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Evaluating the levels of interleukin-6 and interleukin-10 as potential biomarkers for the severity and mortality in COVID-19 patients

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Abstract---Objective: Evaluating the levels of pro-inflammatory cytokine (IL-6) and anti-inflammatory cytokine (IL-10) among patients with COVID-19. Methodology: A study is conducted during the period from 1st November 2021 to 1st April 2022, 110 patients were diagnosed as COVID-19. Their ages range between 20-81 and more years old, Who attended to AL-Amal Specialized Hospital for Communicable Diseases at first week of infection with symptoms (fever, Headache, cough, shortness of breath, diarrhea, loss of taste and smell), and compared with 50 apparently healthy individuals as control group. The diagnosis of each case was established using clinical diagnosis and confirmed by reverse transcriptase polymerase chain reaction (rt-PCR), 5 ml of fresh venous blood samples were collected from COVID-19 infected patients by sterile syringes and saved in serum separator tube (SST) with specific gel to easy sorting the serum, then left for 20 minutes at room temperature. After coagulation, sera were separated by centrifuge 4000xg for 15 minutes and directly stored at -20 °C to be analyzed later for IL-6 and IL-10 assay. Results: In the current study, the prevalence of severe COVID-19 infection according to age are increased in older aged patients, the highest one appeared at the 81 and more age group in 100% followed by (61- 80) age group 92.3%. Seventy diabetic patients are included in this study, 27 (38.6%) of them have severe COVID-19 cases and 31(44.3%) have severe cases with death, the result of statistical analysis demonstrated that statistically significant differences are found in diabetic patients as compared with non-diabetic patients. also the results revealed that there are significant differences in hypertensive patients when compared with non-hypertensive patients (36 *vs.* 22.9) in severe cases, and (40 *vs.* 20) in severe cases with

death. Sixty seven COVID-19 patients have cardiac disease, from them the highest percentage have severe cases with death 27(40.3%), followed by severe cases 25(37.3%). As well as, the number of patients with asthma is very low 25 of 110 patients. Forty COVID-19 patients have kidney disease, from them the highest percentage have severe cases with death 20(50%), followed by severe cases 17(42.5%). Most clinical feature were measured as following fever 89(80.9%), cough 88 (80%), headache 80 (72.7%), shortness of breath 70 (63.6%), Loss of smell and tasting 60 (54.5%), and diarrhea 45 (40.9%) respectively. The mean serum level of IL-6 show a highly significant difference in sera of patient with COVID-19 as compared with control groups (132 *vs.* 9.7 pg/ml), also it show a highly significant differences in severe and severe with death cases when compared with control, as well as it show non-significant differences in moderate and mild cases as compared with controls, in addition, the mean serum level of IL-10 show a highly significant difference in sera of patient with COVID-19 as compared with control groups (645 *vs.* 2 pg/ml), also it show a highly significant differences in severe and severe with death cases when compared with control, as well as it show non-significant differences in moderate and mild cases as compared with controls. Conclusions: The prevalence of severe COVID-19 infection according to age are increased in older aged patients, There is a significant increased in the level of IL-6 and IL-10 in COVID-19 patients, circulating levels of IL-6 and IL-10 might have great potential as biomarkers for the disease severity and mortality in COVID-19 patients. Clinical features more frequent in patients with positive COVID-19 are: fever, cough, headache, shortness of breath, Loss of smell and tasting than others. The most common comorbidities are hypertension, diabetes, cardiovascular diseases, kidney disease and asthma. Recommendations: The proportion mortality among patients infected with COVID-19 varied based on ages, and comorbidities. Special attention should be addressed toward, asymptomatic carriers and workers in health care facilities as they play a key role in disease transmission. There is a needed for measuring the level of cytokines and risk of development to sever disease in non-ICU patients.

Keywords---COVID-19, IL-6, IL-10.

Introduction

COVID-19 is single strain RNA virus with typical crown-like appearance due to the presence of the glycoprotein spikes on the envelope under an electron microscope that infects many host, including human, mainly causes respiratory infections⁽¹⁾. Although most cases had only mild symptoms, 20% of Coronavirus patients develop severe pathology with acute bilateral pneumonia that may evolve to acute respiratory distress syndrome, in addition to multi-organs failure. The risks of the severity of disease and death increase with age and presence of comorbidities⁽²⁾. In general coronaviruses causing widespread respiratory, central

nervous system and gastrointestinal diseases threatening human health and causes economic loss⁽³⁾. Common comorbidities including chronic kidney disease, cardiovascular disease, hypertension and diabetes, are associated with increasing the risk for severe COVID-19⁽⁴⁾.

The immunopathological mechanisms of COVID-19 cytokine storm was take place when virus infects respiratory epithelial tissues and activates the local innate immune cell to release the inflammatory cytokines like IL-1, IL-6, IL-8, IL-12, TNF- α , and chemokines. these inflammatory cytokines and chemokines then recruit more innate immune cells (monocyte, macrophage, neutrophils, DC, and NK cell), and activate adaptive immune cells such as (CD4⁺ and CD8⁺ T cells) from peripheral tissues to produce sustained inflammatory cytokines such as IL-2, tumor necrosis factor alpha (TNF- α) and Interferon gamma (IFN- γ), which induce the myelopoiesis and emergency the granulopoiesis that further aggravate the lung and epithelial damages. In addition, the overproduction of systemic cytokines, specially IL-2, GM-CSF, IFN- γ , and TNF- α , triggers the macrophage activation syndrome (MAS), and erythro-phagocytosis (hemophagocytic lymphohistiocytosis -HLH), resulting in anemia⁽⁵⁾, also causes perturbation of the coagulation and the vascular hemostasis resulting in capillary leak syndrome, thrombosis, and DIC (disseminated intravascular coagulation, these events together lead to acute respiratory distress syndrome (ARDS), multiorgan failure, and death (Figure 1)⁽⁶⁾. The host immunoregulatory system can produce the regulatory cytokines like tumor growth factor- β and IL-10 to antagonize the overactivated immune responses. However, the aggressive inflammatory conditions like CS cannot be calmed by regulatory system's natural ability⁽⁷⁾.

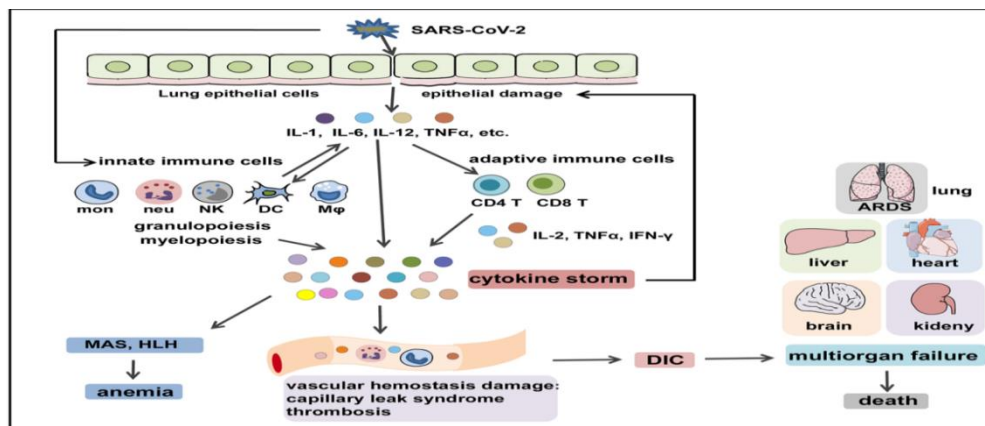


Figure (1): The immunopathological mechanisms of COVID-CS

T lymphocytes, activate neutrophils and macrophages migrate towards and infiltrate alveolar microenvironment releasing the pro-inflammatory cytokines and chemokines. The Th cells response cause release of IL-17 which activate TNF- α , this is in turn enhance the epithelial injury and activate neutrophils to cause degranulation, IL-6 is produced by an alveolar macrophages, which also stimulates neutrophils. Once the epithelial integrity of alveolus is breached epithelial sodium channel and Na/K channels fail to maintain homeostasis eventually leading to increase in the permeability of capillaries causing exudation

of fluids, Treg cells also trigger transforming growth factor beta (TGF- β), which causes fibrosis to the damaged epithelial membrane. Most of the COVID-19 patients cause fibrosis of their lungs ⁽⁸⁾.

Cytokine storm is severe disease associated with lymphopenia and uncontrolled systemic inflammatory responses, which eventually leads to multiple organs failure and death⁽¹⁾. IL-6 is the main cytokine whose development has been linked to many inflammatory diseases⁽⁹⁾. High level of IL-6 were seen in patient with COVID-19, and this level are linked to pulmonary inflammations and severe lung injuries. Previous review show that in the severe COVID-19 disease, high level of serum IL-6 are significantly related to severity of COVID-19 and adverse clinical outcome, which include admission to the intensive care unit, ARDS and death⁽¹⁰⁾. IL-6 as multifunctional mediator of inflammation is widely thought to play pivotal role in COVID-19 induced the cytokine storm and to participate in the interstitial pneumonia observed in severe COVID-19⁽¹¹⁾. In acute phase of inflammations and infections, IL-6 is produced by T-helper 17 (TH17) cell in COVID-19 patient⁽¹²⁾. In addition to, production of this cytokine is also increased by TNF- α as pyrogen cytokine released from immune cell in response to the chronic inflammatory and an autoimmune disease, suggesting that IL-6 as downstream effector of TNF- α ⁽¹³⁾. Besides IL-6 and IL-10 are synthesized from regulatory T cell and even TH 1 cell and have been reportedly implicated in immunoregulation and inflammation, thereby highlighting power of IL-10 to influence immune and inflammatory responses in context of COVID-19⁽¹⁴⁾. Indeed, number of the clinical studies have unveiled that the circulating IL-6 level was elevated in COVID-19 patient, particularly in those with severe stage and was positively associated with the severity and mortality of COVID-19 ⁽¹⁵⁾.

IL-10 is important immunoregulatory cytokine produced by variety of immune cells including Th2 cell, Treg, CD8⁺ T cell, B cell, DC, macrophage, and NK cell. IL-10 exerts anti-inflammatory function by directly limiting innate immune-related function of macrophage ⁽¹⁶⁾, and DC in autocrine and paracrine manner or indirectly via improving Treg development. In addition, IL-10 can activate mast cell and strengthen the function of CD8⁺ T, B, and NK cell ⁽¹⁷⁾.

The generation of effective immune response to infection while also limiting tissues damage require delicate balance between pro- and anti-inflammatory response. IL-10 has potent anti-inflammatory effect and is essential for regulation of the immune responses. However, immunosuppressive properties of IL-10 can also be exploited by the pathogens to facilitate their survival⁽¹⁸⁾. IL-10 elevate earlier than IL-6 in COVID-19 patient. Given long recognized pathological role of IL-6 in cytokine release syndrome (CRS) and resulting mortality. By contrast, clinical significance of highly elevated IL-10 amount in serum of COVID-19 patient has been generally regarded as anti-inflammatory or immune inhibitory mechanism, stimulated by rapid accumulation of proinflammatory cytokines as negative feedback loop⁽¹⁹⁾. Furthermore, recombinant IL-10 has been proposed by many investigators for treating ARDS in COVID-19 patient based on its immunoregulatory and antifibrotic function ⁽²⁰⁾. However, several lines of clinical evidence from human studies suggest that early and dramatic IL-10 elevation upon COVID-19 infection might instead play detrimental pathological role in COVID-19 severity. Accordingly, recent studies also demonstrate immune

activation and inflammation in COVID-19 patient ⁽²¹⁾, which support the hypothesis that IL-10 may play proinflammatory and immune activating role in COVID-19 pathogenesis. Peripheral IL-10 concentration was significantly higher in the intensive care unit (ICU) COVID-19 patient compared to non-ICU patient. Furthermore, IL-10 concentration strongly correlated with IL-6 and other inflammatory markers like C-reactive protein. Similarly to IL-6, high IL-10 expressions can predict poor outcomes in COVID-19 patient⁽²²⁾.

Clinical studies with COVID-19 patient cohorts explored the role of IL-6 alone [19] or IL-6 along with other cytokines including IL-10, IL-2, IL-4, TNF- α and IFN- γ as prognosticator for severe disease [20]. Although IL-10 typically classified as anti-inflammatory and immunosuppressive cytokine, the effects of IL-10 are highly context-dependent and there are several scenarios where IL-10 enhances immune cell activation and proliferation causing the release of pro-inflammatory cytokines. For instance, Lauw *et al.*, were the first to demonstrate the pro-inflammatory effects of IL-10 *in vivo* during human endotoxemia⁽²³⁾.

Materials and Methods

Sample collection and preparation

Samples Collection During the period from 1st November 2021 to 1st April 2022, 110 patients were diagnosed as COVID-19. Their ages range between 20-81 and more years old, 110 sample are collected from COVID-19 patients, Who attended to AL-Amal Specialized Hospital for Communicable Diseases at first week of infection with symptoms (fever, Headache, cough, shortness of breath, diarrhea, loss of taste and smell), and compared with 50 apparently healthy individuals as control group. The diagnosis of each case was established using clinical diagnosis and confirmed by reverse transcriptase polymerase chain reaction (rt-PCR), 5 ml of fresh venous blood samples were collected from COVID-19 infected patients by sterile syringes and saved in serum separator tube (SST) with specific gel to easy sorting the serum, then left for 20 minutes at room temperature. After coagulation, sera were separated by centrifuge 4000xg for 15 minutes and directly stored at -20 °C to be analyzed later for IL-6 and IL-10 assay.

Human Interleukin-6 Enzyme Linked Immunosorbent Assay Kit

Components of Human Interleukin-6 ELISA Kit (Cat. No. SEKH-0013), Beijing Solarbio Science & Technology Co., Ltd. China) for quantitative detection of IL-6 in human serum

Procedure for Detection Interleukin-6

Prepare all reagents and standards as directed. Wash three times before assay. Add 100 μ l standard or samples to each well, incubate 120 minutes, at room temperature (25 $\pm$ 2°C), aspirate and wash 4 times. Add 100 μ l working solution of Biotin-Conjugate anti-human IL-6 antibody to each well, incubate 60 minutes, at room temperature (25 $\pm$ 2°C), aspirate and wash 4 times. Add 100 μ l working solution of Streptavidin-HRP to each well, incubate 30 minutes, at room temperature (25 $\pm$ 2°C), aspirate and wash 5 times. Add 100 μ l Substrate solution

to each well, incubate 30 minutes, at room temperature ($25\pm 2^{\circ}\text{C}$). Protect from light, add 50 μl stop solution to each well, Read at 450 nm within 5 minutes.

Standard Curve

For calculation, average the OD 450 nm readings for each standard, and each sample, and the average control (zero) OD reading. (Relative OD) = (OD of each well) - (OD of zero well) Known concentrations of IL-6 standard and its corresponding reading OD were plotted on the (x-axis) and (y-axis) respectively. The concentration of IL-6 in sample is determined by plotting the sample OD on the Y-axis. The original concentration is calculated by multiplying the dilution factor (Figure 2).

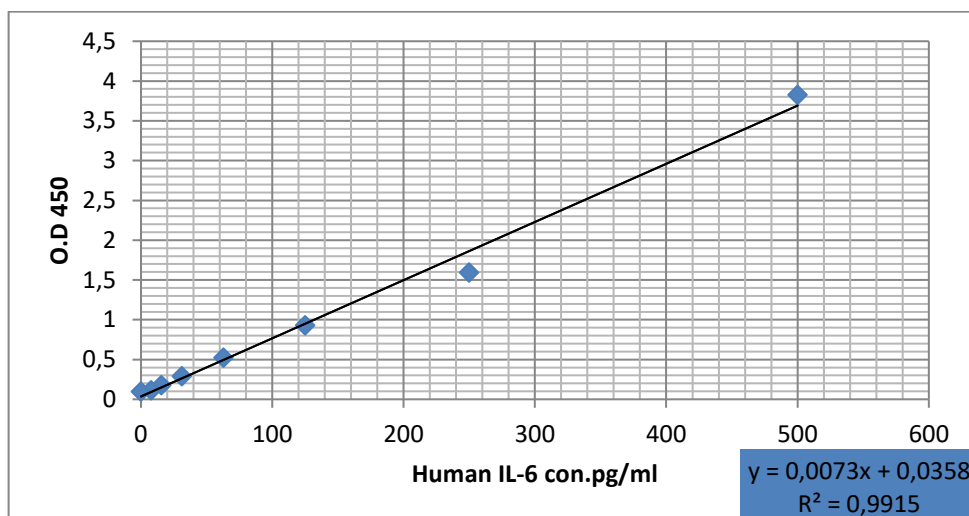


Figure (2): Standard Curve to Determine the Level of Interleukin-6.

Human Interleukin-10 Enzyme Linked Immunosorbent Assay Kit

Components of Human Interleukin-6 ELISA Kit (Cat. No. SEKH-0018), Beijing Solarbio Science & Technology Co., Ltd. China) for quantitative detection of IL-10 in human serum

Procedure for Detection Interleukin-10

Prepare all reagents and standards as directed. Wash three times before assay. Add 100 μl standard or samples to each well, shaking with Micro-oscillator (100 r/min) incubate 120 minutes, at room temperature. Aspirate and wash 4 times, add 100 μl working detector to each well, shaking with Micro-oscillator (100 r/min) incubate 60 minutes, at room temperature, aspirate and wash 5 times, add 100 μl Substrate solution to each well, incubate 30 minutes, at room temperature, Protect from light. Add 50 μl stop solution to each well, Read at 450 nm within 5 minutes.

Standard Curve

For calculation, average the OD 450 readings for each standard, and each sample, and then the average control (zero) OD reading.

(Relative OD) = (OD of each well) - (OD of zero well)

Known concentrations of IL-10 standard and its corresponding reading OD was plotted on the (x-axis) and (y-axis) respectively. The concentration of IL-10 in sample is determined by plotting the sample OD on the Y-axis (Figure 3). The original concentration is calculated by multiplying the dilution factor.

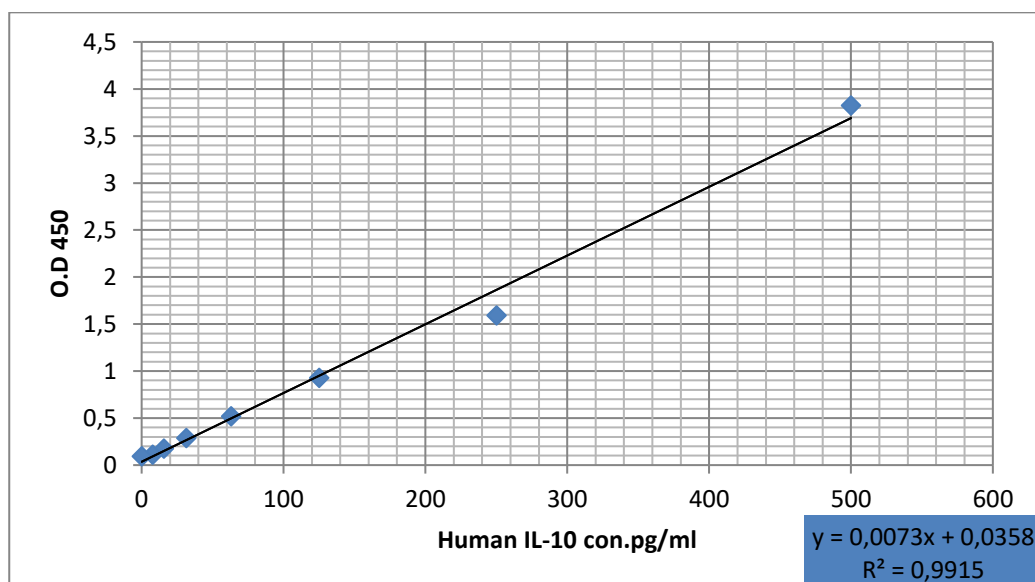


Figure (3): Standard Curve to Determine the Level of Interleukin-10.

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of different factors in study parameters. Chi-square test was used to significantly compare between percentages (0.05 and 0.01 probability) in this study. The results are presented as mean $\pm$ standard deviation (SD) or median and interquartile range. Differences between groups were analysed using the T-test. Statistical analyses were performed using Statistical significance was determined to be $P < 0.05$.

Results

During the period from 1st November 2021 to 1st April 2022 , 110 patients were diagnosed as COVID-19. Their ages range between 20-81 and more years old, and compared with 50 apparently healthy individuals as control group. The diagnosis of each case was established using clinical diagnosis and confirmed by reverse transcriptase polymerase chain reaction (rt-PCR). In the current study, the prevalence of severe COVID-19 infection according to age are increased in older

aged patients, the highest one appeared at the 81 and more age group in 100% followed by (61- 80) age group 92.3%, then the low prevalence frequency was recorded at (41-60) age group in 33.3%, and the low prevalence 29.4 % at the age group (20-40) (table 1).

Table (1): Distribution of Severe cases of COVID-19 patients according to age groups

Age groups Years	Patients No	Severe cases No	Severe cases (%)	P-Value
20-40	17	5	29.4	0.956
41-60	30	10	33.3	0.604
61-80	39	36	92.3**	0.01
81>	24	24	100**	0.001
Total	110	75	68.2	0.05

* Significantly at 0.05, ** Highly Significantly at 0.01 and 0.001.

Seventy Diabetic patients are included in this study, 27 (38.6%) of them have severe COVID-19 cases and 31(44.3%) have severe cases with death , the result of statistical analysis demonstrated that statistically significant differences are found in diabetic patients as compared with non-diabetic patients as shown in table (2). also the results revealed that there are significant differences in Hypertensive patients when compared with non- Hypertensive patients (36 *vs.* 22.9) in severe cases, and (40 *vs.* 20) in severe cases with death.

Sixty seven COVID-19 patients have cardiac disease, from them the highest percentage have severe cases with death 27(40.3%), followed by severe cases 25(37.3%). the results show that there are statistically significant differences in patients with cardiac disease as compared with patients without cardiac disease as shown in table (2),

The result of current study found that the number of patients with asthma is very low 25 of 110 patients. Forty COVID-19 patients have kidney disease, from them the highest percentage have severe cases with death 20(50%), followed by severe cases 17(42.5%). the results show that there are statistically significant differences in COVID-19 patients with kidney disease as compared with patients without kidney disease as shown in table (2).

Table (2): Distribution of study population according to chronic diseases.

Subject groups	Total num ber	Mild		Moderate		Severe		Severe with death		P valu e
		No.	%	No.	%	No.	%	No	%	
Total patients	110	18	30.9	20	30.9	35	29.1	37	9.1	
Diabetic	70	5	7.1	7	10	27	38.6*	31	44.3*	0.0
Non-diabetic	40	13	32.5	13	32.5	8	20	6	15	5
Hypertensive	75	8	10.7	10	13.3	27	36*	30	40*	0.0
Non-	35	10	28.6	10	28.6	8	22.9	7	20	5

hypertensive										
Cardiac disease	67	7	10.4	8	11.9	25	37.3*	27	40.3*	0.05
Without cardiac disease	43	11	25.6	12	27.9	10	23.3	10	23.3	
Kidney disease	40	1	2.5	2	5	17	42.5*	20	50*	0.05
Without kidney disease	70	17	24.3	18	25.7	18	25.7	17	24.3	
Asthma	25	0	0	0	0	11	44*	14	56*	0.05
Without Asthma	85	18	21.2	20	23.5	24	28.2	23	27.1	

* Significantly at 0.05, ** Highly Significantly at 0.01.

Based on clinical features of patients with positive COVID-19, several signs and symptoms were detailed in table (3). Most clinical feature were measured as following fever 89(80.9%), cough 88 (80%), headache 80 (72.7%), shortness of breath 70 (63.6%), Loss of smell and tasting 60 (54.5%), and diarrhea 45 (40.9%) respectively. The statistical analysis found significant differences among clinical features of COVID-19 patients including fever, cough, headache, and shortness of breath.

Table (3): Clinical feature of 110 COVID-19 patients

Clinical signs		Patients No	Patients (%)	P- Value
Fever	Yes	89	80.9*	0.05
	No	21	19.1	
Cough	Yes	88	80*	0.05
	No	22	20	
Shortness of breath	Yes	70	63.6*	0.05
	No	40	36.4	
Diarrhea	Yes	45	40.9	0.60
	No	65	59.1	
Headache	Yes	80	72.7*	0.05
	No	30	27.3	
Loss of smell and tasting	Yes	60	54.5	0.61
	No	50	45.5	

* Significantly at 0.05, ** Highly Significantly at 0.01.

The mean serum level of IL-6 show a highly significant difference in sera of patient with COVID-19 as compared with control groups (132 vs. 9.7 pg/ml), also it show a highly significant differences in severe and severe with death cases when compared with control, as well as it show non-significant differences in moderate and mild cases as compared with controls as shown in table (4), figure (4).

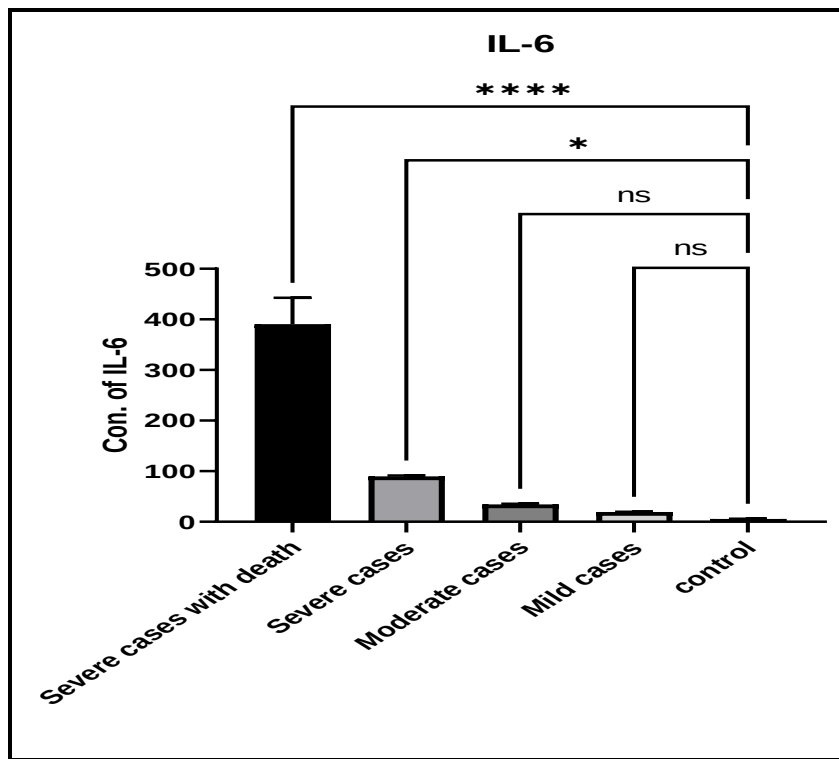


Figure (4): Serum level of IL-6 among Study Population.

Table (4): Serum level of IL-6 among Study Population.

Group		Mean $\pm$ SD (pg/ml)	P-value
Control		9.7 $\pm$ 2.32	<0.001
Patient	Mild	18.5 $\pm$ 3.88	
	Moderate	39.7 $\pm$ 4.86	
	Severe	103.6** $\pm$ 15.53	
	Severe with death	299.7** $\pm$ 44.5	
	Total	132** $\pm$ 18.19	

*Significant at 0.05, ** Highly Significant at 0.01 and 0.001.

The mean serum level of IL-10 show a highly significant difference in sera of patient with COVID-19 as compared with control groups (645 *vs.* 2 pg/ml), also it show a highly significant differences in severe and severe with death cases when compared with control, as well as it show non-significant differences in moderate and mild cases as compared with controls as shown in table (5), figure (5).

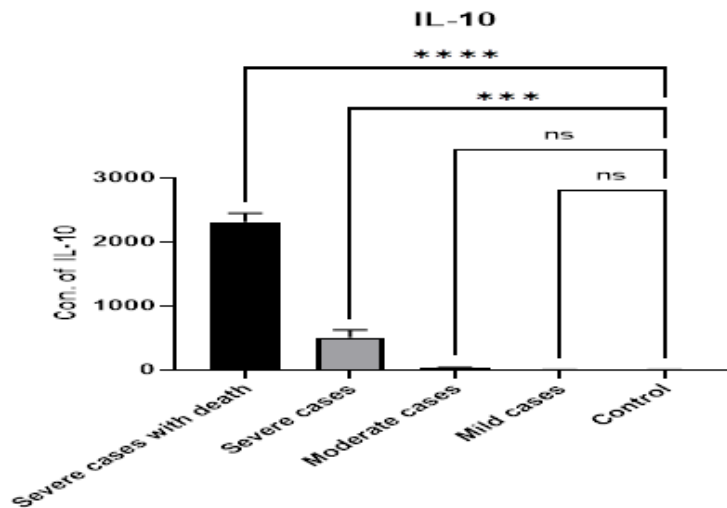


Figure (5): Serum level of IL-10 among Study Population.

Table (5): Serum level of IL-10 among Study Population

Groups		Mean $\pm$ SD (pg/ml)	P-value
Control		2 $\pm$ 1.32	<0.001
Patient	Milled	7 $\pm$ 2.64	
	Moderate	33 $\pm$ 10.9	
	Severe	799** $\pm$ 82.20	
	Severe with death	1759** $\pm$ 102.90	
Total		645** $\pm$ 77.40	

*Significant at 0.05, ** Highly Significant at 0.01 and 0.001.

Discussion

The severity of the clinical picture seems to be correlated with age older than 81 years⁽²⁴⁾. Age rang may play a role in rate of the fatality worldwide, average age of Arab's country was 26.77 years and as February the Arab's country have lowest number of death (8 per million). Also, average age of the Indian population 28 years and fatality rate was (4 per million). This is confirming with report in US, Italy and China which found that the large numbers of deaths were found among the old age cases⁽²⁵⁾. Such observation can be explained by Leij-Halfwerk *et al.* (2019), who reported that the advanced age and comorbidities are related to the impaired nutritional status⁽²⁶⁾, nutritional deficits arise from malabsorption, increased the nutrient losses and augmented metabolic demands, which are frequent in elderly, but also from the reduced dietary intake. Malnutrition is major geriatric syndrome due to the multifactorial etiology, characterized by the muscle wasting and weight loss, which is strongly related to the frailty and negative outcome⁽²⁷⁾.

Due to the age-dependent alterations of the immune system that may be implemented by nutritional deficits, a relevant percentage of elderly patients

progresses to insidious systemic inflammation, mainly affecting the lung, heart, renal function and coagulation system, this stage of hyperinflammation is characterized by massive production of pleiotropic cytokines (e.g., IL-6) by lung resident (e.g., macrophages) and circulating immune cells ⁽²⁸⁾.

Also these results are in an agreement with previous study of COVID-19 patients percentage with comorbidity diseases such as hypertension, diabetic mellitus and asthma were (14.10%, 16.66%, and 3.84%) respectively, the results are in an agreement with study done by Huang *et al.*, (2020) ⁽²⁹⁾, who reported that 41 patients with SARS-COV-2 that less than half number of patients with comorbidities as following including diabetes mellitus 20%, hypertension 15%, and cardiovascular disease 15% in Wuhan City. Guan *et al.*, (2020) ⁽³⁰⁾ reported that among 1099 patients, 179 had severe disease with comorbidity hypertension 23.7%, diabetes mellitus 16.2%, coronary heart diseases 5.8%, and cerebrovascular disease 2.3%. According to result of several studies on presence of comorbidities disease recorded that 20-51% of SARS-COV-2 at time of admission had at least one disease; these include 10-15% hypertension, 10-20% diabetic mellitus and 7-40% cardiovascular disease ⁽³¹⁾. Also agreed with study of Zhang *et al.*, (2020) ⁽³²⁾ demonstrates that 30% of the 140 SARS-COV-2 patients were admitted to the hospital had hypertension and 12% had diabetes. High-risk patients requiring hospitalization for SARS-COV-2 infections are those with common comorbidities including hypertension as their percentage 58%, cardiovascular disease 59%, and diabetes 71% ⁽³³⁾. This may be due to immune status, atrophies and declines in functions of the thymus gland during aging, which is a primary lymphoid organ responsible for the production of immunocompetent T cell ⁽³⁴⁾, which is critically important to the immune system, which serves as the body's defense mechanism, providing surveillance and protection against diverse pathogens⁽³⁵⁾. As well as the results are in agreement with the study of Clerkin *et al.*, who reported that the Cardiovascular comorbidities are common in patient with COVID-19 and have higher risk of morbidity and mortality⁽³⁶⁾. Mechanistically, interaction between the S protein and ACE2 have central role in disease pathogenesis, especially in the cardiovascular manifestations of this disease, and this interaction is potential target for prevention and treatment of COVID-19⁽³⁷⁾. CVD was common comorbidity in patient with COVID-19 predecessors SARS and MERS⁽³⁸⁾. In SARS, prevalence of DM and CVD was 11% and 8% respectively and presence of either comorbidity increased risk of death 12 fold⁽³⁹⁾. DM and hypertension were prevalent in 50% of cases of MERS, CVD was present in 30% of patient⁽⁴⁰⁾. Increased presence of the cardiovascular comorbidities holds true for COVID-19, especially with more severe disease. COVID-19 entry into cells is ACE2 dependent ⁽⁴¹⁾.

The result of current study found that the number of patients with asthma is very low (25 of 110). This finding is comparable with result of a study done by Li *et al.*, (2020) ⁽⁴²⁾ who found that low prevalence of asthma constituted 0.9% (5 of 548) in patients with SARS-COV-2 in Wuhan. It's likely that SARS-COV-2 sensitivity in asthma patients is decreased by Th2-mediated mechanisms. Chronic kidney disease (CKD) seems to be associated with enhanced risk of severe COVID-19 infections and mortality, Cheng *et al.* evaluated association between marker of renal impairments and death in cohort of 701 COVID-19 patients ⁽⁴³⁾. This significant association of Chronic kidney disease with severe COVID-19 infection

was observed by Michael *et al.*, (2020) ⁽⁴⁴⁾, this can be explained by pro-inflammatory state and by alterations of innate and adaptive immune response associated with CKD, this immune profile increases the susceptibility to all infections ⁽⁴⁵⁾.

The results based on clinical features of patients with COVID-19, is an agreement with several neighboring countries as Barry *et al.*, (2020) ⁽⁴⁶⁾, who recorded that the most common clinical sign was fever 67.7%, cough 60.6%, dyspnea 43.4%, fatigue 26.3%, diarrhea 19.2% and loss of smell 9.1% among 99 hospitalized SARS-COV-2 patients in Saudi Arabia. Tian *et al.*, (2020) ⁽⁴⁷⁾ found that the most common symptoms was fever 82.1%, cough 45.8%, fatigue 26.3%, dyspnea 6.9% and headache 6.5% among 262 cases in China.

The result of IL-6 level concentration show a highly significant difference in COVID-19 patients, such observations are in agreement with the result of Kim *et al.*, who reported that IL-6 contributes to the host defense against infection and tissue injury however, the exaggerated, excessive synthesis of IL-6 while fighting COVID-19 leads to acute severe systemic inflammatory response cytokine storms ⁽⁴⁸⁾. Thus, more clinic studies need to be conducted to find right balance between the immune activation and the inflammatory inhibition⁽⁴⁹⁾. These results are in agreement with the result of Huang *et al.*, who reported that IL-6 and IL-10 are the first two markers should be preferentially evaluated for early diagnosis of patient with more severe disease, especially under heavy burden of the medical care in hospitals ⁽²⁹⁾. Cytokine storm and coagulopathy are two main complications of COVID-19, and are more profound and serious in those with more severe form of disease⁽⁵⁰⁾. Upon viral infection, both innate and adaptive immune systems are involved. When innate system, body's first barrier against external agents, cannot eliminate virus, provoke immune system, causing release of more cytokines, which leads to phenomenon termed cytokine storm ⁽⁵¹⁾, this storm, commonly observed among patient with COVID-19, occurs most frequently among severely ill specially those who suffer the lethal consequences. There is direct correlation between immune and coagulation systems, thus immunological and coagulation disturbances are common in COVID-19, both innate and adaptive immune systems are affected in COVID-19 infection ⁽⁵²⁾. Since inflammation has direct effect on coagulation, We found that those with high level of inflammatory cytokines may experience more profound coagulation disturbance, thrombotic risk and fatal consequences. IL-6 concentration has been shown to be higher in patient with severe COVID-19 ⁽⁵³⁾. In our study, we found that the rate of mortality was higher in patients with elevated cytokines.

Ethical Clearance

All experimental protocols were approved under the Faculty of science.

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