 levels and the severity of COVID-19 in Iraqi patients.
2. Study the mechanisms of Vitamin D3 action in patients with COVID-19 and discus how it will be used in reduction the severity of this disease.
3. Study the level of Cathelicidin as a marker of innate immune system in Iraqi patients with COVID 19.
4. Study the association between Cathelicidin and vitamin D3 levels which may support use in treatment of Iraqi patients with COVID 19.

Chapter Two

Materials & Methods

2. Subjects, Materials and Methods

2.1. subjects

case control study for 90 Iraqi individuals with covid19 .60 blood samples from patients presented with sign and symptoms of COVID-19. Patients Classified into three severity levels depending on clinical manifestations: mild, moderate, and severe disease depended on pulmonary imaging, however, respiratory characteristics such as respiratory rate, oxygen saturation, and lesion development and 30 control individuals. clinical data collected by use of specific questionnaire including age, gender, BMI and having any current chronic diseases. They were also exposed to medical examination for signs and symptoms of COVID-19 by specialized doctor based on the World Health Organization (WHO) criteria. All patients were subjected to the full clinical history, clinical examination, and relevant laboratory investigations.

2.2. sample collection

Samples Collection were Started from October to December 2020 from Imam Hussein Medical city in Karbala and from Private Labs from patient's of COVID-19 and control. Five mls of whole blood were collected, blood was left for (15 min) at room temperature in gel tube. Serums were separated by centrifuging for 10 minutes at approximately 4000 xg. Serum samples were separated into two Eppendorf tubes and Store at (-20 C) to avoiding multiple freezing-thawing cycle. Grossly hemolyzed or grossly lipemic specimens were not used.

Parameter were measured include:

- vitamin D3
- cathelicidin
- Hb& (WBC & LYMPHOCYTE)
- D-dimer
- ferritin

2.3. Material and instruments and tools

Table 2.1: Kits

No.	kits	Source (country)
1	D_dimer kit	German
2	Ferritin kit	French
3	human cathelicidin _1 Elisa kit	China
4	maglumi 25_oH vitamin D (CLlA) Kit	Shanghai Korain

Table 2.2: The instruments and tools used in the study

No.	Instruments	Manufacturer
1	Centrifuge	Hettich (Germany)
2	Cobas e 411 fully automated analyzer	China
3	Deep freezer	Hitachi (Japan)
4	Disposable Syringe	China
5	Distilled water	United States
6	Eppendorf tube	China
7	Fully automated chemiluminescence immunoassay analyzer	snibe diagnostic (China)
8	fully automated ELISA analyzer	Germany
9	Gel tube	China
10	Micropipette	Germany
11	Micropipette tips	Salmed _Germany
12	Mini vidus	German
13	Test tube	China
14	XP-300™ Automated hematology analyzer device	Japan

2.4.Methods

2.4.1. Determination of cathelicidin

Principles of Assay an Enzyme-Linked Immunosorbent Assay is included in this kit (ELISA). A Human CATHL1 antibody has been pre-coated on the plate. CATHLI was added to the sample and binds to the antibodies coated on the wells. The biotinylated Human CATHLI Antibody was then added to the sample, which binds to CATHLI. The Streptavidin-HRP was then added, which binds to the CATHLI antibody that has been biotinylated. During the washing stage after incubation, any unbound Streptavidin-HRP was rinsed away. The solution was added to the substrate, and the color developed in proportion to the amount of Human CATHL. For reaction termination, an acidic stop solution was applied, and absorbance was measured at 450nm.

Table 2.3: Reagent Provided

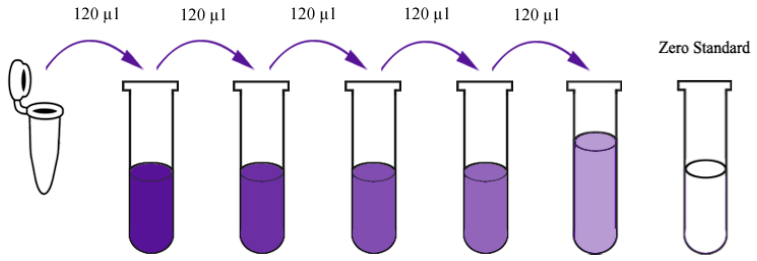
Components	Quantity
Standard Solution (400ng/ml)	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Biotinylated Human CATHLI Antibody	1ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

Reagent Preparation

all reagents were brought at room temperature before using. The standard's 120 µl I(400ng/ml) was Standard To make a 200ng/ml standard stock solution, reconstituted with 120 µl of standard diluent. Before making dilutions, the standard was allowed to sit for 15 minutes with gentle agitation. There were duplicate standard points. To make the 100ng/ml, 50ng/ml, 25ng/ml, and 12.5ng/ml solutions, dilute the standard stock solution (200ng/ml) 1:2 with standard diluent. The zero standards are made up of standard diluent (0 ng/ml). Any leftover solution should be frozen at -20°C and used within one month of freezing. The following is a suggested dilution of standard solutions:

Table 2.4: Preparation of standard solutions

200ng/ml	Standard No.5	120ul Original Standard+ 120µl Standard Diluent
100ng/ml	Standard No.4	120ul Standard No.5+ 120µl Standard Diluent
50ng/ml	Standard No.3	120ul Standard No.4+ 120µl Standard Diluent
25ng/ml	Standard No.2	120ul Standard No.3 + 120µl Standard Diluent
12.5ng/ml	Standard No.1	120ul Standard No.2+ 120µl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
400ng/ml	200ng/ml	100ng/ml	50ng/ml	25ng/ml	12.5ng/ml

Prepare wash buffer

20mL Ix Wash Buffer was made by diluting 25x Concentrate in 480 mL deionized or distilled water. The crystals were gradually mixed until they dissolved entirely.

Assay Procedure

1. All of the reagents, standard solutions, and samples were made according to the instructions. Before use, all reagents were brought to room temperature. The experiment was carried out at room temperature.
2. For the assay, the number of strips was determined. For use, the strips were put into the frames. The unused strips should be kept at a temperature of 2-8°C.
3. A standard well with a 50ul standard was added. Because the standard solution already contains biotinylated antibodies, no antibody was added to a standard well.
4. 40ul sample was applied to sample wells, followed by 10ul anti-CATHLI antibody, 50ul streptavidin-HRP to sample wells, and finally 50ul streptavidin-HRP to standard wells (Not blank control well). It was a mixed bag. A sealer was applied to the plate. At 37°C, incubate for 60 minutes.
5. The plate was cleaned five times with wash buffer after the sealer was removed. For each wash, wells were immersed in at least 0.35 ml wash buffer for 30 seconds to 1 minute. All wells were aspirated and washed five times with wash buffer for automated washing; the well was overfilled with wash buffer. Paper towels or other absorbent material were used to map the plate.
6. 50 µl of substrate solution A was added to each well, followed by 50ul of substrate solution B. For 10 minutes at 37°C in the dark, the incubator plate was covered with a new sealer.
7. A 50 µl Stop Solution was applied to each well, and the blue hue was quickly turned to yellow.
8. Within 10 minutes of adding the stop solution, the optical density (OD value) of each well was assessed using a microplate reader set to 450 nm.

Typical Data

This standard curve is just meant to be used as an example. With each assay, a standard curve should be created.

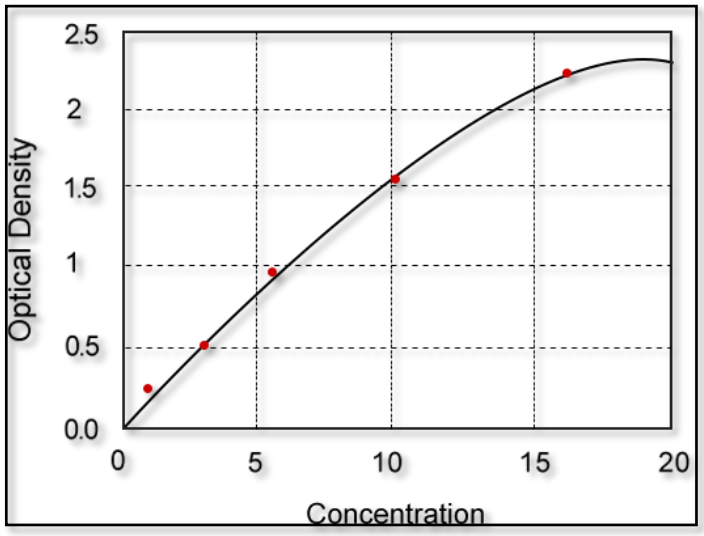


Figure (2.1) standard curve

2.4.2. determination of vitamin D3

Principle

The 25-OH Vitamin D assay was a chemiluminescence immunoassay with a competitive design. The 25-OH Vitamin D assay was a chemiluminescence immunoassay with two incubations for the quantification of human serum. The displacing reagent separated the 25-OH vitamin D from its binding protein in the first incubation, and the 25-OH vitamin D antibody on the magnetic microbeads binds to the 25-OH vitamin D antibody, generating an antibody-antigen complex. The 25-OH Vitamin D labeled ABEI were added after a second incubation. During a wash cycle, the remaining loose material was removed. Then, to start a flash chemiluminescent reaction, Starter 1+2 was applied. The chemiluminescent reaction that resulted was quantified in relative light units (RLUS). This is inversely proportional to the amount of 25-OH Vitamin D in the sample (or, if applicable, the calibrator/control)

Table 2.5: Kit Component

Components	Contents
Magnetic Microbeads	Magnetic microbeads coated with 25-OH Vitamin D monoclonal antibody, containing BSA, NaN3 (<0.1%).
Calibrator Low	Containing BSA and 25-OH Vitamin D antigen, NaN3 (<0.1%).
Calibrator High	Containing BSA and 25-OH Vitamin D antigen, NaN3 (<0.1%).
Displacing Reagent	Acidic buffer.
ABEI Label	25-OH Vitamin D antigen labeled with ABEI.
Internal Quality Control	Containing BSA and 25-OH Vitamin D antigen, NaN3 (<0.1%).
All reagents are provided ready-to-use.	

Test Procedure

Preparation of the Reagent

When the kit was successfully loaded, the magnetic microbeads were automatically resuspended, ensuring that the magnetic microbeads were homogeneously resuspended before usage. Follow the operating instructions for the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer to ensure proper test performance. An RFID CHIP on the Reagent kit was used to identify each test parameter.

Calculation of Results

The analyzer calculated the 25-OH Vitamin D concentration in each sample automatically using a calibration curve prepared by a 2-point calibration master curve process. The data were given in nanograms per milliliter

Interpretation of Results

A review of the literature indicated the following ranges based on factors such as dietary intake, race, UV exposure, and season.

Table 2.6: classification of 25_OH vitamin D status

Vitamin D status	25-OH Vitamin D
Deficiency	< 10 ng/mL
Insufficiency	10-29 ng/mL
Sufficiency	30-100 ng/mL
Toxicity	>100 ng/mL

2.4.3 determination of ferritin:

Principle

The assay uses a one-step enzyme immunoassay sandwich approach with a fluorescent detection step at the end (ELFA). The Solid Phase Receptacle (SPR) functions as both the solid phase and the assay's pipetting mechanism. The assay's reagents are pre-dispensed and ready to use in sealed reagent strips. The equipment carried out all of the assay stages automatically. The reaction media was cycled in and out of the SPR several times. The substrate (4-Methyl- umbelliferyl phosphate) was cycled in and out of the SPR during the final detection stage. The conjugate enzyme catalyzed the hydrolysis of this substrate. whose fluorescence was measured at 450 nanometers. The fluorescence intensity is proportional to the a mount of antigens present in the sample. Results were automatically calculated by an equipment with the calibration curve recorded in memory at the end of the assay and then printed out times.

Table 2.7: Content of The Kit

60 FER strips	SPR	Ready-to-use.
60 FER SPRS 2 x 30	STR	Ready-to-use. Interior of SPRS coated with monoclonal anti-ferritin immunoglobulins (mouse).
FER control 1 x 2 ml (liquid)	C 1	Ready-to-use. Tris buffer (0.1 mol/l) pH 7.4 + human ferritin + bovine albumin + protein and chemical stabilizers. The confidence interval in ng/ml is indicated on the MLE card after the following mention: "Control C1 Dose Value Range".
Calibrator 1 x 2 ml (liquid)	S 1	Ready-to-use. Tris buffer (0.1 mol/l) pH 7.4 + human ferritin + bovine albumin + protein and chemical stabilizers. The concentration in ng/ml is indicated on the MLE card after the following mention "Calibrator (S1) Dose Value". The confidence interval in "Relative Fluorescence Value" is indicated on the MLE card after the following mention: "Calibrator (S1) RFV Range".
FER dilution buffer 1 x 25 ml (liquid)	R1	Ready-to-use. Tris buffer (0.1 mol/l) pH 7.4 + bovine albumin + protein and chemical stabilizers.

Procedure

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

2. For each sample, control, or calibration to be evaluated, use one FER strip and one FER SPR. After removing the appropriate SPRS, make careful to reseal the storage pouch.

3. To enter the test code, type or choose FER on the instrument. "S1" must be used to identify the calibrator, and it must be tested twice. "C1" should be used to identify the control if it has to be tested.

4. A Vortex-type mixer was used to mix the calibrator, control, and samples.

5. In the sample well, 100 ul of calibrator, sample, or control were pipetted.

6. In the instrument, the SPRS and strips were inserted. Check that the color labels on the SPRS and the Reagent Strips match the assay code.

7. The equipment carried out all of the assay stages automatically. The assay will take around 30 minutes to complete.

8. The SPRS and strips were removed from the instrument when the assay was completed.

9. SPRS and reagent strips that have been utilized were discarded in the proper container.

Result and Interpretation

The findings of the assay were automatically examined by the computer once it was completed. For each material examined, fluorescence was measured twice in the reading cuvette of the Reagent Strip. The first reading is a background reading taken before the SPR was placed into the substrate cuvette. After incubating the substrate with the enzyme remaining on the interior of the SPR, the second reading was taken. By removing the background reading from the final result, the RFV (Relative Fluorescence

Value) is calculated. On the result sheet, you'll see this computation. The concentrations were expressed in ng/ml (preparation NIBSC 80/578). The results were automatically generated by the instrument using calibration instrument curves that were saved by the (4-parameter logistics model). After dilution to 1/10 or 1/100 in FER dilution buffer, samples with ferritin contents greater than 1,200 ng/ml must be retested (R1) To determine the sample titer, multiply the value by the dilution factor if the dilution factor was not entered when the Work List was established (see User Manual). The patient's history, as well as any other tests performed, should be considered when interpreting test results.

Range of expected values

These figures are provided as a guide; each laboratory should generate its own reference values based on a carefully selected population. "We used 206 samples from clinically healthy, hematologically normal adults with no liver diseases to establish expected values." When utilizing the percentage technique to characterize the population seen, the following results are obtained:

Men:

Range of values Prevalence	0-68 ng/ml 5%	68-208 ng/ml 45%	208 - 434 ng/ml 45%	Mean 236 ng/ml

Normal menstruating women:

Range of values Prevalence	0-9.3 ng/ml 5%	9.3- 45 ng/ml 45%	45 - 159 ng/ml 45%	Mean 58 ng/ml

Menopausal women:

Range of values Prevalence	0- 24.4 ng/ml 5%	24.4- 118 ng/ml 45%	118- 278 ng/ml 45%	Mean 151 ng/ml

2.4.4 Measurement of D-dimer:

Principle of the test

The sandwich chemiluminescence immunoassay D-Dimer is a sandwich chemiluminescence immunoassay. The sample (or calibrator/control, if applicable), ABEI labeled with anti-D-Dimer monoclonal antibody, buffer, and magnetic microbeads coated with another anti-D-Dimer monoclonal antibody were thoroughly mixed and incubated to form a sandwich; after precipitation in a magnetic field, decant the supernatant, and run a wash cycle. After that, we added Starter 1+2 to start a flash chemiluminescent reaction. A photomultiplier was used to measure the light signal as relative light units (RLUs), which were proportionate to the amount of D-Dimer in the sample (or calibrator/control, if appropriate).

Table 2.8: Kit Components

Components	Contents	100 tests (REF:13060 6008M)
Magnetic microbeads	Magnetic microbeads coated with anti-D-Dimer monoclonal antibody, containing BSA, NaN3 (<0.1%).	2.5mL
Calibrator Low	D-Dimer antigen containing BSA, NaN3 (<0.1%).	2.5mL
Calibrator High	D-Dimer antigen containing BSA, NaN3 (<0.1%).	2.5mL
Buffer	containing BSA, NaN3 (<0.1%).	6.5 mL
ABEI Label	Anti-D-Dimer monoclonal antibody labeled with ABEI, containing BSA, NaN3 (<0.1%).	6.5 mL
Internal Quality control	D-Dimer antigen containing BSA, NaN3 (<0.1%).	2 mL

Test Procedure

Preparation of the Reagent

When the kit is successfully loaded, the magnetic microbeads are automatically resuspended, ensuring that the magnetic microbeads are homogeneous before usage.

Dilution

This reagent kit does not include sample dilution by analyzer. Manual dilution of samples with concentrations over the measurement range is possible. The value was multiplied by the dilution factor after manual dilution.

Calculation of Result

The analyzer estimated the D-dimer concentration in each sample automatically using a calibration curve prepared using a 2-point calibration master curve process. The outcome was measured in FEU/ml.

2.4.5 Determination of Complete blood cells CBC:

CBC measurements were made by XP-300™ Automated hematology analyzer device.

Analysis Principle:

· WBC: DC detection method

· LYM: DC detection method

· HGB: Non-cyanide hemoglobin analysis method

Parameters:

Whole blood mode, Pre-diluted mode WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT LYM%, MXD%, NEUT%, LYM#, MXD#, NEUT# RDW-SD, RDW-CV, PDW, MPV, PCT, P-LCR

Sample volum:

· WB mode Approx. 50µL PD mode Approx. 20µL

2.5 Statistical analysis

All the data was analyzed with descriptive statistical analysis using SPSS software version 26.0. Independent _sample t test has been used to determine the significant difference between two groups, p values less than 0.05 is considered significant. One-way anova has been used to compare among biochemical parameters in more than two groups. Roc analysis also has been used to determine the sensitivity and specificity of Vitamin D ,Cathelicidin and D-dimer.

Chapter Three

Results & Discussion

3. Result & Discussion

3.1 Demographic characteristics of patients and controls:

The demographic characteristics and laboratory parameters of both patient groups and the healthy control group were summarized in Table (3.1). Age of participants which was within the age group of (20-74) years old. The descriptive table also shown an adjustment of other risk factors which were collected through the self-reported technique (student questionnaire), these factors included: hypertension, DM, BMI and smoking.

Table (3.1): Demographic characteristics of patients and controls

Characteristics	Patient group N=60		Control group N=30	
Age(mean)	50		47	
BMI (Mean Kg/m2)	31.91		30.94	
Gender% (male/female)	N	Percent	N	Percent
	32/28	53.33% / 46.67%	16/14	53.33% / 46.67%
DM(Yes/No)%	28/32	32.22%/34.44%		–
hypertension (Yes/No)%	39/21	43.33%/23.33		–
Smoking state (Yes/No)%	29/31	48.33% /51.67%	18/12	60% /40%

Gender distribution among the studied groups were: 53.33% male, 46.67% female for patients and control group as shown in figure 3.1&3.2.

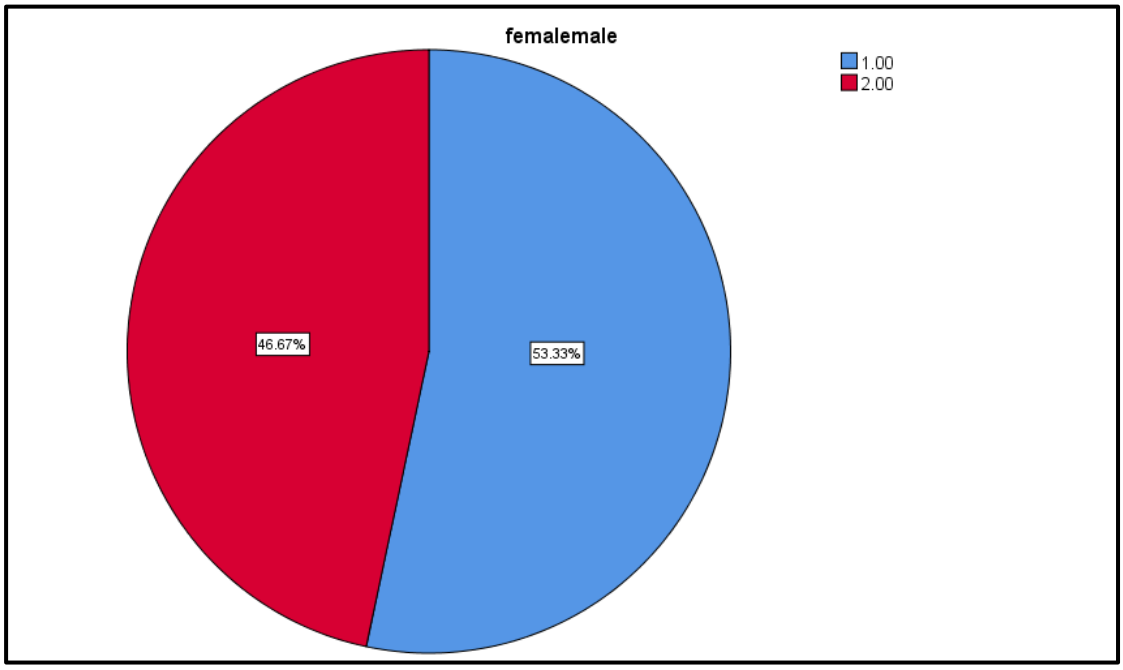


Figure (3.1) percentage of male and female among COVID19 patients.

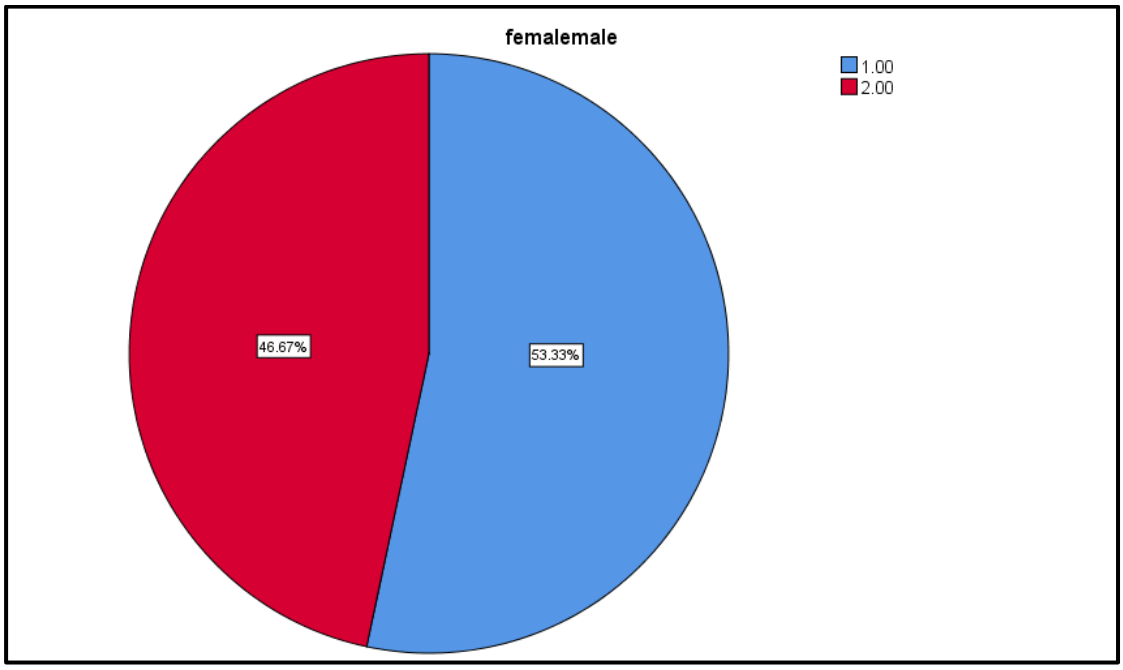


Figure (3.2) percentage of male and female among control subjects.

The present study showed that the higher frequency of COVID19 occurs in men (53.3%), while its frequency in Women was Lower (46.67%). This result is in agreement with (Klein and Morgan, 2020) who showed that (COVID-19) trend to effect man more than woman.

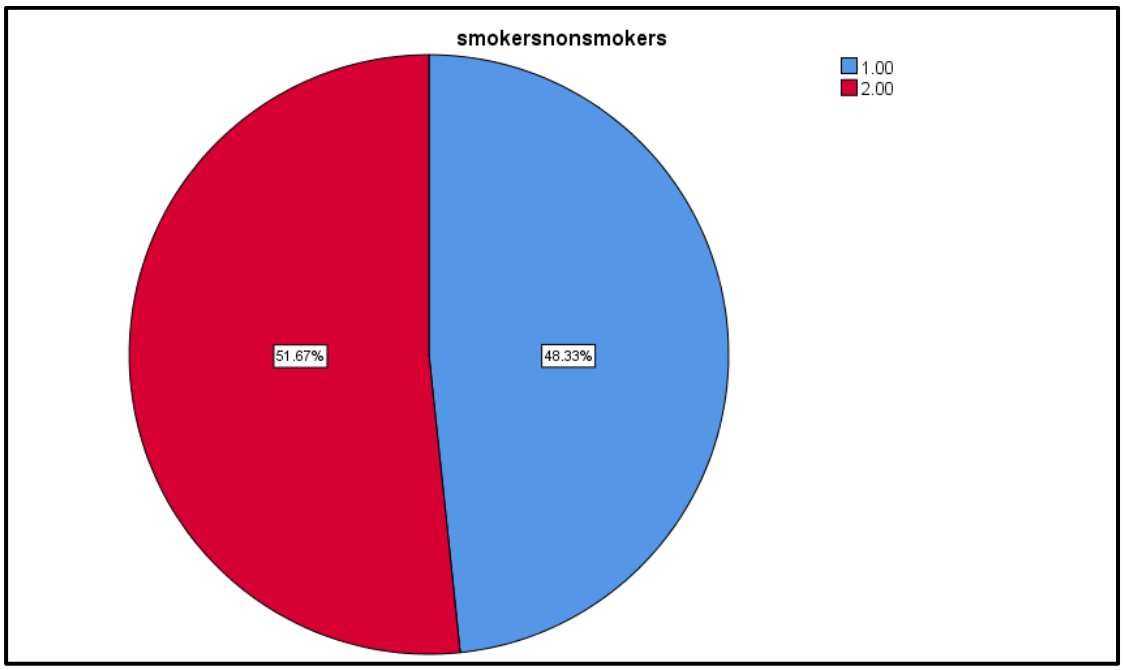


Figure (3.3) percentage of smokers and nonsmoker among COVID19patients.

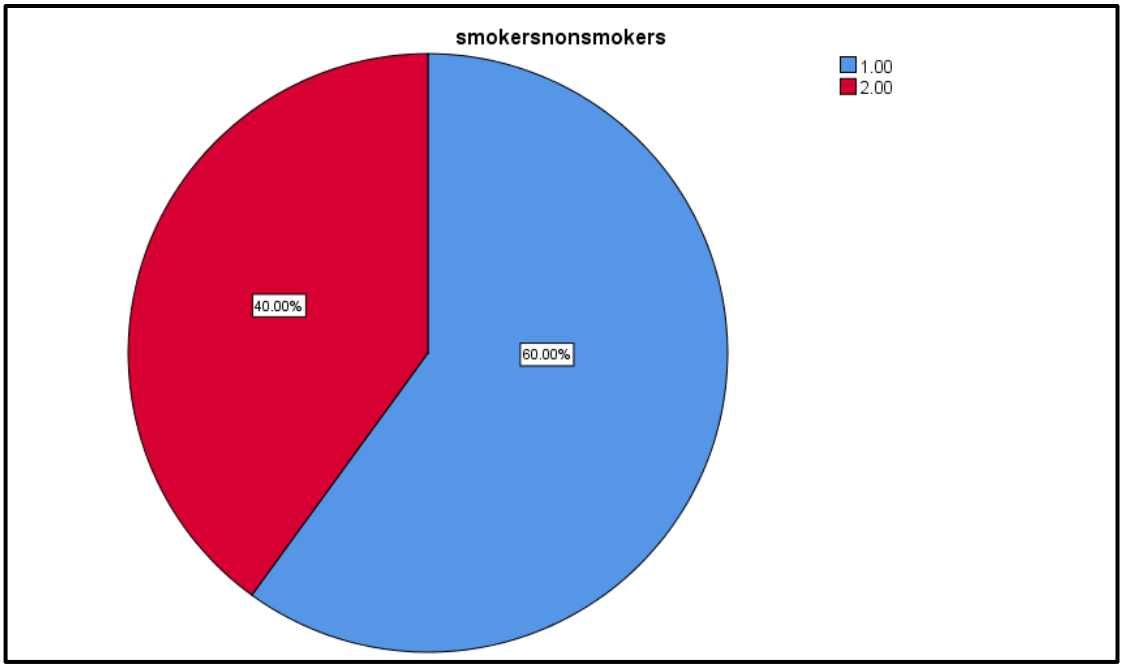


Figure (3.4) percentage of smokers and nonsmoker among control group.

As shown in figure3.3 &3.4 smoking distribution among the studied groups were: 48.33% smokers,51.67% non smokers in COVID19 patients, while 60% smokers and 40 % nonsmokers in control group .

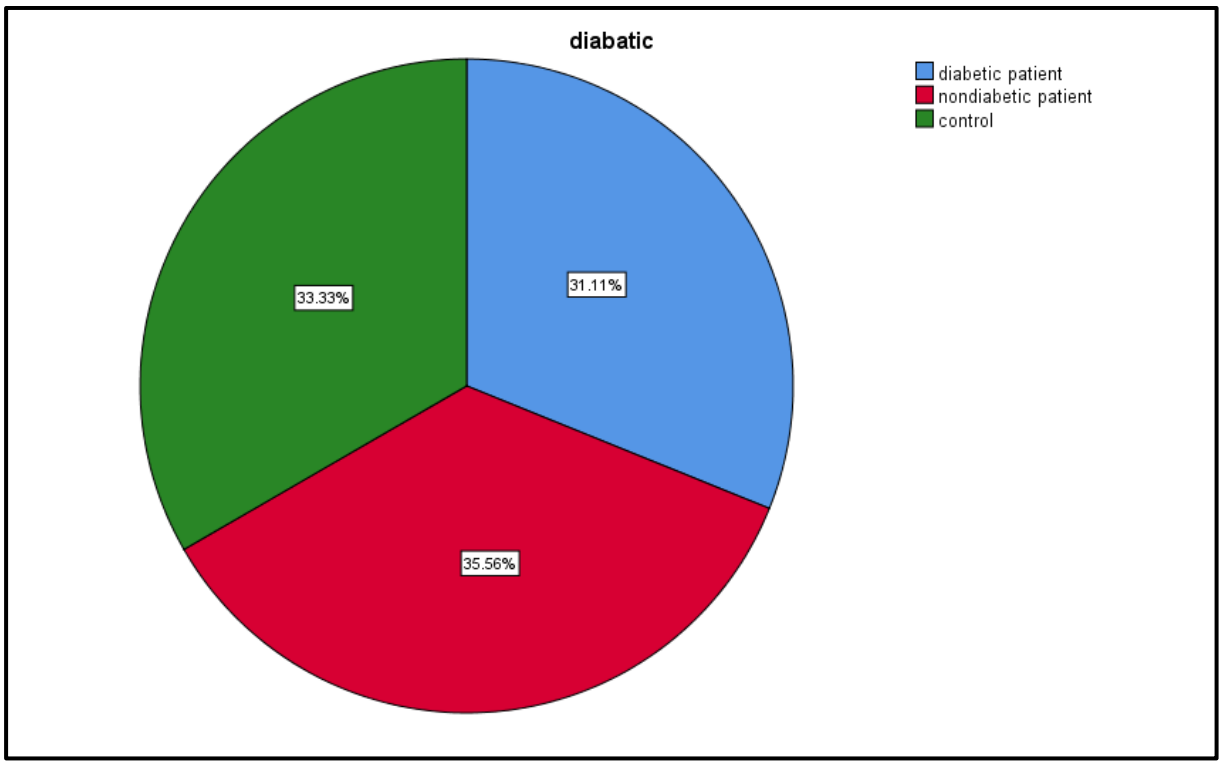


Figure (3.5) percentage of Diabetic and non Diabetic among studied subject.

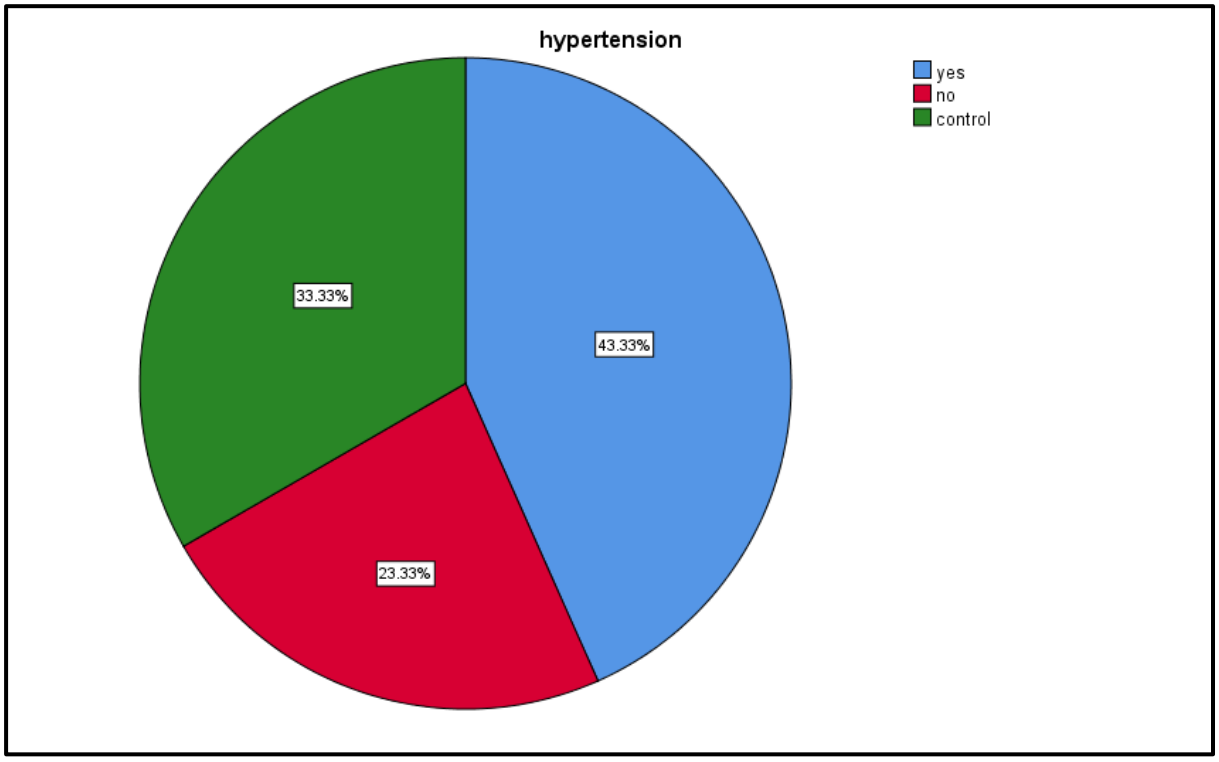


Figure (3.6) percentage of Hypertension and without hypertension among studied subject.

As shown in figure3.5 DM distribution among the studied groups were: 31.11% DM,35.56 non DM, while 43.3% with hypertension and 23.3 % without hypertension as shown in figure 3.6.

The current study included 90 subjects (60 subjects with COVID19 and 30 control individuals). Biochemical characteristics of the recruited individuals are presented in table 3.2 show significant differences in lymphocyte, catheliciden, vitamin D3, D dimer and ferritin in patient group when compared with those of control group. However, no significant difference was seen in WBC and HB. In patients group the mean levels of HB, WBC, Lymphocyte count, D-dimer ,ferritin ,cathelicidin and vitamin D3 were12.7,16.3,4.5,1799.7,1542.1,51.8, and 15.3 respectively. In controls, the mean levels of HB, WBC , Lymphocyte count , D-dimer ,ferritin ,cathelicidin and vitamin D3 were 11.9, 14.87, 24.9, 327.8, 204.8, 49.4and 17.05 respectivel.

Table 3.2: biochemical parameters in covid19 patients in comparison with controls

Parameters	Patient(N)=60 Mean ±SD	Control (N)=30 Mean +SD	P_value
Hb g\dl	12.7± 2.1	11.9±1.7	> 0.05
WBC \mm^3	16.3± 501.2	14.87.1±102.3	> 0.05
Lymph\mm^3	4.5± 2.5	24.9±7.2	< 0.05
D-dimer ng\ml	1799.7 ± 1823.6	327.8±66.4	< 0.05
Ferritin ng\ml	1542.1 ± 2220.06	204.8±103.2	< 0.05
cathelicidin ng\ml	51.8±8.04	49.4 ± 9.1	<0.05
Vitamin D3 ng\ml	15.3 ± 6.1	17.05 ± 6.4	< 0.05

The present study showed that the mean levels of HB was higher in patient group when compared with those of control group . This result is in agreement with (Kuno *et al.*, 2021) who showed that high hemoglobin is also associated with in-hospital mortality of COVID-19 in this study is valuable. High Hb levels themselves might cause systemic thrombosis .Lack of oxygen due to lung disease or living in high places this leads to increase in the concentration of red blood cells and hemoglobin (the protein responsible for transporting oxygen to body tissues).The present study showed that the mean levels of WBC and lymphocyte count were lower in patient group when compared with those of control group. This result is

in agreement with (Huang and Pranata, 2020) who showed that the association between lymphopenia and severe COVID-19 . Other than lymphocyte infiltration and sequestration in the lungs, gastrointestinal mucosa may be a direct target of SARS-CoV-2 infection, and a rise in pro-inflammatory cytokines in COVID-19, particularly IL-6, may promote additional lymphocyte decrease. In COVID-19, lymphopenia can be utilized as a prognostic indicator. The present study showed that mean level of ferritin and D-dimer were higher in patient group when compared with those of control group .This result is in agreement with (Al Meani *et al.*, 2020) who showed that slightly higher levels of D-dimer and Ferritin level in serum of COVID-19 patients. H and L are the two subunits that make up ferritin. H-ferritin is thought to operate as an immune modulator with both proinflammatory and immunosuppressive properties, according to previous research. Increased ferritin levels could be a sign of a severe inflammatory response triggered by viral entrance into the human body and its effects on iron metabolism. Elevated serum ferritin levels have been found to significantly correlate with disease severity in COVID-19 infected patients. D-dimer levels have been linked to the severity of COVID-19 infection as well as mortality. D-dimer is a cross-linked fibrin breakdown product, signaling enhanced thrombin production and fibrin dissolution by plasmin. High D-dimer levels are common in acute illness individuals with a number of infectious and inflammatory diseases. The coagulation system is active in critically sick patients, according to the study, and D-dimer levels are linked to the activation of the proinflammatory cytokine cascade, which leads to CSS. Venous thromboembolism is thought to be caused by coagulation irregularities caused by high D-dimer levels, which may contribute to raspatory impairment caused by COVID-19 infection (Qeadan *et al.*, 2021).The present study showed that the mean levels of cathelicidin was higher when compared with those of control group. This

result is in agreement with (Pahar *et al.*, 2020) who showed that AMPs are really expressed in normal skin, but their levels rise when the skin is damaged by external stimuli such as trauma, inflammation, or infection. Human Cathelicidin (LL-37) possesses antibacterial and antiviral properties. The antibacterial mechanism is most likely based on the peptide's unique membrane penetrating properties. By targeting the steps that occur before the virus enters the cell, LL-37 can prevent viral infection. It has the ability to: (i) produce pores in the viral envelope; (ii) promote extracellular aggregation of viral particles, blocking virus access and increasing virus absorption by phagocytes; and (iii) hinder the virus's attachment to its receptor on the cell surface. LL-37 and defensins can also disrupt viral replication at the intracellular stage. AMPs, on the other hand, do not work in isolation, especially when it comes to their role as powerful immune modulators, where they interact with toll-like receptors (TLRs) and chemokine receptors. Both of these receptors have been implicated in the pathophysiology of COVID-19 (Laneri et al., 2021). Respiratory Viruses are combated by antimicrobial peptides. Bacteria and viruses are common causes of lung illness in humans, with respiratory viruses accounting for a disproportionately higher part of the etiological role. They have a significant impact on mortality and economy around the world, as the World Health Organization has documented and tracked. Influenza A virus (IAV), respiratory syncytial virus (RSV), parainfluenza virus (PIV), metapneumovirus (MPV), human rhinovirus (HRV), human adenovirus (HAdV), bocavirus (BoV), and coronavirus are the most prevalent respiratory viruses (CoV). Antiviral activity of defensins and LL-37 has been proven against a number of viruses, including coronaviruses (Ghosh and Weinberg, 2021) .The present study showed that the mean levels of vitamin D3 was lower when compared with those of control group. This result is in agreement with (Bayramoğlu *et al.*, 2021) This study found out

association between vitamin D deficiency and clinical severity of COVID-19 (Bayramoğlu *et al.*, 2021). Vitamin D deficiency affects immunological activities because it acts as an immunomodulator, enhancing innate immunity by secreting antiviral peptides, which increases mucosal defenses. Low levels of serum vitamin D have been linked to acute respiratory tract infections such epidemic influenza in clinical investigations. Recent studies have revealed some of the mechanisms by which vitamin D reduces the incidence of microbial infections. Vitamin D works through a variety of methods to reduce the risk of viral infection and mortality. Vitamin D works through three channels to minimize the chance of catching a cold: the physical barrier, cellular natural immunity, and adaptive immunity. Vitamin D has also been shown to reduce the risk of COVID-19 infections and mortality in a recent review. These include maintaining cell connections and gap junctions, improving cellular immunity by lowering the cytokine storm via interferon- and tumor necrosis factor effect, and controlling adaptive immunity by limiting T helper cell type 1 responses and encouraging T cell induction. (Ali, 2020)

Table 3.3: Comparison of biochemical parameters according to smoking in patients and controls

Biochemical parameter		N	Mean ± Std. Deviation
Hb **g\dl**	smoker patient	29	13.24 ± 2.30 *a,b
	non smoker patient	31	12.19 ± 1.90 *c
	smoker control	18	12.87 ± 1.49
	non smoker control	12	10.45 ± 1.03
WBC**\mm³**	smoker patient	29	16.76 ± 5.63*a,b
	non smoker patient	31	15.93 ± 4.628
	smoker control	18	8.59 ± 1.76*c
	non smoker control	12	8.25 ± 2.39
Lymph**\mm³**	smoker patient	29	4.52 ± 2.5573*a,b
	non smoker patient	31	4.39 ± 2.5734
	smoker control	18	25.03 ± 6.36*c
	non smoker control	12	24.78 ± 8.64
D-dimer **ng\ml**	smoker patient	29	1838.63 ± 1782.03*a,b
	non smoker patient	31	1716.28 ± 1878.82
	smoker control	18	338.38 ± 72.48*c
	non smoker control	12	312.08 ± 55.23
Ferritin **ng\ml**	smoker patient	29	1853.85 ± 2959.64 *a,b
	non smoker patient	31	1208.85 ± 881.85
	smoker control	18	261.08 ± 97.58*c
	non smoker control	12	120.4833 ± 21.13794
cathelicidin **ng\ml**	smoker patient	29	55.104 ± 9.44
	non smoker patient	31	49.88 ± 9.09
	smoker control	18	49.64 ± 7.28
	non smoker control	12	48.99 ± 8.32
vitamin D3 **ng\ml**	smoker patient	29	16.23 ± 6.27*a,b
	non smoker patient	31	17.88 ± 6.64
	smoker control	18	14.17 ± 6.87*c
	non smoker control	12	15.57 ± 5.21

*:p-value <0.05,a(significant between smoker patient and non smoker patient)

b (significant between smoker patient and smoker control)

c (significant between smoker control and non smoker control)

According to smoking, table 3.3 shows significant differences between smoking covid19 patients and non smoking control, between non smoking

covid19 patients and nonsmoking control (p-value <0.05)in hematological parameters. The present study showed Mean levels of hematological parameters were higher in smoker than nonsmoker in patient and control. This result is in agreement with the (Malenica *et al.*, 2017) . Cigarette smoking increased the number of red blood cells, white blood cells, and hemoglobin in the blood. Hematological markers (e.g., hemoglobin, WBC, lymph count) are severely affected by cigarette smoking. Smoking had negative health effects and was a risk factor for the development of a variety of pathological conditions and diseases, including chronic obstructive pulmonary disease (COPD), cancer, pancreatitis, gastrointestinal disorders, periodontal disease, metabolic syndrome, and a few autoimmune diseases. Cigarette smoking has been linked to an increased risk of cardiovascular disease. Furthermore, the taxing effect of cigarette smoke on the respiratory tree, as well as the resultant inflammation, can lead to an increase in leukocyte numbers. It's been proven that inflammatory stimulation of the respiratory tract causes an increase in inflammatory markers in the bloodstream, particularly cytokines, which influence the quantity of leukocytes (Tapson, 2005) .This study showed that current cigarette smokers had better d-dimer levels than ex-smokers, who in turn had higher levels than individuals who had never smoked evidence implicates smoking immediately in the pathogenesis of thrombophilia states in the less platelet-dependent venous side of the circulation, there are strong suggestions of culpability. Similarly, there is no doubt about the causal relationship between smoking and the improvement of chronic obstructive pulmonary disease (COPD). Nevertheless, Despite clinical trial results linking smoking and COPD to venous thromboembolism (VTE), there is no evidence that irregular blood coagulation has a role in the pathogenesis or mortality of COPD(Lee et al., 2011) .This study showed that Serum ferritin levels were greater in

smokers, and serum ferritin levels are linked to smoking status. Cigarette smoke particles have been accumulating for a long time, and these particles have a systemic effect on iron homeostasis. The lung's iron homeostasis is disrupted by cigarette smoke particles in particular (Lee et al., 2011).The present study showed that smokers had significantly lower levels of serum 25OHD. This result is in agreement with (Brot, Jorgensen, and Srensen, 1999) . The metabolism of vitamin D is significantly affected by smoking smokers had a 14-fold greater mortality rate than non-smokers. As a result, the fact that smoking is the major cause of COPD, along with new research claiming that smoking increases the risk of COVD19, have refocused attention on stopping smoking (Kayhan Tetik, Gedik Tekinemre, and Taş, 2021).

Table 3.4: Comparison of biochemical biochemical parameters according to diabetes mellitus in patients and controlS

Biochemical parameter		N	Mean ± Std. Deviation
Hb **g\dl**	diabetic	28	13.04 ± 2.23034
	non diabetic	32	12.40 ± 2.07562
	control	30	11.90 ± 1.77763
WBC**mm**^{3}	diabetic	28	16.28 ± 4.8103^{*a,b}
	non diabetic	32	16.38 ± 5.4402
	control	30	7.84 ± 2.0257
Lymph **\mm**^{3}	diabetic	28	4.41 ± 2.4808^{*a,b}
	non diabetic	32	4.60 ± 2.6401
	control	30	24.93 ± 7.2160
D-dimer **ng\ml**	diabetic	28	1971.90 ± 2221.22^{*a,b}
	non diabetic	32	1611.15 ± 1381.62
	control	30	327.86 ± 66.40
Ferritin **ng\ml**	diabetic	28	1046.23 ± 1028.66
	non diabetic	32	1975.98 ± 2835.90
	control	30	204.84 ± 103.24
cathelicidn **ng\ml**	diabetic	28	50.13 ± 9.68
	non diabetic	32	51.82 ± 8.04
	control	30	48.79 ± 8.86
vitamin D3 **ng\ml**	diabetic	28	15.73 ± 4.66^{*a,b}
	non diabetic	32	15.73 ± 6.16
	control	30	18.21 ± 7.51

*:p-value <0.05

a(significant between DM patient and non DM patient)

b (significant between DM patient and control)

Table 3.5: Comparison of biochemical biochemical parameters according to hypertension in patients and controls

Biochemical parameter		N	Mean±Std. Deviation
Hb **g\dl**	yes	39	12.45 ± 1.66
	no	21	13.16 ± 2.84
	control	30	11.90 ± 1.77
WBC**mm³**	yes	39	15.79 ± 5.26*a,b
	no	21	17.34 ± 4.77
	control	30	7.847±2.0257
Lymph**mm³**	yes	39	4.54 ± 2.59*a,b
	no	21	4.45 ± 2.51
	control	30	24.93 ± 7.21
D-dimer **ng\ml**	yes	39	1993.37± 1878.37*a,b
	no	21	1939.45 ±1723.82
	control	30	327.86 ± 66.40
Ferritin **ng\ml**	yes	39	1554.14 ± 2611.66*a,b
	no	21	1519.74 ± 1256.715
	control	30	204.84 ± 103.24
Cathelicidin **ng\ml**	yes	39	51.70 ± 9.003
	no	21	47.037 ± 9.29
	control	30	50.827 ± 8.049
vitamin D3 **ng\ml**	yes	39	16.24 ± 5.14*a,b
	no	21	18.57 ± 8.21
	control	30	15.33 ± 6.16

*p-value <0.05a(significant between hypertension patients and non hypertension patients), b (significant between hypertension patients and control).

In Table (3.4) and (3.5) the present study showed significant differences in WBC, Lymphocyte count, d-dimer and vitamin D3 but no significant difference in catheliciden, ferritin and HB according DM. However according to hypertension there are significant differences in WBC, Lymphocyte count, D_dimer, ferritin and vitamin D3. No significant difference in catheliciden and HB. In COVID-19 patients, diabetes is a prevalent co-morbidity, a risk factor, and an independent prognostic factor. high blood pressure, arterial and venous thromboembolism, kidney

disease, neurologic disorders, and diabetes mellitus) are all common in COVID-19 patients, and hypertension appears to be one of the strongest predictors of COVID-19–related death (Sardu et al., 2020). Diabetes mellitus is frequently linked to cardiovascular disease and hypertension, which are commonly accompanied by obesity and, in some cases, smoking. DM is clearly one of the primary risk factors for COVID-19, according to the evidence. Diabetes and COVID-19 are linked by three pathophysiological mechanisms. The first pathway has a higher risk of COVID-19 because Angiotensin-converting enzyme 2 is dysregulated. Liver dysfunction and chronic systemic inflammation are the other two crucial physiological linkages between diabetes and COVID-19. ACE2 was first discovered in human heart failure and lymphoma cDNA libraries. Later, it was discovered to be the receptor for the SARS-CoV virus. Recently, it was shown that the virus SARS-CoV-2 uses ACE2 as a cellular entry point, and that SARS-CoV-2 has an even stronger affinity for ACE2 than SARS-CoV. The expression and distribution of ACE2 in the human body may potentially reveal the infection's capacity pathways and the organs most vulnerable to SARS-CoV-2. In different tissues and organs, ACE2 is expressed in diverse ways. High ACE2 expression was seen in lung alveolar cells, esophageal epithelial cells, ileum and colon absorptive enterocytes, cardiac cells, kidney proximal tubule cells, bladder urothelial cells, and oral mucosa epithelial cells. Acute Respiratory Distress (ARD) and Acute Renal Failure (ARF) are the most common acute diseases with high ACE2 expression in the lungs and kidneys (ARF). ACE2 alteration expression and distribution changes with age and is influenced by a variety of illnesses. The expression of ACE2 in diabetic patients changes as the disease progresses. ACE2 is elevated in the early stages of diabetes (Marhl et al., 2020). Low vitamin D3 levels are linked to diabetes and hypertension (Heaney, 2008).

table 3.6 shows no significant differences in Hb, WBC and Cathelicidin according to severity of COVID-19, while this table showed significant differences in lymphocyte count, d-dimer, ferritin and vitamin D3 between mild, moderate and severe group in covid19 patients.

Table 3.6: Comparison of biochemical parameters according to severity of disease in patients and controls

Biochemical parameter		N	Mean±Std. Deviation
Hb **g\dl**	mild	20	13.27 ± 1.74
	moderate	20	12.2100 ± 2.80
	sever	20	12.62 ± 1.71
	control	30	11.90 ± 1.77
WBC**\mm³**	mild	20	16.50 ± 5.69
	moderate	20	16.11 ± 4.48
	sever	20	16.40 ± 5.34
	control	30	7.84 ± 2.02
	Total	90	13.50 ± 5.90
Lymph**\mm³**	mild	20	4.66 ± 2.25*a
	moderate	20	4.67 ± 2.62*b
	sever	20	4.15 ± 2.83*c
	control	30	24.93 ± 7.21
D-dimer **ng\ml**	mild	20	379.44 ± 157.94*a
	moderate	20	1175.61 ± 418.85*b
	sever	20	3783.45 ± 1831.20*c
	control	30	327.86 ± 66.40
Ferritin **ng\ml**	mild	20	1322.29 ± 3262.43*a
	moderate	20	1276.77 ± 1144.87*b
	sever	20	2027.24 ± 1725.67*c
	control	30	204.84 ± 103.24
cathelicidin **ng\ml**	mild	20	46.86 ± 8.68
	moderate	20	51.54 ± 6.60
	sever	20	52.85 ± 11.11
	control	30	48.82 ± 8.04
vitamin D3 **ng\ml**	mild	20	16.78 ± 6.39*a,
	moderate	20	16.34 ± 4.04*b
	sever	20	15.25 ± 8.11*c
	control	30	18.33 ± 6.16

*: p-value <0.05

a (significant between mild and moderate)

b (significant between moderate and severe)

c (significant between severe and control)

the present study showed That the patients with the severe COVID19 were more likely to develop lymphopenia than mild patients and moderate

patients. This result is in agreement with (Huang *et al.*, 2020) who showed that severe patients have higher rates of lymphopenia. The present study showed that D-dimer level was higher in severe patients than mild and moderate patients. this result is in agreement with (Zhang,H. *et al.*, 2020) who discovered that the D-Dimer levels in severe patients were much greater than in other groups. D-dimer testing is used as part of a diagnostic strategy to rule out diagnostic thrombosis caused by any mechanism. D-dimer levels have been linked to the severity of COVID-19 and clinical outcome in numerous studies. An increased D- dimer level on admission (> 2.14 mg/L), patients at higher risk for mortality and hence tell clinicians about appropriate candidates for extended care and early intervention (Tang *et al.*, 2020) (Riyahi, Dev1 and Behzadi, 2021) (Connors and Levy, 2020). Ferritin levels were significantly higher in severe patients than mild and patients. this study is in agreement with (Zeng *et al.*, 2020) They discovered that COVID-19 patients in the severe group had greater serum ferritin levels than those in the non severe group. Inflammation, liver illness, and cancer all cause a rise in serum ferritin, which is a surrogate sign of stored iron. All of these findings show that an exaggerated inflammatory response is linked to the severity of COVID-1 infection. Serum ferritin is a well-known inflammatory marker that can spike in response to systemic and pulmonary inflammation, as well as a variety of disorders including COVID-19. The mechanisms underlying the link between increased ferritin levels and infection severity in COVID-19 patients are unknown; however, there are a number of possibilities, including pro-inflammatory cytokines that may increase ferritin synthesis, cellular damage caused by an inflammatory process that promotes intracellular ferritin leakage, and iron liberation from ferritin. Hepatic dysfunction in severe COVID-19 is associated with increased activation of coagulative and fibrinolytic pathways, including depressed platelet counts,

rising neutrophil counts and neutrophil to lymphocyte ratios, and high ferritin levels, according to Wang et al. This shift in immunological balance has been shown to increase with age, therefore older patients may fare worse since they rely more on this pathway. (Lin *et al.*, 2020) (Ruddell, 2009) (Kell and Pretorius, 2014) (Reyt *et al.*, 2015) (Ahmed and Al-Mousawi, 2020).

Vitamin D3 deficiency was also shown to be considerably higher in severe patients compared to mild and moderate patients in this study. This result is in agreement with (Radujkovic et al., 2020), which found a link between Vitamin D deficit and the severity of COVID-19. Patients who were low in vitamin D were more likely to be admitted to the hospital and required more (intense) oxygen therapy. Vitamin D deficiency has been linked to a variety of clinical problems, including increased vulnerability to infectious disease. Vitamin D supplementation protects individuals with very low Vitamin D levels (25(OH)D 10 ng/mL) from acute respiratory tract infections. Vitamin D supplementation as a potential therapeutic method for COVID-19 has been shown to have a positive influence on mortality from SARS-CoV-2 infection Nipith and Michael , 2020).

Table 3.7 showed correlation between vitamin D 3 and Cathelicidin was significant at the 0.05 level in covid19 patients . In patients group (r= - o. 27,p =0.03) negative correlation.

Table 3.7: Correlation vitamin D3 with cathelicidin in COVID19 patients

	patient
r	- 0.27*
p	0.03

This result showed correlation between vitamin D 3 and Cathelicidin levels in covid19 patients as shown in figure 3.5. This result is in agreement with (Herr *et al.*, 2011) who showed that links vitamin D with innate

immunity Activities of Vitamin D3 Many genes, including the hCAP18 gene, which encodes cathelicidin, a precursor to LL37, are transcriptionally controlled in part by vitamin D. LL37 is active against bacteria and viruses after the cathelicidin peptide is cleaved. LL37 also modulates immunological responses and works in tandem with toll-like receptors and other immune system components. LL37 has been shown to inhibit the replication of a variety of viruses, including enveloped RNA viruses like the Corona Virus 2 of the Severe Acute Respiratory Syndrome. (SARS-CoV-2) that causes COVID-19 illness (Crane-Godreau *et al.*, 2020)

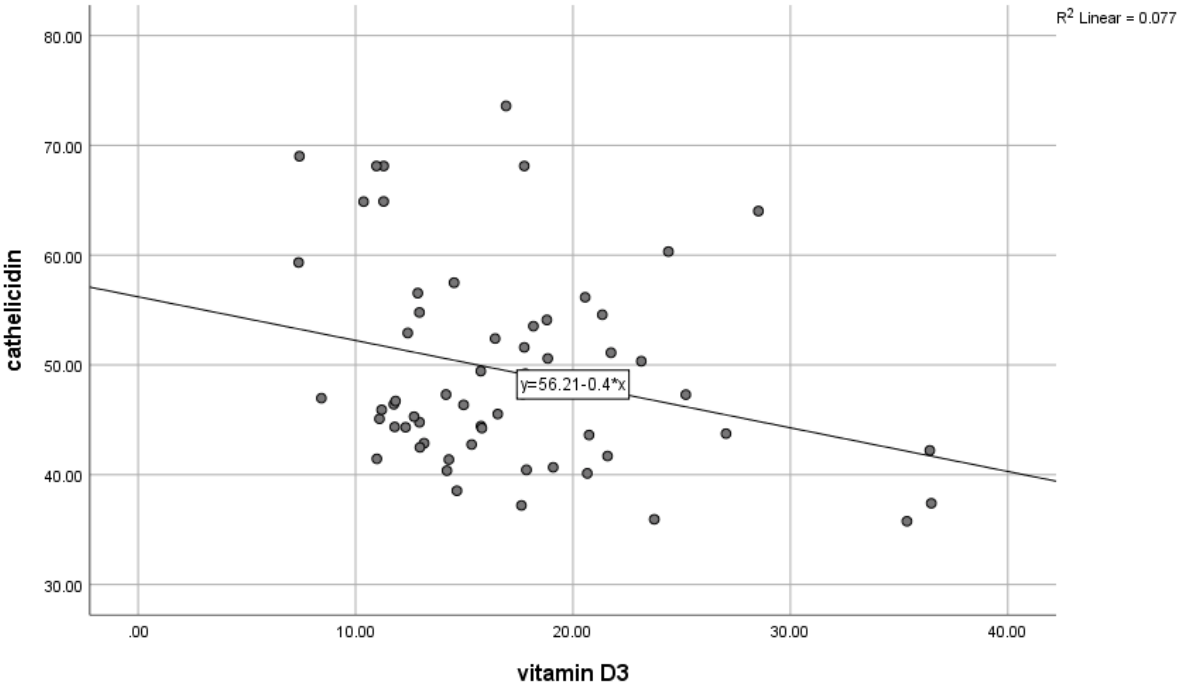


figure (3.7) Correlation of cathelicidin with vitamin D3 in patients group .

3.2. Receiver operator characteristics (ROC):

Receiver operating characteristic (ROC) curve analysis of cathelicidin, vitamin D3 was performed. The best area under the ROC curve (AUC) was for cathelicidin levels (AUC = 0.39, p <0.001) and for vitamin D3 levels (AUC=0.59, P >0.001).

As shown in Figure 3.6, ROC analysis indicated that cathelicidin level > 38.11 ng/ml and vitamin D3 levels >8.74 /ml with 93% sensitivity, 96% specificity for cathelicidin while for vitamin D3 95% sensitivity, 96% specificity. The best area under the ROC curve (AUC) was for D-dimer levels (AUC = 0.87, p >0.001). ROC analysis indicated that D_dimer levels> 219.5ng /ml was predictive of increasing covid-19 with 93% sensitivity, 96% specificity as shown in Table (3.8).

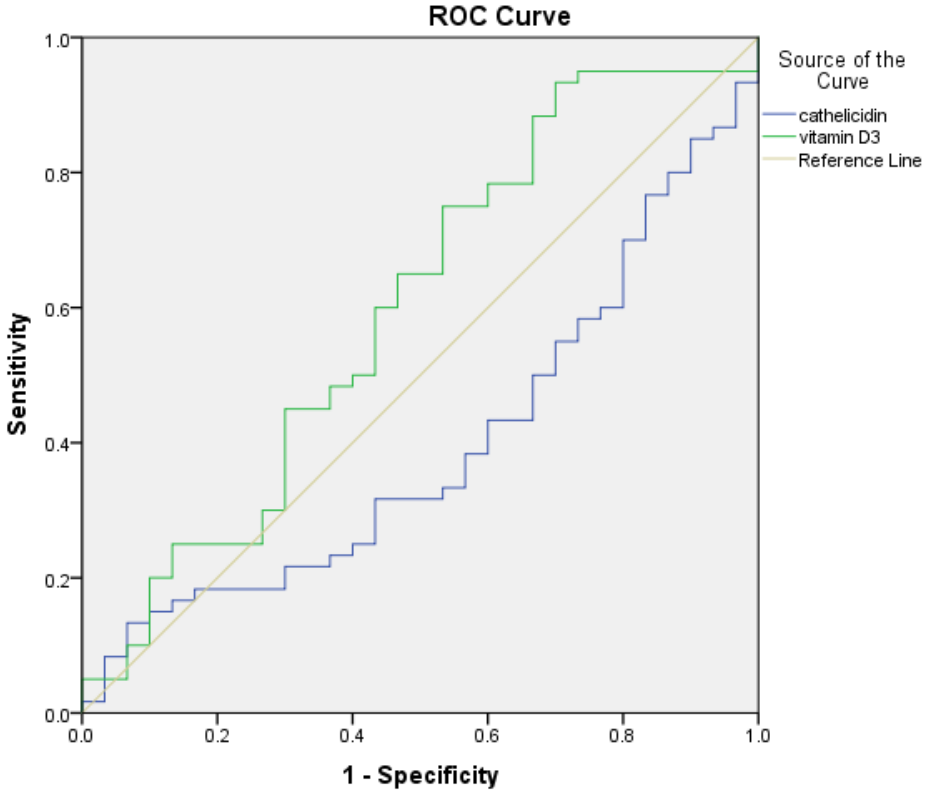


Figure (3.8) ROC analysis of cathelicidin and vitamin D3

Table 3.8: Differentiation power (area under the ROC Curve, Sensitivity % and Specificity %) of cathelicidin and vitamin D3 levels in covid19 patients.

Test Result Variable(s)	Cut off value	AUC	Asymptotic Sig.[b]	Sensitivity	1 - Specificity
Cathlicidine	38.11	0.39	>0.01	.93	.96
Vitamin D3	8.74	0.59	> 0.01	.95	.96
D-dimer	219.5	0.87	< 0.01	.93	.96

Chapter Four

Conclusion
&
Future work

4. Conclusions & Future work

4.1. Conclusions

1. vitamin D 3, and D-dimer are factors for severity of COVID-19 in the studied subjects.
2. Vitamin D3 deficiency is more susceptible for development of COVID-19 diseases by affecting on innate immunity.
3. Covid-19 infection lead to increase D- dimer and the serum ferritin level, so it can be depended in the early diagnosis of COVID-19 infection.
4. The current study suggest monitoring cathelicidin, vitamin D3 and D-dimer levels as a good biomarkers in assessing prognosis and intensive treatment of COVID-19.

From this conclusions we recommended:

4.2. future work

1. Measurement of level of IL-6 cytokine which controls the immune response and its association with severity of COVID-19 illness.
2. Study the gene expression for cathelicidin and its receptor in COVID19 patients to monitor its level.

References

References

- Abdin, S. M., Elgendy, S. M., Alyammahi, S. K., Alhamad, D. W., & Omar, H. A. (2020). Tackling the cytokine storm in COVID-19, challenges, and hopes. *Life sciences*, p.118054.
- Adhikari, S. P., Meng, S., Wu, Y., Mao, Y., Ye, R., Wang, Q., ... and Zhou, H. ,2020. A scoping review of 2019 Novel Coronavirus during the early outbreak period: Epidemiology, causes, clinical manifestation and diagnosis, prevention and control. pp. 1–12.
- Ahmed, T. H. and Al-Mousawi, N. H.,2020. Post-hospitalization, levels of D-dimer, C-reactive protein, ferritin, and lactate dehydrogenase in recovered COVID-19 Iraqi patients. *Systematic Reviews in Pharmacy*, PP.92-99.
- Al Meani, S. A., Abdulkareem, A. H., Ibrahim, M. O., Ahmed, M. M., and Mukhlif, M. Y. ,2020. Assessment the Correlation of D-dimer and Ferritin Level in Patients Infected with Covid-19 in Anbar Governorate of Iraq. *Syst. Rev. Pharm*, *11*(9), pp.1130-1133.
- Alexander, J., Tinkov, A., Strand, T. A., Alehagen, U., Skalny, A. and Aaseth, J. ,2020. Early nutritional interventions with zinc, selenium and vitamin D for raising anti-viral resistance against progressive COVID-19. *Nutrients*, *12*(8),p. 2358.
- Ali, N. ,2020. Role of vitamin D in preventing of COVID-19 infection, progression and severity. *Journal of infection and public health*, *13*(10),pp. 1373-1380.

References

- Awadasseid, A., Wu, Y., Tanaka, Y. and Zhang, W., 2020. Initial success in the identification and management of the coronavirus disease 2019 (COVID-19) indicates human-to-human transmission in Wuhan, China. *International journal of biological sciences*, *16*(11), pp.1846.

- Baeke, F., Takiishi, T., Korf, H., Gysemans, C. and Mathieu, C. ,2010. Vitamin D: modulator of the immune system. *Current opinion in pharmacology*, *10*(4),pp 482-496.

- Balla M, Merugu GP, Patel M, Koduri NM, Gayam V, Adapa S, et al.,2020. COVID-19, modern pandemic: a systematic review from a front-line health care providers' perspective. *J Clin Med Res.*12(4)pp.215–229.

- Batah, S. S. and Fabro, A. T., 2020. Pulmonary pathology of ARDS in COVID-19: A pathological review for clinicians. *Respiratory medicine.5(7)*, pp. 106.

- Bayramoğlu, E., Akkoç, G., Ağbaş, A., Akgün, Ö., Yurdakul, K., Duru, H. N. S. and Elevli, M. ,2021. The association between vitamin D levels and the clinical severity and inflammation markers in pediatric COVID-19 patients: single-center experience from a pandemic hospital. *European journal of paediatrics*.11(8)aQ, pp. 1-7.

- Biesalski, H. K. ,2020. Vitamin D deficiency and co-morbidities in COVID-19 patients–A fatal relationship. *Nfs Journal*, *20*, p.10.

- Bikle, D. D. ,2009. Vitamin D and immune function: understanding common pathways. *Current osteoporosis reports*, *7*(2), pp. 58-63.

- Brot, C., Jørgensen, N. R., & Sørensen, O. H. ,1999. The influence

of smoking on vitamin D status and calcium metabolism. *European journal of clinical nutrition*, *53*(12),pp. 920-926.

- Burki, T. K. (2020). The Russian vaccine for COVID-19. *The Lancet Respiratory Medicine*, *8*(11),pp. e85-e86.
- Christakos, S., Ajibade, D. V., Dhawan, P., Fechner, A. J., & Mady, L. J. (2012). Vitamin D: metabolism. *Rheumatic Disease Clinics*, *38*(1),pp. 1-11.
- Connors, J. M., & Levy, J. H. (2020). COVID-19 and its implications for thrombosis and anticoagulation. *Blood, 135*(23), pp. 2033-2040.
- Cowbrough, K. (2015). The importance of vitamin D. *British Journal of Midwifery*, *23*(Sup7), pp.3-4.
- Crane-Godreau, M. A., Clem, K. J., Payne, P. and Fiering, S. ,2020. Vitamin D deficiency and air pollution exacerbate COVID-19 through suppression of antiviral peptide LL37. *Frontiers in public health*, *8*, p.232.
- Duan, G,2020. Intuition on virology, epidemiology, pathogenesis, and control of COVID-19. *Nov. Res. Microbiol. J.*, 4 (5), pp. 955–967.
- Emperador, D. , Dittrich, S. , Domen, J. , Sra, H. , V. D. B. A, and C. C.,2020 .Diagnostic, care or hospital outpatient settings has COVID-19 disease (Review).22(2),PP.5-4.
- Földi, M., Farkas, N., Kiss, S., Zádori, N., Váncsa, S., Szakó, L., ... and KETLAK Study Group. 2020. Obesity is a risk factor for developing critical condition in COVID-19 patients: a systematic review and meta-analysis. *Obesity Reviews*, *21*(10), pp. e13095.

References

- Ganz, T. ,2003. The role of antimicrobial peptides in innate immunity. *Integrative and comparative biology*, *43*(2), pp. 300-304.
- Gao, F., Zheng, K. I., Wang, X. B., Sun, Q. F., Pan, K. H., Wang, T. Y., ... and Zheng, M. H. ,2020. Obesity is a risk factor for greater COVID-19 severity. *Diabetes care*, *43*(7), pp. e72-e74
- Gasmi, A., Noor, S., Tippairote, T., Dadar, M., Menzel, A.and Bjørklund, G., 2020. Individual risk management strategy and potential therapeutic options for the COVID-19 pandemic. *Clinical Immunology*, *215*, p.108409.
- Ghosh, S. K.and Weinberg, A.,2021. Ramping up AMPs against SARS-CoV-2. *Frontiers in Molecular Biosciences*, *8*, p.547.
- Hafeez, A., Ahmad, S., Siddqui, S. A., Ahmad, M. and Mishra, S. ,2020. A review of COVID-19 (Coronavirus Disease-2019) diagnosis, treatments and prevention. *EJMO*, *4*(2), pp. 116-125.
- Hamid, I. S. (2021). Antiangiogenic Effect of Ficus Carica Linn Leaf Extract on Total Blood Vessels, Matrix Metalloproteinase-9 Expression and Macrophages in the Chick Embryo Chorioallantoic Membrane. *Systematic Reviews in Pharmacy*, *12*() ,pp. 1282-1286.
- Harapan,H.,2020.Coronavirus disease 2019 (COVID-19): A literature review, *J. Infect. Public Health*, 13(5), pp. 667–673.
- He, F., Deng, Y. and Li, W., 2020 .Coronavirus disease 2019: What we know?. *Journal of Medical Virology*, 92(7), pp. 719–725.
- Heaney, R. P., 2008. Vitamin D in health and disease. *Clinical Journal of the American Society of Nephrology*, 3(5), pp. 1535–1541.

References

- Hendaus, M. A. ,2020. Remdesivir in the treatment of coronavirus disease 2019 (COVID-19): a simplified summary. *Journal of Biomolecular Structure and Dynamics*.35(12), pp. 1-6.
- Herr, C., Greulich, T., Koczulla, R. A., Meyer, S., Zakharkina, T., Branscheidt, M., ... and Bals, R.,2011. The role of vitamin D in pulmonary disease: COPD, asthma, infection, and cancer. *Respiratory research*, *12*(1), pp. 1-9.
- Herr, C., Shaykhiev, R. and Bals, R., 2007. The role of cathelicidin and defensins in pulmonary inflammatory diseases. *Expert opinion on biological therapy*, *7*(9), pp.1449-1461.
- Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., ... and Cao, B. ,2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The lancet*, *395*(10223), pp.497-506.
- Huang, I., & Pranata, R.,2020. Lymphopenia in severe coronavirus disease-2019 (COVID-19): systematic review and meta-analysis. *Journal of intensive care*, *8*, pp. 1-10.
- Hung, I. F. N.,2020.Treatment of coronavirus disease 2019,*Curr. Opin. HIV AIDS*, 15 (6), pp. 336–340.
- Jain, A., Chaurasia, R., Sengar, N. S., Singh, M., Mahor, S. and Narain, S., 2020. Analysis of vitamin D level among asymptomatic and critically ill COVID-19 patients and its correlation with inflammatory markers. *Scientific reports*, *10*(1), pp.1-8.
- Jin, Y., Yang, H., Ji, W., Wu, W., Chen, S., Zhang, W. and Duan, G.,2020. Virology, epidemiology, pathogenesis, and control of COVID-19. *Viruses*, *12*(4), p. 372.

References

- Jothimani, D., Kailasam, E., Danielraj, S., Nallathambi, B., Ramachandran, H., Sekar, P., ... and Rela, M., 2020. COVID-19: Poor outcomes in patients with zinc deficiency. *International Journal of Infectious Diseases*, *100*, pp. 343-349.

- Kamen, D. L. and Tangpricha, V. ,2010. Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. *Journal of molecular medicine*, *88*(5), pp.441-450.
- Kell, D. B., & Pretorius, E. ,2014. Serum ferritin is an important inflammatory disease marker, as it is mainly a leakage product from damaged cells. *Metallomics*, *6*(4), pp. 748-773.
- Kermali, M., Khalsa, R. K., Pillai, K., Ismail, Z., and Harky, A. ,2020. The role of biomarkers in diagnosis of COVID-19 – A systematic review. *Life Sciences*, 254(May), p. 117788.
- King, E. ,2020. The Role of Vitamin D deficiency in COVID-19 related deaths in BAME, Obese and Other High-risk Categories.
- Klein, S. L. and Morgan, R. ,2020.The impact of sex and gender on immunotherapy outcomes. *Biology of Sex Differences*, 11(1), pp. 1–13.

- Kubiak, J., Thorsby, P. M., Kamycheva, E. and Jorde, R. ,2018. Vitamin D supplementation does not improve CVD risk factors in vitamin D-insufficient subjects. *Endocrine connections*, *7*(6), pp.840-849.
- Kumar, A., Gupta, P. K. and Srivastava, A. ,2020. A review of modern technologies for tackling COVID-19 pandemic. *Diabetes &Metabolic Syndrome: Clinical Research & Reviews*, *14*(4), pp.569-573.

References

- Kuno, T., So, M., TaTashi, M., and Egorova, N. N. ,2021. U shape association of hemoglobin level with in-hospital mortality for COVID-19 patients. *Journal of thrombosis and thrombolysis*.56(4),pp. 1-5.
- Kuroda, K., Okumura, K., Isogai, H., & Isogai, E. ,2015. The human cathelicidin antimicrobial peptide LL-37 and mimics are potential anticancer drugs. *Frontiers in oncology*, *5*, p.144.
- Laneri, S., Brancaccio, M., Mennitti, C., De Biasi, M. G., Pero, M. E., Pisanelli, G., ... and Pero, R. ,2021. Antimicrobial Peptides and Physical Activity: A Great Hope against COVID 19. *Microorganisms*, *9*(7) ,pp. 1415.
- Lee, S. H., Kim, E. Y., Lee, S. K., Hong, J. Y. and Kim, Y. S.,2011. The relationship between serum ferritin levels, smoking, and lung function in Korean: analysis of the Fourth Korea National Health and Nutrition Examination Survey (KNHANES IV). *Chest*, *140*(4),pp. 568A.
- Li, X., Geng, M., Peng, Y. and Meng, L. ,2020. Lu Sh. *Molecular immune pathogenesis and diagnosis of COVID-19, J Pharmaceutical Analysis*, *10*(2), pp.102-8.
- Liao, D., Zhou, F., Luo, L., Xu, M., Wang, H., Xia, J., ... and Hu, Y. ,2020. Haematological characteristics and risk factors in the classification and prognosis evaluation of COVID-19: a retrospective cohort study. *The Lancet Haematology*, *7*(9), pp. e671-e678.
- Lin, Z., Long, F., Yang, Y., Chen, X., Xu, L., and Yang, M. 2020. Serum ferritin as an independent risk factor for severity in COVID-19 patients. *Journal of Infection*, *81*(4), pp.647-679.

References

- Lisboa Bastos, M., Tavaziva, G., Abidi, S. K., Campbell, J. R., Haraoui, L. P., Johnston, J. C., ... and Ahmad Khan, F. ,2020. Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. BMJ 370: m2516.
- Maghbooli *, Z. ,2020* .Vitamin D sufficiency, a serum 25-hydroxyvitamin D at least 30 ng/mL reduced risk for adverse clinical outcomes in patients with COVID-19 infection. *PLoS One*, 15(9 September), pp. 1–13, 2020.
- Malenica, M., Prnjavorac, B., Bego, T., Dujic, T., Semiz, S., Skrbo, S., ... band Causevic, A. ,2017. Effect of cigarette smoking on haematological parameters in healthy population. *Medical Archives*, *71*(2), p.132.
- Marhl, M., Grubelnik, V., Magdič, M. and Markovič, R. ,2020. Diabetes and metabolic syndrome as risk factors for COVID-19. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, *14*(4), pp. 671-677.
- McCullough, P. J., Amend, J., McCullough, W. P., Repas, S. J., Travers, J. B. and Lehrer, D. S.,2020. The essential role of vitamin D in the biosynthesis of endogenous antimicrobial peptides may explain why deficiency increases mortality risk in COVID-19 infections. *Preprints.org*, May, pp. 1–23.
- Meltzer, D. O., Best, T. J., Zhang, H., Vokes, T., Arora, V. and Solway, J. ,2020. Association of vitamin D status and other clinical characteristics with COVID-19 test results. *JAMA network open*, *3*(9),pp. e2019722-e2019722.

References

- Meltzer, D. O., Best, T. J., Zhang, H., Vokes, T., Arora, V., and Solway, J. ,2020. Association of Vitamin D Status and Other Clinical Characteristics With COVID-19 Test Results. *JAMA network open*, 3(9), p. e2019722.
- Merzon, E., Tworowski, D., Gorohovski, A., Vinker, S., Golan Cohen, A., Green, I. and Frenkel-Morgenstern, M. ,2020. Low plasma 25 (OH) vitamin D level is associated with increased risk of COVID-19 infection: an Israeli population-based study. *The FEBS journal*, *287*(17), pp.3693-3702.
- Montano-loza, M. E. A. J. ,2020. Perspective : improving vitamin D status in the management of. *European Journal of Clinical Nutrition*, pp. 856–859.
- Mousavizadeh, L. and Ghasemi, S. ,2020.Genotype and phenotype of COVID-19: Their roles in pathogenesis.*Journal of Microbiology, Immunology and Infection*, 10 (2020), pp. 0–4.
- Name, J. J., SouzaR, A. C. R., Vasconcelos, A. R., Prado, P. S., and Pereira, C. P. M. ,2020. Zinc, Vitamin D and Vitamin C: perspectives for COVID-19 with a focus on physical tissue barrier integrity. *Frontiers in Nutrition*, *7*, pp,295.
- Nipith, Ch. and Michael, F.,2020. Immunologic effects of vitamin D on human health and disease. *Nutrients*, *12*(7), p.2097.
- Pahar, B., Madonna, S., Das, A., Albanesi, C., and Girolomoni, G., 2020. Immunomodulatory role of the antimicrobial LL-37 peptide in autoimmune diseases and viral infections. *Vaccines*, *8*(3), pp.517.

References

- Pascarella, G.,2020. COVID-19 diagnosis and management: a Jcomprehensive review. *Journal of Internal Medicine*, 288(2), pp. 192–206.
- Phoswa, W. N., & Khaliq, O. P., 2020. Is pregnancy a risk factor of COVID-19?. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *252*, pp.605-609.
- Phua, J.,2020 Intensive care management of coronavirus disease 2019 (COVID-19): challenges and recommendations. Lancet Respir. Med. 8,pp. 506–517.
- Qeadan, F., Tingey, B., Gu, L. Y., Packard, A. H., Erdei, E., and Saeed, A. I. ,2021. Prognostic Values of Serum Ferritin and D-Dimer Trajectory in Patients with COVID-19. *Viruses*, *13*(3), pp. 419.
- Radhi, H. T., ABIM, S. and Khalaf, S. ,2013. Prevalence and Risk Factors of Vitamin D Deficiency among Men. *Bahrain Medical Bulletin*, *35*(3),PP7-9.
- Radujkovic, A., Hippchen, T., Tiwari-Heckler, S., Dreher, S., Boxberger, M. and Merle, U. , 2020. Vitamin D deficiency and outcome of COVID-19 patients. *Nutrients*, *12*(9),p. 2757**.**
- Reinholz, M., Ruzicka, T. and Schauber, J. ,2012. Cathelicidin LL-37: an antimicrobial peptide with a role in inflammatory skin disease. *Annals of dermatology*, *24*(2), pp.126-135.
- Reyt, G., Boudouf, S., Boucherez, J., Gaymard, F., and Briat, J. F., 2015. Iron-and ferritin-dependent reactive oxygen species distribution: impact on Arabidopsis root system architecture. *Molecular plant*, *8*(3), pp.439-453.

References

- Rhodes, J. M., Subramanian, S., Laird, E., Griffin, G. and Kenny, R. A. ,2021. Perspective: Vitamin D deficiency and COVID-19 severity–plausibly linked by latitude, ethnicity, impacts on cytokines, ACE2 and thrombosis. *Journal of internal medicine*, *289*(1), pp.97-115.
- Riyahi, S., Dev1, H. and Behzadi, A., 2021. Pulmonary Embolism in Hospitalized Patients with COVID-19.45(2),PP.56-58.
- Rothan, H. A. and Byrareddy, S. N. ,2020. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *Journal of autoimmunity*, *109*, pp. 102433.
- Ruddell, R. G., 2009. Ferritin functions as a proinflammatory cytokine via iron-independent protein kinase C zeta/nuclear factor kappaB - Regulated signaling in rat hepatic stellate cells. *Hepatology*, 49(3), pp. 887–900.
- Saponaro, F., Saba, A. and Zucchi, R. ,2020. An update on vitamin D metabolism. *International Journal of Molecular Sciences*, *21*(18), pp.6573.
- Sardu, C., Gambardella, J., Morelli, M. B., Wang, X., Marfella, R. and Santulli, G. ,2020. Hypertension, thrombosis, kidney failure, and diabetes: is COVID-19 an endothelial disease? A comprehensive evaluation of clinical and basic evidence. *Journal of clinical medicine*, *9* (5), p.1417.
- Shi, Y., Wang, G., Cai, X. P., Deng, J. W., Zheng, L., Zhu, H. H., ... and Chen, Z. ,2020. An overview of COVID-19. *Journal of Zhejiang University-SCIENCE B*, *21*(5), pp. 343-360.

References

- Shrestha, R. and Shrestha, L.,2020. Coronavirus disease 2019 (Covid-19): A pediatric perspective. *J. Nepal Med. Assoc.*, 58(227), pp.525–532.
- Singhal, T. ,2020. Uma revisão da doença de Coronavírus-2019 (COVID-19). *Indian J Pediatr*, *87*, pp. 281-286.
- Sitaram, N., & Nagaraj, R. ,2002. Host-defense antimicrobial peptides: importance of structure for activity. *Current pharmaceutical design*, *8*(9), pp.727-742.
- Song, C. Y., Xu, J., He, J. Q., & Lu, Y. Q. ,2020. Immune dysfunction following COVID-19, especially in severe patients. *Scientific reports*, *10*(1), pp. 1-11.
- Strzałkowska N. and Jo, A.,2012 "Cathelicidins : family of antimicrobial peptides . A review,".18(2), pp. 10957–10970.
- Tang, N., Bai, H., Chen, X., Gong, J., Li, D., and Sun, Z., 2020. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *Journal of thrombosis and haemostasis*, *18*(5), pp. 1094-1099.
- Tapson, V. F. ,2005.The role of smoking in coagulation and thromboembolism in chronic obstructive pulmonary disease. *Proceedings of the American Thoracic Society*, 2(1), pp. 71–77.
- Tetik, B. K., Tekinemre, I. G., & Taş, S. ,2021. The effect of the COVID-19 pandemic on smoking cessation success. *Journal of Community Health*, *46*(3), 471-475.

References

- van Doremalen, N., 2020 .Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. *New England Journal of Medicine*, 382(16), pp. 1564–1567.
- Velavan, T. P.and Meyer, C. G. ,2020. La epidemia de COVID-19. *Trop Med Int Health*, *25*(3), pp. 278–280.
- Wiersinga, W. J., 2020. Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. *JAMA - Journal of the American Medical Association*, 324(8), pp. 782–793.
- Winter, A. K.and Hegde, S. T., 2020. The important role of serology for COVID-19 control. *The Lancet Infectious Diseases*, *20*(7), pp. 758-759.
- Zapata, I., 2020.Vitamin d and inflammation-potential implications for severity of COVID-19.*Irish Medical Journal*, 113(6), pp. 1–3.
- Zeng, F., Huang, Y., Guo, Y., Yin, M., Chen, X., Xiao, L. and Deng, G., 2020. Association of inflammatory markers with the severity of COVID-19: a meta-analysis. *International Journal of Infectious Diseases*, *96*, pp.467-474.
- Zhang, H.,2020.Potential Factors for Prediction of Disease Severity of COVID-19 Patients.43(11),PP.5-10.
- Zhang, J., Lu, Y., Kwong, J. S. W., Li, X., Zheng, W. andHe, R., 2020. Quality Assessment of the Chinese Clinical Trial Protocols Regarding Treatments for Coronavirus Disease 2019. *Frontiers in Pharmacology*, 11(August), pp. 1–11.

Appendix

Study questionnaire

Sample data:

No.	Date	Pre or post-operative	Date of operation

ID:

Name	Age:	Gender:	Mobile:
Marital status:	Blood group:	Occupation	Educational level

Risk factor:

Hypertension
Smoking
Diabetes mellitus
BMI

الخلاصة

المقدمة: ظهر الالتهاب الرئوي الناجم عن عدوى فيروس كورونا (**SARS-CoV 2**) في مدينة ووهان ، مقاطعة هوبي ، الصين في ديسمبر 2019. بحلول 11 فبراير 2020 ، أطلقت منظمة الصحة العالمية (**WHO**) رسميًا اسم المرض الناتج عن الإصابة بفيروس **(SARS-CoV 2)** مثل مرض فيروس كورونا 2019 (**COVID-19**)، فيروس كورونا المتلازمة التنفسية الحادة الوخيم (**SARS-CoV 2**) المسؤول عن عدوى **COVID-19**.

فيتامين **D3** هو فيتامين قابل للذوبان في الدهون وله وظيفة مناعية ، لا سيما في تعديل الاستجابة الالتهابية للعدوى الفيروسية، ينظم فيتامين **D3** المناعة التكيفية والفطرية على المستوى الخلوي.

الكاثليسيدين هو الببتيدات المضادة للميكروبات والمكونات الرئيسية للمناعة الفطرية.

الهدف من الدراسة: للعثور على الارتباط بين مستويات فيتامين **D3** وشدة **COVID-19** في موضوع مدروس ، وتحليل كيفية آليات فيتامين **D3** في مرضى **COVID-19** وكيفية استخدامه في الحد من شدة هذا المرض. دراسة الارتباط بين مستويات الكاثليسيدين وفيتامين **D3** لإثبات أهمية دورهما في علاج المرضى العراقيين المصابين بفيروس **COVID-19**.

المواد وطريقة العمل: المواد والطرق: تكونت دراسة الحالة والشواهد هذه من 90 عينة ، 60 منها مصابة بـ **COVID-19**، ينقسم المريض إلى ثلاث مجموعات 20 حالة خفيفة ، 20 متوسطة ، 20 حالة شديدة من **COVID-19**، 30 منهم من الافراب الضابطين وكانت الفئة العمرية للأفراد (20-70). تم إجراء الكشف عن مستويات الكاثليسيدين بواسطة (**ELISA**)، تم إجراء مستويات فيتامين **D3** بواسطة **chemiluminescence immunoassay analyzer**. تم اختبار **D-dimer** باستخدام **Cobas e 411** وتم اختبار مستوى الفيريتين باستخدام نظام **Mini-Vidas** الآلي. تم إجراء قياسات **CBC** بواسطة جهاز تحليل الدم الآلي **XP 300** ™.

النتائج:. من فيتامين **D3** و **D-dimer** و **ferritin** في المجموعات المدروسة أظهروا أهمية عالية في المرضى الحادون ومرتبطون بشدة **COVID-19** في الأشخاص الخاضعين للدراسة (قيمة **p** <0.05)، تمت زيادة مستويات **D-dimer** و **ferritin** بشكل ملحوظ ، بينما انخفض

فيتامين **D3** في مريض **COVID-19**، أظهرت الدراسة الحالية وجود علاقة سلبية بين فيتامين **D3** والكاثيليسيدين في مرضى **COVID-19**، أظهر فيتامين **D3** والكاثيليسيدين و **D-dimer** خصوصية وحساسية عالية في تحليل **ROC**.

الخاتمة: يعتبر فيتامين **D3** والكاثيليسيدين و **D-dimer** عوامل تنبؤية لشدة **COVID-19** في العناصر الفرعية المدروسة ونقص فيتامين **D3** أكثر عرضة للإصابة بأمراض **COVID-19** التي تؤثر على المناعة الفطرية. لذلك ، يمكن أن تشير هذه الدراسة إلى مراقبة مستويات الكاثليسيدين وفيتامين **D3** و **D-dimer** كمؤشر بيولوجي جيد للتشخيص وعلاج **COVID-19**.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء/ كلية الطب

فرع الكيمياء والكيمياء الحياتية

ارتباط فيتامين د 3 والكاثيليسيدين بخطورة مرض كوفيد 19 في عينة من المرضى العراقيين

رسالة مقدمة

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

من قبل

منتهى محمود عبد

2018 – بكالوريوس تحليلات مرضية – جامعة كربلاء

اشراف

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2021 م