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# KL6 as Diseased Severity Biomarker in Covid 19 Patients

**A Research paper**

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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

{وَيَسۡـَٔلُونَكَ عَنِ ٱلرُّوحِۖ قُلِ ٱلرُّوحُ مِنۡ أَمۡرِ رَبِّى وَمَآ أُوتِيتُم مِّنَ ٱلۡعِلۡمِ إِلَّا قَلِيلًا }

صَدَقَ اللهُ العلي العَظيم

سورة الاسراء ( الآية 85 )

# Certification

I certify that this research paper was prepared under my supervision at the department of Biology, College of science, University of Babylon, as a partial requirement for the Degree of Higher Diploma in Forensic Evidence.

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In view of available recommendation, I forward this research paper for debate by the Examination Committee.

Signature:

Name: **Assist. Prof. Dr. Adi Jassim Abd AL-Rezzaq**

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Date:

# Dedication

I dedicate this work for soul of my mother, you will always be in my heart and mind.

For soul of my father, who made me the man I am.

For my lovely family my wife and my children

For my dear brother Jihad N. Alshammary.

Mohammed

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Also my gratitude goes to all the patients for their cooperation in achieving this research.

mohammed

## Summary:

COVID-19 is the most aggressive pandemic of 21st century. First pandemic era the tendency of infection may restricted to non-child age groups, especially elderly. Progressively it is age groups expanded to cover all groups. The aim of the current study was to investigate the serum level of Lactate dehydrogenase (LDH) and Krebs Von Den Lungen - 6 (KL6) among COVID-19 patients.

The number of patients included in the current study were 60 (36 female and 24 male) with Age Mean±SD (57.86±15.85 for female and 54.5±14.89 for male) during a period of one month June 2021 from Merjan Medical city, Hilla-Iraq. The results revealed that old age adults (>=60 years) were most frequent among COVID-19 patients. All patients have positive C-Reactive Protein and lymphocytopenia as screening diagnostics assay for COVID-19 patients' suspicion. Actually, the elderly has medical problems like hypertension, diabetes mellitus and cardiovascular diseases collectively can call old-age diseases (OADs).

The results revealed that OADs compile (46.66%) and (28.33%) in female and male COVID-19 patients respectively. Additionally, the lung involvement by Computed tomography scan were highly significant in compare with same patients without OADs (Sig. value 0.0001) and also the oxygen saturation Spo2 was decline significantly in OADs compared to non-OADS (Sig. value 0.0001). The results revealed that, there is a significant difference between LDH serum level in control when compared to male COVID-19 patients (Sig. value 0.0001) and female COVID-19 patients (Sig. value 0.0077). While non-significant differences were observed between COVID-19 patients and control. Concern KL6 levels the results revealed that, the level of KL6 is same in COVID-19 patients and healthy control. The study concludes: elderly female was highly prevalent among those with old-age diseases with worsens high score lung involvement and low oxygenation, Slight increased level of LDH may indicate mild COVID-19 cases or convalescent

and non-significant difference in level of MUC1 may be related to age or COVID-19 stage, mild or convalescent.

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## List of Abbreviations

| Symbol | Description |
|---|---|
| ACE2 | Angiotensin converting enzyme- 2 |
| APCs | Antigen-presenting cells |
| BALF | Broncho alveolar lavage fluid |
| BALT | Bronchus-associated lymphoid tissue |
| C0VID-19 | Corona virus disease 2019 |
| CBC | Complete blood count |
| CD4+ | Cluster of differentiation 4 |
| CD8 | Cluster of differentiation 8 |
| CRP | C- reactive protein |
| ELISA | Enzyme linked immunosorbent assay |
| HEV | High endothelial venule |
| HRP | Horse radish peroxidase |
| IgA | Immunoglobulin A |
| IL-1 | Interleukin-1 |
| IL-12 | Interleukin-12 |
| IL-6 | Interleukin-6 |
| IL-8 | Interleukin-8 |
| ILD | Interstitial lunge disease |
| MERS | Middle East respiratory syndrome |
| NCIP | Novel corona virus-infected pneumonia |
| NK | Natural Killer |
| OD | Optical density |
| SARS-COV-2 | Severe acute respiratory syndrome-coronavirus-2 |
| TLRs | Toll- like receptor |
| TNF-α | Tumor necrosis factor- alpha |
| WHO | World health organization |

# Chapter One

# Introduction

## *1.Introduction and literatures review*

### 1.1. Introduction:

The rapid outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) infection, has plainly become a public health emergency of international concern. Despite a wide range of clinical presentation and varying degrees of severity, more than 887.814 people worldwide have now recovered from this viral lung infection. Though the number of recoveries does provide solace, there is still little information about how these patients and their lung integrity will evolve throughout the post-infectious healing process (Frix et al., 2020).

Understanding the clinical and epidemiological characteristics of the disease is important for informing public health decision making, which would enable improvement of surveillance and effective planning of treatment (D'Alessandro et al., 2021). Although SARS-CoV2 infection is associated with different clinical patterns, the lung proved to be specifically targeted and interstitial pneumonia represents the most frequent clinical feature of pulmonary involvement (Scotto et al., 2021). Interstitial lung damage induced by viral infection conventionally leads to respiratory symptomatology associating cough, chest pain and dyspnea. Dyspnea may in some cases persist for several weeks due to lung parenchymal sequelae. Coronavirus are known for their potential to induce lung fibrosis (Frix et al., 2020).

Krebs von den Lungen-6 (KL-6) was recently proposed as a diagnostic biomarker for differentiating Interstitial Lung Disease (ILD). It is predominantly expressed in the lung by injured and regenerating alveolar type II cells ("Krebs von Den Lungen-6 as a Biomarker for Disease Severity Assessment in Interstitial Lung Disease. It has also been proposed as a prognostic marker of ARDS: high KL- 6 concentrations have been demonstrated in ventilated patients and showed a correlation with risk of mortality (Sato et al., 2004).

Serum levels of KL-6 are elevated in a variety of respiratory and non-respiratory conditions, including breast and pancreatic cancer and diabetes mellitus. However, most attention deserves its role in different interstitial lung diseases (ILDs), such as interstitial pneumonia, alveolar proteinases, pulmonary sarcoidosis and radiation pneumonitis. In fact, KL-6 levels in the serum of patients with interstitial pneumonia are significantly higher compared to those of healthy people and patients with other respiratory diseases (Scotto et al., 2021).

Severity of Covid-19 was different among patients Thus, the main aim of this study was to determine some of immunological parameters such as LDH, KL6 and CRP relatedness to severity of disease by the flowing objectives:

1- Collection of blood samples from Covid-19 patients.
2- Demographic and clinical history of patients.
3- Comparing level of CBC, CRP, LDH, KL6 in patients' serum and control.

## 1-2: Literatures Review

### 1-2-1: Coronaviruses

Coronaviruses are a large family of viruses which may cause disease in animals or humans. Seven coronaviruses can produce infection in people around the world but commonly people get infected with these four human coronaviruses: 229E, NL63, OC43, and HKU1. They usually cause a respiratory infection ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and severe acute respiratory syndrome (SARS) and the most recently discovered coronavirus (COVID-19) causes infectious disease (WHO, 2020). This zoonotic disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The WHO originally called this infectious disease Novel Coronavirus-Infected Pneumonia (NCIP) and the virus had been named 2019 novel coronavirus (2019-nCoV). On 11th Feb 2020, the (WHO) officially renamed the clinical condition COVID-19 (a shortening of Corona Virus Disease-19), which was announced in a tweet. An outbreak of COVID-19 caused by the 2019 novel coronavirus (SARS-CoV-2) began in Wuhan, Hubei Province, China in December 2019, the current outbreak is officially a pandemic (Murphy *et al*.,2020) Since knowledge about this virus is rapidly evolving, readers are urged to update themselves regularly (Figure 1-1).

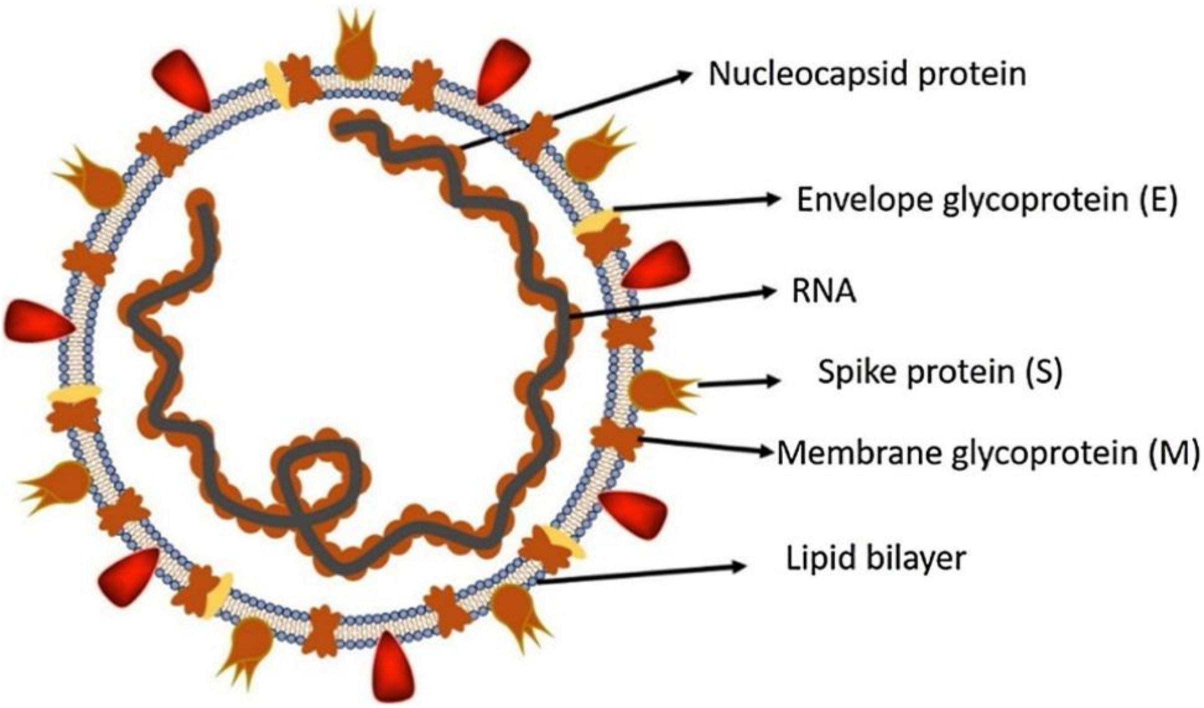


**Figure 1-1. A structure of Respiratory Syndrome (SARS) coronavirus**

**1-2-2: Role of KL6 glycosylation in infection and immunity**

In order to survive the external environment, most mammals, including humans use complex molecules that make up a thick layer of mucus and act as a protective barrier. This epithelial barrier is made up of the mucin proteins that act as the first line of defense and is part of our innate immunity, in which glycans play a critical role in cell–cell adhesion and communication (Zhao *et al*., 2008). Mucins are mainly produced by surface goblet cells and glandular epithelial cells, that are connected to other parts of the innate and adaptive immune systems. Mucins are heavy transmembrane and secreted heterodimeric glycoproteins, and their degree of glycosylation determines their protective function (Bose and Mukherjee, 2020).

Mucins are present on almost all epithelial cells lining the respiratory, gastrointestinal, and reproductive organs. They are mainly made up of O-glycosylated repeats which bind water and give them their characteristic gel-like properties (Corfield, 2015). Some mucins are membrane-bound having a hydrophobic membrane-spanning domain that favors adherence to the plasma membrane. Most mucins are secreted as gel-forming mucus or to form a component of saliva. Mucins are composed of an extracellular N-terminal domain that is glycosylated and participates in ligand binding and cell-cell adhesion, and an intracellular C-terminal domain that has highly conserved phosphorylation sites and binds to various proteins and transcription factors thus playing major roles in downstream signaling (Pinzón Martín *et al.,* 2019). They undergo biochemical changes both in the extracellular domain and the cytoplasmic domain during bacterial, viral and parasitic infections and directly influence pro-inflammatory and anti-inflammatory responses (Bose and Mukherjee, 2020, Dhar, P., and McAuley *et al*., 2019).

KL6 sense ligands of pathogenic origin and pass this information downstream by activating immunomodulatory pathways. Currently, 22 human mucin genes have been identified which are divided into two major types, membrane-bound and secretory. In humans, membrane-bound mucins include MUC1, MUC3A, MUC3B, MUC4, MUC12, MUC13, MUC16, MUC17, and MUC22, and secreted mucins include MUC2, MUC5B, MUC5AC, MUC6, MUC7, MUC19, and MUC20 (Seeberger, 2019).

Human mucin genes MUC21 and MUC22 have been recently identified and are located near to each other (Norman et al., 2017). Mice lacking both copies of mucins like MUC1, MUC2, MUC3, and MUC16 have been shown to have increased bacterial infection and inflammation (Linden et al., 2008). Previously, it has been shown that MUC1 double knockout mice infected with Influenza-A virus were more prone to infection compared to wild-type mice (McAuley *et al.*, 2017). This study shows that MUC1 is a critical component of the host immune response that keeps the severity of influenza in check. An increase in MUC15 expression was found to coincide with the peak of immune activation during influenza (Chen *et al.*, 2019).

Theories of glycobiology state that changes in host glycosylation can cause inflammation, and inflammatory signaling induced by infection may lead to changes in host glycosylation. This is described as the "glycoevasion hypothesis" (Kreisman and Cobb, 2012). These studies provide the rationale for studying mucins to elucidate mechanisms of infectious disease pathologies. Many knockout mouse models with glycosylation changes have been generated over the decades. In mice, some of these knockouts are embryonic or newborn lethal (Marek *et al*., 1999; Shafi *et al.*, 2000; Wang *et al*., 2001;Z. Ye and Marth, 2004), which is also seen in the human population by the low survival rate and severe pathologies associated with the congenital disorder of glycosylation (CDG) diseases (Freeze and Ng, 2011;JaAeken, 2010). These data show the physiological relevance of glycans in general and that the disturbance of glycosylation enzymes generates immune-associated pathologies. Sialylated core 1 O-glycans are considered a hallmark of naïve CD8+ T cells present in the thymus; however, the loss of the sialylated core 1 and the synthesis of core 2 O-glycans are found during the activation of CD8+ T cells in the periphery (Chervenak and Cohen, 1982; Piller *et al.*, 1988).The O-glycans revert to the core 1-dominated phenotype after the differentiation into CD8+ memory cells, (Galvan *et al*.,1998) and those effector CD8+ T cells still having core 2 O-glycans undergo apoptosis. Animals without the ST3 Gal-I sialyltransferase enzyme were reported to lack the sialylatedcore 1 O-glycans on naïve CD8+ T cells. Also, the loss of ST3 Gal-I enhanced synthesis of the core 2 O-glycans on naïve T cells in the periphery, thus leading to apoptosis in the absence of immune challenge (Priatel *et al*., 2000). These results show that glycoprotein sialylation is a homeostatic mechanism for CD8+ T cell-dependent immune responses. The removal of the ST6 Gal-I enzyme in mice resulted in a severe B cell-centered phenotype where IgM levels and proliferation of B cells were significantly reduced upon stimulation via IgM or CD40 cross-linking. Antibody responses were also reduced for both T-independent and T-dependent antigens (Hennet *et al*., 1998). The evidence of bacterial infection-mediated glycosylation changes is many and have been reviewed previously (Kreisman and Cobb, 2012). However, even viruses are known to interact with glycans to enter into host cells (Bose and Mukherjee, 2020). Hemagglutinin is a viral protein that binds to glycans containing sialic acids on the target cell surface and aids viral adherence to the cell. The viral-encoded enzyme neuraminidase cleaves these host sialic acid molecules upon entry, leading to the detachment of viral particles into the cell. This is a crucial event for the viral lifecycle, since neuraminidase inhibition can be an effective therapy (Grienke *et al*., 2012). In this context, it becomes imperative that glycosylation patterns of mucins influence susceptibility to infection, magnitude of immune response, and to some extent response to therapy.

The severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) belongs to the Coronaviridae family and is the causative agent of pneumonia, defined as coronavirus disease 2019 (COVID-19), which first emerged in the Hubei province in China (Henss *et al*., 2021). The virus rapidly spread worldwide, and the SARS-CoV-2 pandemic was declared by the World Health Organization (WHO) on 11 March 2020. Coronaviruses can cause different diseases in humans. Four endemic human coronaviruses, OC43, 229E, HKU1, and NL63, are the causative agents of common colds. Two other coronaviruses, the severe acute respiratory syndrome virus coronavirus and the Middle East respiratory syndrome virus, have a high pathogenic potential, with 15%–30% mortality rates in humans and have caused small epidemics of severe pneumonia (Cui *et al*., 2019).

### 2.1.2 Coronavirus diversity

Coronaviruses are members of the subfamily Coronavirinae in the family Coronaviridae and the order Nidovirales (International Committee on Taxonomy of Viruses). This subfamily consists of four genera Alpha coronavirus, Beta coronavirus, Gamma coronavirus and Delta coronavirus on the basis of their phylogenetic relationships and genomic structures. The alpha coronaviruses and beta coronaviruses infect only mammals. The gamma coronaviruses and delta coronaviruses infect birds, but some of them can also infect mammals(Woo *et al.*, 2012). Alpha coronaviruses and beta coronaviruses usually cause respiratory illness in humans and gastroenteritis in animals. The two highly pathogenic viruses, SARS-CoV and MERS-CoV, cause severe respiratory syndrome in humans, and the other four human coronaviruses (HCoV- NL63, HCoV-229E, HCoV- OC43 and HKU1) induce only mild upper respiratory diseases in immunocompetent hosts, although some of them can cause severe infections in infants, young children and elderly individuals(Masters and Perlman, 2013; Su *et al.*, 2016). Alpha coronaviruses and beta coronaviruses can pose a heavy disease burden on livestock; these viruses include porcine transmissible gastroenteritis virus (Baric and Sims, 2005), porcine enteric diarrhea virus (PEDV) (Lin *et al.*, 2016) and the recently emerged swine acute diarrhea syndrome coronavirus (SADS-Cov) (Zhou *et al*., 2018). On the basis of current sequence databases, all human coronaviruses have animal origins: SARS-CoV, MERS- CoV HCoV- NL63 and HCoV-229E are considered to have originated in bats; HCoV- OC43 and HKU1 likely originated from rodents (Cui *et al*., 2019). Immunohistochemical studies showed that KL-6 is a sialoglycoprotein antigen with a molecular weight equal to or greater than 1000 kDa strongly expressed on type II pneumocytes (reported in 1985 by Kohno et al.), and serum KL-6 levels are regarded as an index of alveolar epithelial cell damage and subsequent regeneration (Kohno *et* *al*., 1993; Kuwano *et al*., 2002). Moreover, the expression of KL-6 protein seems to correlate with altered alveolar-capillary permeability (Inoue *et al*., 1997), suggesting a link between high KL-6 serum levels and alveolar epithelial barrier dysfunction, and the subsequent onset of acute respiratory distress syndrome (ARDS). A previous study examined KL-6/MUC1 levels in the serum and pulmonary epithelial lining fluid (ELF) or Broncho alveolar lavage fluid (BALF) of patients with ARDS or acute lung injury (ALI) (Iashizaka *et al*., 2004; Sato *et al*., 2004). These studies reported that the KL-6/MUC1 levels in these samples were significantly higher in non-survivors than in survivors. A recent study evaluated the levels of KL-6/MUC1 in ELF and serum obtained at multiple time points from patients with ARDS. A comparison of the kinetics of KL-6/MUC1 levels in ELF and serum between survivors and non-survivors revealed that only the KL-6/MUC1 levels in ELF on days 0–3 after the diagnosis of ARDS were significantly higher in non-survivors than in survivors (Scotto *et al*., 2021). Three coronaviruses have crossed the species barrier to cause deadly pneumonia in humans since the beginning of the 21st century: severe acute respiratory syndrome coronavirus (SARS-CoV) (Drosten *et al*., 2003; Ksiazek *et al*., 2003)

# Chapter Two

# Materials and Methods

## 2- Materials and Methods:

### 2.1: Materials:

#### 2.1.1: Instruments and Equipment

The equipment and instruments used in the current study were listed in the table (2-1) below.

**Table (2-1): Instruments and Equipment in this study**

**Table (2-1): Materials and Kits**

| Instruments and Equipment | Origin | Company |
|---|---|---|
| Autoclave | Japan | Hirayama |
| Centrifuge | Germany | Hittech |
| Chemistry analyzer | Japan | Fujifilm |
| Disposables | China | Homecare |
| ELISA reader+Washer | USA | Bioteck |
| Freezer | Lebanon | Concord |
| Incubator | Germany | Memmert |
| Tubes | China | Citoglass |

#### 2.1.2: kits

**Table (2-2): Study kits**

| Kit | Origin | Company |
|---|---|---|
| CRP kit | Spain | Spainreact |
| LDH kit | Japan | Fujifilm |
| Mucin ELISA kit | China | Elabscience |

## 2.2: Methods:

### 2.2.1: Patients and Control Group

This case- control study involves 60 (36 female and 24 male) patient COVID 19 who's admitted to COVID 19 Wards in Merjan Medical City during June to July 2021 in Babylon Province. All patients were diagnosed based on previous clinical report and clinical examination. These cases are compared with 28 healthy control subjects, all subject with ages ranged from (15- >55) years, all of them is asked to fill a questionnaire and all had no family history of any disease. All patients suffering from Covid-19 pneumonia were included and excluded other type of respiratory disease.

### 2.2.2: Ethical considerations

The approvals were obtained from all the participants (patients and healthy) and after obtaining the fundamental approvals form the official authorities, the following information is recorder (patient name, age, sex, date infection, chronic disease). As well as record the percentage of oxygen Sop2, computed tomography scan. and whether the treatment is given (plasma, Remdesivir, Altamira).

### 2.2.3: Collection of samples

Fife milliliters of venous blood sample was taken from all subjects. A tourniquet is applied directly on the skin around the arm. The skin over the vein is sterilized with 70% ethyl alcohol from the subjects before blood collection. Then the blood samples are transferred into Gel tube for serum separation, the blood left for about 30 minutes in room temperature for clotting and then centrifuged at 3000 rpm for 2 minutes. Then the serum is collected in sterile Eppendorf tube in four repeaters and kept frozen at -20 C°.

### 2.2.4: Immunological study

#### 2.2.4.1: Estimation of serum Human KL6

ELISA kit applied to the in vitro quantitative determination of Human KL6 concentrations in serum.

**A. Test principle**

ELISA kit uses Sandwich-ELISA as a method. The 96 micro titer plates provided in this kit has been adsorbed with an antibody specific to Human KL6. Samples or Standards were added to the appropriate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for perforin were added to well and allowed to bind. Avidin-Horseradish Peroxidase (HRP) conjugate were added to each well and incubated. Free components are washed away after that chromatogenic substrates are added to all wells and allowed to incubated. Only those wells that contain KL6, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value was proportional to the concentration of perforin. The concentration perforin in the samples by comparing the OD of the samples to the standard curve.

**B. Reagent preparation**

1-All samples and kit components are brought from refrigerator before use.

**C. Assay procedure**

1. Sample: 100µL of standard, blank, or sample per well is added. The blank well is added with reference standard and sample diluent. The plate covered with plate sealer, then incubated for 90 min at 37℃.

2. Biotinylated Detection Ab: The liquid of each well was removed, without washed. Immediately 100µL of biotinylated detection Ab working solution was added to each well. The plate covered with plate sealer. Gently tap the plate to ensure full mixing, then incubated for 1 h at 37°C.

3. Wash: Each well aspirated and washed, repeating the process three times. Washing done by filling each well with wash buffer. Complete removal of liquid at each step is essential. After the latter wash, the remaining wash buffer was removed by aspirating or decanting. The plate was upturned and putted it against thick clean absorbent paper.

4. HRP Conjugate: 100µL of HRP conjugate working solution is added to each well. The plate covered with the plate sealer, then incubated for 30 min at 37°C.

5. Wash: The washing process is repeated for five times as conducted in step 3.

6. Substrate: 90µL of substrate solution was added to each well, the plate covered with a new plate sealer, then incubated for about 15 min at 37°C. The plate was protected from light, the reaction time can be shortened or extended according to the actual color change, but not more than 15 min.

7. Stop: 50µLof stop solution is added to each well, then the color turns to yellow immediately. The order to add stop solution should be the same as the substrate solution.

8. Optical density (OD): The optical density of each well was determined at once, using a micro-plate reader set to 450 nm. Concentration of unknown samples and control groups are calculated from the standard curve.

2-Preparation of Standard Solution: Reconstitute the lyophilized recombinant protein to make 50 pg/ml of perforin solution by 1ml standard and sample diluents buffer was added to a tube of lyophilized protein. The tube has been shacked gently with vortex, taking care not to foam and then kept at room temperature for 10 min. and mixed thoroughly.

**Dilution method:** Take 7 EP tubes, 500 µl of Reference Standard &Sample Diluent was added to each tube. 500 µl from 10 pg/ml mucin Working solution was pipetting to the first tube and mix up to produce 5 pg/ml working solution. Pipette 500 µl of the solution from the former tube in to the latter one according to this step. Then 500 µl is moved from each tube to another to prepared series dilute. Each tube has been thoroughly before next transfer.

3. Wash Buffer – 30 ml of Concentrated Wash Buffer added to 750 ml of deionized H2O to prepared Wash Buffer.

4. Biotinylated Detection Ab Working Solution: The stock tube was centrifuged before use, then diluted by Biotinylated Detection Ab Diluent (1:100). The required amount was calculated before experiment (100µL/well).

5. Concentrated HRP Conjugate – Concentrated HRP Conjugate were diluted by adding concentrated HRP Conjugate Diluent (1:100).

6. Substrate Reagent- The needed dosage of the reagent can be aspirated with sterilized tips and the unused residual reagent shouldn't be dumped back into the vial again because it is sensitive to light and contaminants.

### 2.2.4.2: Detection LDH

Dry chemistry test for LDH by Fujifilm (Dri-Chem NX500i)

1- Opining the foil and withdraw the test chip.

2 -Putting the chip in chamber inside the analyzer.

3 -Placing the 1ml tube containing serum sample in sample holder along with tip.

4-Starting the analyzer automatically and the results will appear 5 minutes later.

### 2.2.5: Statistical Analysis

Graph Pad prism version 7.05. Numerical data were tested for normal distribution using the T test. data were presented as the mean ± standard deviation of different parameters.

### 2.2.6: Biosafety and Hazard Material Disposing

Biosafety aspects followed during the work include disposing of all contaminated supplies by autoclaving and then incineration. All benches cleaned with alcohol before and after the work.

# Chapter Three

# Results and Discussion

## Results and discussion:

The number of patients included in the current study were 60 (36 female and 24 male) with Age Mean±SD (57.86±15.85 for female and 54.5±14.89 for male). The results showed that the old age adults (>=60 years) were most frequent among COVID-19 patients (Table 1). Results of figure (1) showed that all patients have positive CRP and lymphocytopenia as screening diagnostics assay for COVID-19 patients' suspicion. Actually, the elderly has medical problems like hypertension, diabetes mellitus and cardiovascular diseases collectively can call old-age diseases (OADs). The results revealed that OADs compile (46.66%) and (28.33%) in female and male COVID-19 patients respectively. Additionally, the lung involvement by CT were highly significant in compare with same patients without OADs (Sig. value 0.0001) and also the Spo2 was decline significantly in OADs compared to non-OADS (Sig. value 0.0001) (Table 2).

**Table (1): Distribution of COVID-19 patients among age group**

| Age group (Years) | Female (n=36 (%) | Male (n=24 (%) |
|---|---|---|
| young adult (<=39) | 4 (6.64) | 3 (4.98) |
| middle age adults (40-59) | 14 (23.24) | 11 (18.26) |
| old age adult (>=60) | 18 (29.88) | 10 (10.66) |
| Age Mean±SD | 57.86±15.85 | 54.5±14.89 |

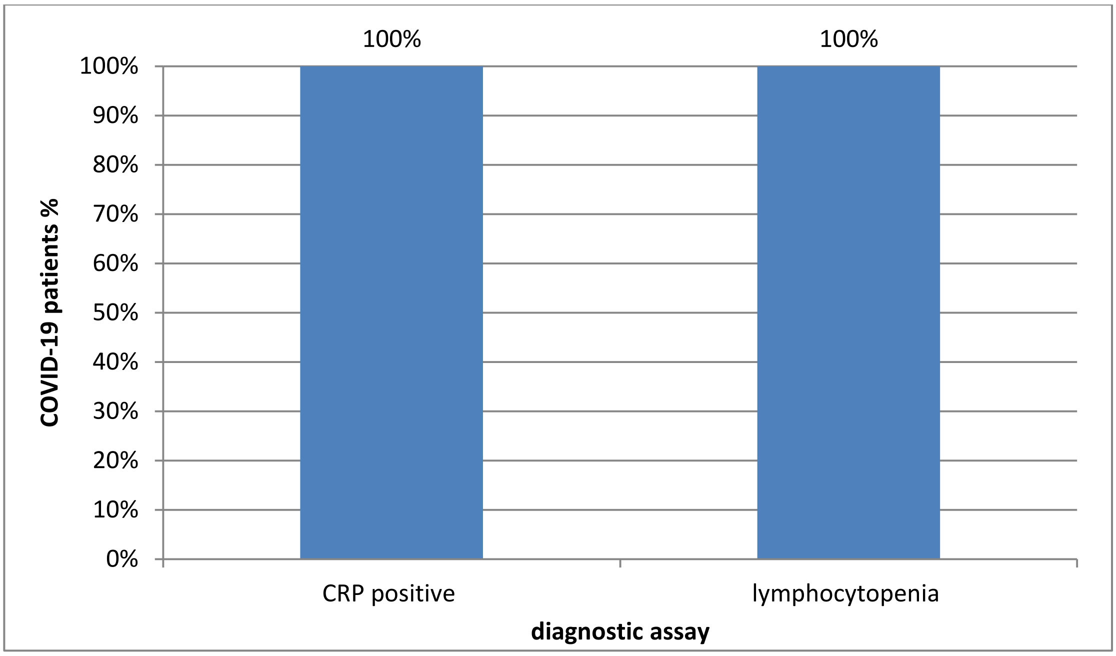


Figure (1): Percentage of COVID-19 patients for CRP and lymphocytopenia

**Table (2): Clinical investigations of COVID-19 patients**

| **Gender** | **Status** | **no. of patients** | **lung involvement (CT%) Mean±SD** | **Spo2 (Mean)** | **CRP** |
|---|---|---|---|---|---|
| Female | Old-age diseases | 28 (46.66%) | 41±0.15 | 88±0.05 | Positive |
| | Non-Old-age diseases | 8 (13.33%) | 21±0.06 | 93±0.04 | Positive |
| Sig. Value | - | - | 0.0001* | 0.0001* | |
| Male | Old-age diseases | 17 (28.33%) | 40±0.17 | 91±0.06 | Positive |
| | Non-Old-age diseases | 7 (11.66%) | 24±0.12 | 93±0.03 | Positive |
| Sig. Value | - | - | 0.0001* | 0.0001* | |

*Significant differences at P 0.05

The elderly COVID-19 patients have clear disruption of the innate and adaptive immune system, subsequent leading to an extensive production of cytokines and inflammatory mediators the so-called inflammation process as well as a more profound depletion of CD4+ cells that consequently lead to a disproportionate cytokine storm and a reduced virus clearance (Aw *et al.*, 2007; Napoli *et al.*, 2020). Moreover, the elderly was significantly associated with more extensive lung involvement, lower oxygen saturation levels, and higher need for oxygen supplementation. Consequently, these patients had more severe COVID-19 and required mechanical ventilation and ICU admission more often than younger patients (Guo *et al*., 2020; Lian *et al*., 2020).

Elderly people are possibly less compliant than younger subjects to follow authorities' recommendations such as wearing a face mask or social distancing (Daoust, 2020), the risk of developing a severe form increases with the number of comorbidities (Fu *et al*., 2020).

CRP is an acute-phase protein that serves as an early marker of inflammation or infection. The CRP serum level is routinely measured in early diagnosis of pneumonia, and some Chinese publications have reported the prognosis value of CRP (Warusevitane *et al*., 2016; Liu *et al*., 2020).

Lymphopenia was detected in severe COVID-19 patients (85%) and suggested as a severity predictor. They also reported that low lymphocyte count and poor prognosis were related to aging. Lymphopenia could have occurred in COVID-19 patients via four mechanisms: (a) viral attachment to the cell surface receptor ACE2 infect lymphocytes that lead to lymphocyte death; (b) the possible role of coronavirus in the destruction of lymphoid organs; (c) induction of lymphocyte apoptosis by the production of tumor necrosis factor-α and interleukin-6, and (d) inhibition of lymphocyte production during metabolic acidosis. Although the pathogenesis of COVID-19 remains unclear, lymphopenia was observed in most of the patients. Aging and chronic illness lead to endothelial dysfunction that dismounts cell-cell adhesions, promotes endothelial cell death, extravasation that resulted in lymphopenia (Liao *et al*., 2002; Bermejo-Martin *et al*., 2018; Elhassadi *et al*., 2020; Tan *et al*., 2020; Xu *et al*., 2020).

**KL6 and LDH level**

The results revealed that, there is a significant difference between LDH serum level in control when compared to male COVID-19 patients (Sig. value 0.0001) and female COVID-19 patients (Sig. value 0.0077). While non-significant differences were observed between COVID-19 patients and control. Concern Mucin1 levels the results revealed that, the level of Mucin1 is same in COVID-19 patients and healthy control (Table 3,4,5).

**Table (3): Difference of LDH and KL6 level among COVID19 Male patients and control**

| Test | | COVID-19 Male (n=24) | Control (n=28) | Sig. Value |
|---|---|---|---|---|
| LDH | Mean | **279.12** | **177.42** | 0.0001* |
| | S.D. | **123.12** | **42.23** | |
| MUC1 | Mean | **1.74** | **1.51** | 0.4405 |
| | S.D. | **1.21** | **0.92** | |

**Table (4): Difference of LDH and KL6 level among COVID19 female patients and control**

| Test | | COVID-19 female (n=36) | Control (n=28) | Sig. Value |
|---|---|---|---|---|
| LDH | Mean | **317.22** | **177.42** | 0.0077* |
| | S.D. | **265.51** | **42.23** | |
| MUC1 | Mean | **2.28** | **1.51** | 0.1200 |
| | S.D. | **2.45** | **0.92** | |

**Table (5): Difference of LDH and KL6 level among COVID19 Male and female patients**

| Test | | COVID-19 Male (n=24) | COVID-19 female (n=36) | Sig. Value |
|---|---|---|---|---|
| LDH | Mean | **279.12** | **317.22** | 0.5143 |
| | S.D. | **123.12** | **265.51** | |
| MUC1 | Mean | **1.74** | **2.28** | 0.3217 |
| | S.D. | **1.21** | **2.45** | |

The results agreed partially with this of Martha *et al*., (2021) who found that elevated LDH was present in 44% (34%–53%) of the patients and so elevated LDH was associated with poor prognosis in patients with COVID-19. Twenty-eight studies reported LDH levels in severe vs. non-sever groups (Szarpak *et al*., 2020). Elevated LDH and CRP serum concentrations are associated to respiratory failure in CoVID-19 patients (Poggiali et al., 2020). Akdogan *et al*., (2021) found that CRP and LDH levels were positively correlated with lung lesions in early-stage COVID-19, potentially reflecting disease severity. Because LDH and CRP levels can potentially reflect the pulmonary function, they can be potential predictors of COVID-19- related respiratory failure. For avoiding poor prognosis; LDH and CRP should be considered as potential predictors for identifying the need for thoracic CT scans, close monitoring of pulmonary function, and aggressive supportive therapy early in the course of COVID-19

Lactate dehydrogenase (LDH) is one of the biomarkers used for determination of prognosis in patients with COVID-19 (Tao *et al*., 2018). LDH is an intracellular enzyme found in cells in almost all organ systems, which catalyzes the interconversion of pyruvate and lactate, with concomitant interconversion of NADH and NAD+. Although LDH has been traditionally used as a marker of cardiac damage since the 1960s, abnormal values can result from multiple organ injury and decreased oxygenation with upregulation of the glycolytic pathway. The acidic extracellular pH due to increased lactate from infection and tissue injury triggers the activation of metalloproteases and enhances macrophage mediated angiogenesis (Hsu and Sabatini, 2008; Martinez-Outschoorn *et al*., 2011). Severe infections may cause cytokine-mediated tissue damage and LDH release. Since LDH is present in lung tissue (isozyme 3), patients with severe COVID-19 infections can be expected to release greater amounts of LDH in the circulation, as a severe form of interstitial pneumonia, often evolving into acute respiratory distress syndrome, is the hallmark of the disease (Patschan *et al*., 2006; Zhang *et al*., 2014). A meta-analysis study found that elevated LDH values were associated with 6-fold increase of severe COVID-19 disease (Henry *et al.*, 2020).

Most mammals, including humans use complex molecules that make up a thick layer of mucus and act as a protective barrier. This epithelial barrier is made up of the mucin proteins that act as the first line of defense and is part of our innate immunity, in which glycans play a critical role in cell–cell adhesion and communication (Zhao *et al.*, 2008). Mucins are mainly produced by surface goblet cells and glandular epithelial cells, that are connected to other parts of the innate and adaptive immune systems. Mucins are heavy transmembrane and secreted heterodimeric glycoproteins, and their degree of glycosylation determines their protective function (Bose and Mukherjee, 2020). Mucins are present on almost all epithelial cells lining the respiratory, gastrointestinal, and reproductive organs. They are mainly made up of O-glycosylated repeats which bind water and give them their characteristic gel-like properties (Corfield, 2015).

Mucin1 or KL-6 is a sialoglycoprotein antigen which proved elevated in the serum of patients with different interstitial lung diseases, especially in those with a poorer outcome. Given that interstitial pneumonia is the most common presentation of SARS-CoV2 infection (Scotto *et al.*, 2021). Pathogens, including viruses of the upper respiratory tract, utilize mucin proteins to enter into host cells. KL6 are critical components of innate immunity and also play important roles in infectious disease progression. Their expression is regulated by different cytokines during infection and inflammation (Bose *et al.*, 2021)

The results of current study shown on significant difference in KL6 level between COVID-19 patients and healthy control. These results may explain as the COVID-19 patients under study may be not severe or being convalescent.

The clinical stages of COVID-19 can be divided into three phases based on the cells that are likely infected: (i) the asymptomatic state (the initial 1 to 2 days of infection); (ii) the upper airway and conducting airway response (the next few days); and (iii) hypoxia, ground glass opacity, and progression to acute respiratory distress syndrome (ARDS) (Mason, 2020).

A recently studies reported markedly increased levels of KL6 and MUC5AC in the sputum aspirated from the trachea of patients with severe COVID-19 symptoms (Lu *et al*., 2020). One of the severe complications of COVID-19 is acute respiratory distress syndrome (ARDS). COVID-19-associated ARDS is often fatal, especially in the presence of several preexisting conditions (Ruan *et al*., 20202; Zhou *et al*., 2020). Kost-Alimova *et al*., (2020) found that elevated KL6 levels predict the development of acute lung injury (ALI). Al-bataineh *et al*., (2017) found that the ischemia can leading to hive level of KL6.

# Conclusion and Recommendation

## Conclusion

1- Elderly female was highly prevalent among those with old-age diseases with worsens high score lung involvement and low oxygenation.
2- Slight increased level of LDH may indicate mild COVID-19 cases or convalescent.
3- Non-significant difference in level of KL6 may be related to age or COVID-19 stage, mild or convalescent.

## Recommendation

The current study highly recommended that following:

1- Studying the KL6 level in relation to proinflammatory cytokines.
2- Studying the correlation of KL6 with moderate and severe COVID 19 stages and age

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## الخلاصة :

كوفيد- 19 هو الجائحة الاكثر عدوانية في القرن الحادي والعشرون في حقبة الجائحة الاولى. قد يقتصر ميل العدوى على الفئات العمرية غير الاطفال وخاصة" كبار السن تدريجيا " يتم توسيع الفئات العمرية لتشمل جميع الفئات .

الهدف من الدراسة الحالية هو التحقق في مستوى LDH وkl6 في مصل مرضى كوفيد- 19 وكان عدد المرضى المشمولين في الدراسة الحالية هو 60 مريض (36 انثى 24 ذكر )مع متوسط العمر هو 57.86±15.85 للإناث و 54.5±14.89 للذكور) خلال شهر حزيران 2021 من مدينة مرجان الطبية في/ حلة / العراق.

واظهرت النتائج ان كبار السن (60عاما")كانوا اكثر شيوعا" بين مرضى كوفيد -19 .جميع المرضى لديهم بروتينات الطور الحاد و وصورة الدم الكاملة ايجابي الفحص وقلة اللمفاويات كمقياس تشخيص للمرضى كوفيد- 19المشتبه بهم . في الواقع يعاني كبار السن من مشاكل طبية مثل ارتفاع ضغط الدم والسكري وامراض القلب والاوعية الدموية جميعها تسمى امراض الشيخوخة .

كشفت النتائج ان امراض الشيخوخة تجتمع في (46.66% ) و (28.33%) في الذكور والإناث في مرضى كوفيد -19على التوالي . بالإضافة الى ذلك كانت فحص الرئة بواسطة التصوير المقطعي ( CT . ) Scan ذات دلالة عالية بالمقارنة في نفس المرضى الذين لا يعانون من امراض الشيخوخة ذات قيمة (0.0001 ) وايضا" انخفاضSPO2 بشكل ملحوظ مقارنة بالذين لا يمتلكون امراض شيخوخة بذات قيمة .(0.0001)

واوضحت النتائج وجود فروق ذات دلالة احصائية بين مستوى LDH المصلي في السيطرة بالمقارنة مع مرضى كوفيد-19 الذكور ذات القيمة (0.0001) ومرضى الإناث كوفيد- 19 ذات القيمة (0.0077) بينما لوحظت فروق غير معنوية بين مرضى كوفيد -19 ومجموعة السيطرة . وكشفت النتائج ان مستوى ال KL6 هو نفسه في مرضى كوفيد-19 والسيطرة الاصحاء.

وخلصت الدراسة ان الاناث المسنات كانت منتشرة بشكل كبير بين اولئك الذين يعانون من امراض الشيخوخة مع تفاقم تأثر الرئة بدرجة عالية وانخفاض الاوكسجين. وقد تشير الزيادة الطفيفة في مستوى LDH الى حالات كوفيد -19 الخفيفة او قد يشير الاختلاف غير الملحوظ في مستوى الKL6 ان تكون مرتبطة في العمر او بمرحلة الكوفيد -19 او النقاهة.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
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# KL6 كمؤشر حيوي لشدة المرض في مرضى كوفيد-19

بحث

مقدم الى كلية العلوم / جامعة بابل

كجزء من متطلبات نيل شهادة الدبلوم العالي في العلوم / الأدلة الجنائية

من قبل

محمد عباس كريم قاسم

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