Expression of IL-8 is associated with disease severity in Egyptian COVID-19 patients

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ABSTRACT

Background: The coronavirus disease, or COVID-19, caused considerable morbidity and mortality all over the world. It was noticed that patients with severe and complicated forms of COVID-19 have a dysregulated hyper-inflammatory immune response. The role of Interleukin-8 (IL-8) as a biomarker in COVID-19 is under current debate, conflicting data have been shown concerning the association of IL-8 levels with the disease severity of patients. In the current study, we evaluate the expression level of IL-8 in a group of infected coronavirus patients and analyze the relationship between the IL-8 profile and patient outcomes depending on the severity of the disease course. **Patients and Methods:** One hundred and forty symptomatic and asymptomatic COVID-19 patients and 70 healthy individuals were included. We implemented a cytokine assay to measure IL-8 and evaluate its expression, analyzed the correlation between IL-8 levels and the outcomes of severely infected patients. **Results:** Infected coronavirus patients showed an elevation in IL-8 expression both in serum and plasma, protein level of IL-8 was increased in infected patients when compared to healthy volunteers. Receiver Operating Curve (ROC) showed that IL-8 has a diagnostic efficiency with cutoff point of >5.85, sensitivity of 100.0%, specificity of 100.0%, and an area under curve (AUC)= 1. Statistical analysis revealed that the relative expression levels of IL-8 were positively correlated with disease severity. The results revealed that IL-8 levels could be used as a prognostic marker for prediction of ICU patients admission and that its level was a risk factor for mortality. **Conclusion:** IL-8 exhibited an early elevation response to coronavirus infection supporting its role as an early predictor of severe disease and that it might be a sensitive and specific prognostic indicator for infection and disease severity and clinical outcomes such as respiratory failure and ICU dmission.

Keywords: coronavirus, interleukin-8, disease severeity.

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INTRODUCTION

The Chinese government just informed WHO of several cases of pneumonia with unclear etiologies before the end of 2019 **(Huang et al., 2020).** In Wuhan, China, the infection began at the Hunan seafood market which include various animal such as bats, birds, snakes and others, and swiftly spread to more than 50 people **(Dhama et al., 2020).** In January 2020, The Chinese National Health Commission shared more details about outbreak and viral pneumonia. Based on the analysis of sequence isolated from patients the virus is confirmed as a novel coronavirus **(Shereen et al., 2020).** Chinese patients who had pneumonia caused by the Wuhan coronavirus were initially thought to have visited a seafood market where animals were sold or eaten contaminated animals or birds **(Asselah et al., 2021).** However, further research showed that some people caught the virus even though they had no record of going to the fish market **(Shereen et al., 2020).** As a result, it was recorded in more than 100 different nations. The virus spreads from human to other human through the mouth or nose. These aerosols can get inside of a person's body and get into their lungs **(Riou & Althaus, 2020).**

Coronavirus is an enveloped, non-segmented, positive-sense RNA virus which considered as β-coronavirus. It categorized in four genera, including α−, β−, γ−, and δ-coronavirus where β- and α- coronavirus have the ability to infect mammals while γ− and δ- coronaviruses can infect birds **(Shereen et al., 2020).** Previous most known coronaviruses are SARS and MERS, which produce serious and potentially fatal respiratory tract diseases were belonged to β−coV **(Holmes et al., 2021).** It has been demonstrated that the sequence of SARS-CoV-2 genome was 96.2% identical to RaTG13 (bat coronavirus) and 79.5% with SARS-CoV. So, it has been speculated that the bat is the virus' native host according to the findings of the genome sequencing of virus and research, and that SARS-CoV-2 may transfer from

bats to humans via undiscovered intermediary animals **(Guo et al., 2020).**

Diagnosis for COVID-19 patients includes two main methods, nucleic acid testing for detection of SARS-CoV-2 RNA using quantitative real-time (RT-qPCR) in nasopharyngeal and oropharyngeal samples **(Pascarella et al., 2020**). Despite the high specificity of RT-qPCR, occasionally contaminated samples or technical errors might cause false negative results **(Ai et al., 2020)**. The other method is the serological assay for detection of specific IgG and IgM antibodies against SARS-CoV-2 **(Ravi et al., 2020).** Other detection methods were developed and are now in use to get around some of these restrictions and offer quick diagnosis turnaround times **(Chilamakuri & Agarwal, 2021).**

Interleukin-8 is one of the chemokine family, it occupies 5.25 kbp of DNA on the 4q12-21 chromosome. It has three introns and four exons, and it shares DNA with other C-X-C chemokines in a cluster. It is represented by a 1.8 kb single mRNA transcript **(Sharma et al., 2015**). It is released by activated monocytes and macrophages and encourages the directional migration of neutrophils, basophils, and T lymphocytes **(Palomino & Marti, 2015**). It was discovered to be crucial in autoimmune, inflammatory, and infectious illnesses. IL-8 is carefully controlled due to its strong pro-inflammatory characteristics, and its expression is minimal or nonexistent in normal tissues **(Ghasemi et al., 2011).** IL-8 displays significant compartmentalized response in the response of cells, are consistent with the lungs. when taking into account the immune substance's well-known function in drawing neutrophils to the lungs during acute pulmonary inflammation **(Ronit et al., 2021).**

The aim of this study is to to highlight the role of IL-8 as a biomarker and prognostic factor for COVID-19 and describe its signature in patients with severe COVID-19 and to investigate its expression level influence on severity of the infection.

SUBJECTS AND METHODS:

Ethics statement:

The study was approved by the Ethics Committee of National Hepatology and Tropical Medicine Research Institute (NHMRI, Egypt) , and written informed consents were signed by all participants to use their specimens for research purposes , in accordance with the institutional guidelines. All the experimental procedures in this study complies with the latest version of the Declaration of Helsinki and general guidelines for good clinical practice.

Study design and participants:

We designed for a longtudinal study of one hundred and forty symptomatic and asymptomatic COVID-19 patients, fulfilling the incusion criteria of both sex and of age between the age of 20 to 70 years. Health care worker (medical, nursing, allied health, ancillary worker, visiting doctor) working in an environment with direct exposure to patients with confirmed COVID-19 infection were included. All patients had a covid positive report from the hospital by RT-PCR.

Pregnant subjects and lactating females, patients with cancer, liver diseases, immunosuppression, ischemic heart disease, chronic kidney disease, atrial fibrillation, heart failure, rheumatoid arthritis or osteoarthritis, acquired hypothyroidism, Alzheimer disease and related disorders or senile dementia, depression, osteoporosis, and asthma were excluded from the study.

To enhance validity, 70 healthy individuals matched for age, sex, were included as controls.

We grouped patients with fever and slight upper respiratory tract symptoms as mild cases; patients with shortness of breathing, constant pain, or pressure in the chest and respiratory difficulties, and/or radiological findings of pneumonia as moderate cases; and patients with respiratory failure requiring intensive care units (ICU) as severe COVID-19 cases.

Clinical data collection

Demographic, clinical, laboratory, and outcome data were obtained from electronic medical records using a standard data collection form. All data were checked by 2 physicians and a third physician adjudicated any difference in interpretation between the 2 primary interviewers. Symptoms of cough, fever, chest discomfort, fatigue, diarrhea, and expectoration were recorded. Fever was defined as axillary temperature of at least 37.3°C. Signs of body temperature and SpO2 were recorded. Most of the in-hospital deaths were collected form all the admitted patients in the COVID-19 specialized ICU.

Laboratory Tests:

Throat-swab specimens were obtained for COVID-19 examination. All patients had positive results of COVID-19 examination by real-time reverse transcription-polymerase chain reaction.

Five milliliter of blood samples were collected from 140 adult patients who confirmed COVID-19 based on a positive nasopharyngeal swab test.

A similar amount of blood was sampled from 70 controls, matched for age and sex.

Blood was collected by venipuncture and placed into Vacutainer tubes (Becton Dickinson) containing EDTA. A volume of 2.5 ml of the collected blood was transferred into one PAXgene tube and stored at − 80°C.

Plasma and serum were separated from whole blood within 12 hours of collection for cytokine assay.

Routine blood chemistry examinations were done at the second morning after admission including hemoglobin, white blood cell count (WBC) and platelets, inflammatory markers including C-reactive protein (CRP), and kidney and liver function tests were performed in the hospital central laboratory.

Radiological examination:

Chest CT was performed in symptomatic patients with strict precautions to minimize hazardous exposure of patients and health care professionals to SARS-CoV-2. CT helps to identify complications in addition to pulmonary involvement, as well as to suggestive alternative diagnoses. The CT abnormalities of COVID-19 were recorded.

Cytokine assay:

IL-8 levels in COVID-19 patients and healthy individuals's sera were measured by Enzyme-Linked Immunosorbent Assay (ELISA) method according to manufacturer's instructions using Human assay kit, (Sunlong Biotech Co., Ltd).

Briefly, 100 µl of prepared standards and samples were added to appropriate wells of the ELISA plate and then assayed according to the manufacturer's instructions. The absorbance was measured at 450 nm in a microtest plate spectrophotometer, (Abcamm CA, USA) and IL-8 levels were quantified with a calibration curve. Each standard or sample was assayed in duplicate.

Molecular testing:

Total RNA extraction

Using 200 µl of both serum and plasma, total RNA was extracted using the magnetic beads technique as described according to Abbott mSample preparation system kit (Abbott Molecular, Inc., Des Plaines, IL) according to manufacturer protocol. Samples were extracted in duplicates and the concentration was determined by 260/280 nm absorbance using a Nanodrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, UK).

Quantitative Real-Time Reverse-Transcription Assay (qRT-PCR)

Briefly, 10 µl of the extracted RNAs from all samples were reversely transcribed using reverse transcription kit (Applied Biosystems, San Diego, CA, USA). Upon the completion of 1st strand cDNA synthesis, 5µl of the cDNA were used for the real time PCR amplification step using IL-8 and GAPDH specific (Table 1), Maxima SYBR Green/ROX qPCR Master Mix 2X (Thermo Fisher Scientific, UK) in in Applied Biosystems™ StepOne™ Real-Time PCR System. All reactions were run in duplicates. Finally, the ΔΔCT method was used for the relative quantification of mRNAs in all samples **(Akin et al., 2012).**

Table 1: Primer sequence used for RT-qPCR.

Gene	Primer sequence (5'-3')	PCR product size (bp)
IL-8 (forward)	GTATCGGACGCCTGGTTAC	128bp
Reverse	CTTGCCGTGGGTAGAGTCAT	
GAPDH(forward)	AGAGTGATTGAGAGTGGACC	118bp
Reverse	ACTTCTCCACAACCCTCTG	

Statistical analysis

The data were analysed using Microsoft Excel 2016 and statistical package for social science 'IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA)'. Continuous normally distributed variables were represented as mean±SD. with 95% confidence interval, while nonnormal variables were summarized as median with 25 and 75 percentile and using the frequencies and percentage for categorical variables; a P value < 0.05 was considered statistically significant. To compare the means of normally distributed variables between two groups, the Student's t test was performed, while ANOVA test was used in multigroup. Mann–Whitney tests were used to compare the means between groups of non-normally distributed variables, while kruskal–Wallis H test in multigroup. $\chi 2$ test or Fisher's exact test, were used to determine the distribution of categorical variables between groups. The diagnostic performance of IL-8 was assessed by receiver operating characteristic (ROC) curves. The area under the ROC (AUC) was calculated as an accuracy index for prognostic performance of selected tests. The cutoff for the diagnosis of a group of the study was taken from the point of maximum combined sensitivity and specificity. The risk assessment OR (95% C.I) was done by using the logistic regression analysis.

RESULTS

Demographic and baseline clinical characteristics of patients with COVID-19:

A total of 210 participants were recruited in this study, including 140 infected COVID-19 patients,

and 70 healthy volunteers were included as a control group. Individual demographic and clinical data of the studied groups are shown in (Table 2).
The controls had an equal distribution of men and women and the most common comorbidities were hypertension and diabetes.
The COVID-19 patients included 40 (28.6%) males and 100 (71.4%) females with mean age (50.5±10.9 years). Patient age was a key risk factor in our analysis with odd ratio (95% Confidence Interval) OR (95% C.I) =1.092(1.059- 1.126), P<0.001. Ten (7.1%) patients do not have any symptoms of the disease, 60(42.9%) were suffering from bony aches and headache, while 70(50.0%) patients had symptoms of cough, dyspnea and fever. Forty patients (28.6%) were classified as mild infection, 30(21.4%) were moderate and 70(50.0%) were classified as severe infection. Regarding the clinical outcomes, highly significant difference was observed with OR (95% C.I) = 2.333(1.269 - 4.291), P=0.006, 70(50%) patients died, and remaining 70 patients were discharged after recovery (Table 2).

Table 2: Demographic and clinical data in comparison between control and COVID-19 positive patients.

		Control N=70	Cases N=140	P. value	OR(95% C.I)	P. value
Age		40.3±9.8	50.5±10.9	<0.001**	1.092(1.059-1.126)	<0.001**
Sex	Female	35(50.0%)	100(71.4%)	0.002**	0.400(0.221-0.725)	0.004**
	Male	35(50.0%)	40(28.6%)			
Symptoms	No symptoms	70(100.0%)	10(7.1%)	0.3	-	-
	Bonyaches, headache	0(0.0%)	60(42.9%)	0.5	-	-
	Cough, dyspnea, fever	0(0.0%)	70(50.0%)	0.9	-	-
Outcome	Living	70(100.0%)	70(50.0%)	0.001**	2.333(1.269 - 4.291)	0.006**
	Dead	0(0.0%)	70(50.0%)			
Classification	Mild	0(0.0%)	40(28.6%)	0.01*	-	-
	Moderate	0(0.0%)	30(21.4%)	0.01*	-	-
	Severe	0(0.0%)	70(50.0%)	0.001**	-	-
IL-8 in Serum $x10^{-4}$		0.141(0.032-0.53)	2.34(1.45- 4.21)	0.001**	1.623(0.809-3.618)	0.001**
IL8 in Plasma $x10^{-4}$		0.133(0.047-0.62)	2.22(1.17- 3.98)	0.01*	1.845(0.843-3.922)	0.001**
IL-8 Protein ng/µl		0.92±0.67	17.2±6.3	<0.0001**	6.934(2.568-12.905)	0.001**

Age and Protein of IL-8 ng/µl are represented as Mean±SD; the data were analyzed by t test. While Sex, Symptoms, Outcome, Classification are represented as frequency and percent F (%); the data were analyzed by paired X^2 test. IL-8 in Serum, and Plasma are represented as Median and interquartile range (IQR); the data were analyzed by Mann-Whitney U test.
OR; Odd Ratio, C.I; Confidence Interval, P value calculated depend on log linear regression analysis.
* P value <0.05 is significant, ** P value <0.01 is highly significant.

Expression level of IL-8 in serum and plasma

Mann-Whitney U test was used to analyze the data, the expression levels of IL-8 are represented as median with Interquartile range (25% -75%). Infected COVID-19 patients showed an elevation in IL-8 expression level both in serum and plasma with a significant difference between the patients and the healthy individuals and with medians and IQR, OR (95% C.I) = =2.34(1.45- 4.21) and 1.623(0.809- 3.618) for IL-8 in serum, and 2.22(1.17- 3.98) and 1.845(0.843- 3.922) for IL-8 in plasma and P=0.001(Table 2, Fig. 1A).

Protein levels of IL-8 in the studied groups.

Student's t test was used for data analysis. It was noticed that the serum protein level of IL-8 was increased in infected patients when compared to the control group with Mean± SD = 17.2±6.3 and a significant difference was observed between the patients and healthy individuals with OR (95% C.I) =6.934(2.568- 12.905), P = 0.001 (Table 2, Fig. 1B).

A. B.

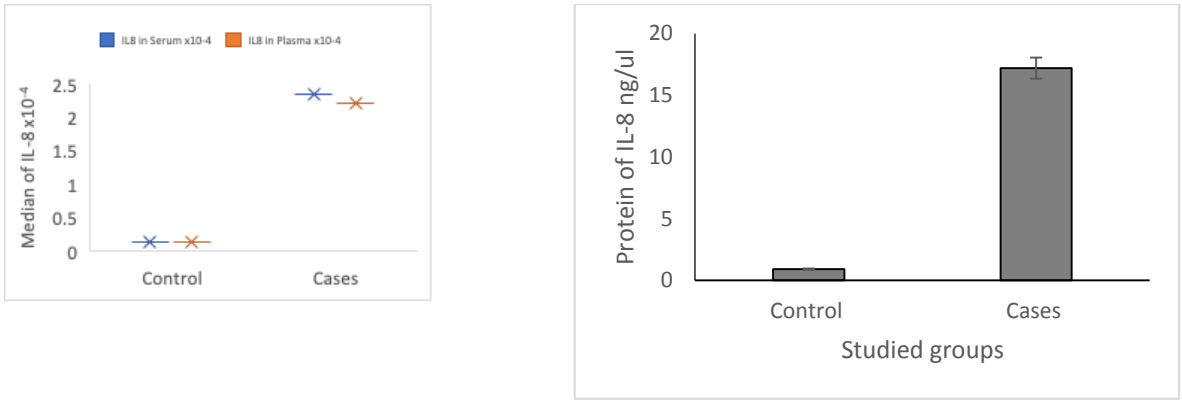

Figure 1: (A): Expression level of IL-8 in the studied groups. (B): Protein level of IL-8 by ELISA.

Diagnostic performance of IL-8

Receiver Operating Curve (ROC) was established to show the diagnostic performance of IL-8 regarding the studied groups. A highly significant difference was observed between the infected patients and the healthy individuals regarding IL-8 levels refers to a good diagnostic performance for IL-8.

it was found that, IL-8 at the cutoff point of >5.85, with sensitivity of 100.0%, specificity of 100.0%, with an area under curve (AUC)= 1, S.E= 0 and 95% C. I =(1.0-1.0), P <0.0001 (Table 3, Fig. 2).

Table 3: Diagnostic performance for the studied genes in the studied samples.

	Cutoff point	Sn.	Sp.	AUC	S. E	Asymptotic 95% C. I		P. value
						Lower Bound	Upper Bound	
IL-8 protein ng/µl	>5.85	100.0	100.0	1.0	0.0	1.0	1.0	<0.0001**

Sn: Sensitivity, Sp: Specificity, AUC Area under curve and C.I: 95% Confidence Interval.
* P value <0.05 is significant, ** P value <0.01 is highly significant.

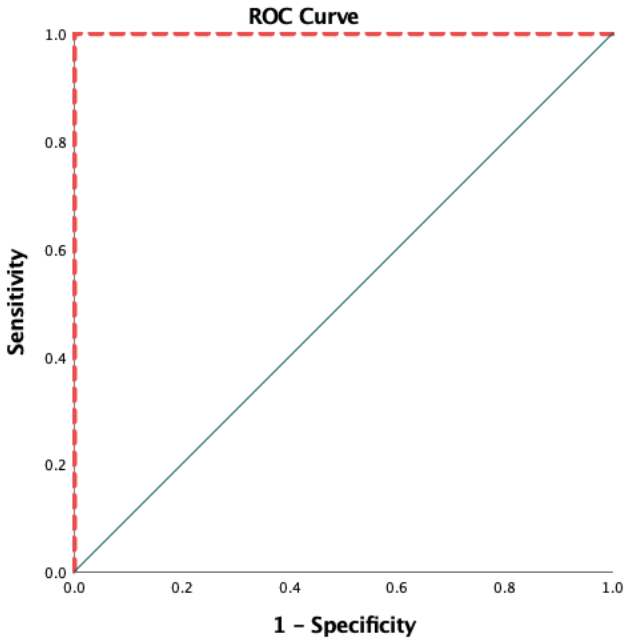

Figure 2: ROC Curve of the IL-8.

The associations between the infection severity and patient outcomes

ANOVA test and paired X^2 test through Linear-by-Linear association were used to analyze the associations between the infection severity and patient outcomes, a highly significant difference was observed between mild, moderate and severely infected patient groups with P<0.001 in terms of age as older patients were classified as severely infected with Mean± SD of 55.8±7.9 years and younger patients were categorized as mild infection with mean age of 40.0±9.4 and patients who were moderately infected had mean age between the mild and severe infection and their mean ages were 52.0±10.1 years.

In general, females had a higher percentage of participation (71.4%) than males (28.6%), and accordingly, regarding their classification with the severity of the infection, a highly significant difference was observed between the classified groups in terms of sex, P<0.001.

Concerning the C-reactive protein, it was observed that its levels increase with the disease severity. Highly significant difference was observed between groups regarding decreased SpO2%, increase respiratory rate and the radiological finding of pneumonia with P<0.001. Blood platelets, total leukocyte count, lymph%, alanine transaminase (ALT), Creatinine and lactate dehydrogenase (LDH), all of these parameters increased with the disease severity P<0.001. Patients who had symptoms of bonyaches and headache were classified as mild and moderate infection, while severe infected patients had the symptoms of cough, dyspnea and fever with significant difference between the 3 groups P<0.001. For patients who admitted to intensive care unit (ICU) and patients outcome, the number of patients who admitted to ICU and dead patients increased with the disease severity P<0.001. Kruskal Wallis test was used to analyze the association between the IL-8 levels in serum and plasma and the disease severity, the results showed that the IL-8 levels increases significantly with the development of the disease severity, similarly, with the regard to the IL-8 protein level that was increased with disease severity, P<0.001 (Table 4).

Table 4: The association between the demographics and baseline laboratory characteristics of patients and disease severity.

		Mild N=40	Moderate N=30	Severe N=70	P. value
Age		40.0±9.4	52.0±10.1	55.8±7.9	<0.001**
Sex	Female	40(100.0%)	10(33.3%)	50(71.4%)	<0.001
	Male	0(0.0%)	20(66.7%)	20(28.6%)	
C-reactive protein	Negative	0(0.0%)	0(0.0%)	0(0.0%)	-

	Positive	40(100.0%)	30(100.0%)	70(100.0%)	
Diabetes	Negative	30(75.0%)	20(66.7%)	40(57.1%)	0.163
	Positive	10(25.0%)	10(33.3%)	30(42.9%)	
Hypertension	Negative	20(50.0%)	20(66.7%)	40(57.1%)	0.378
	Positive	20(50.0%)	10(33.3%)	30(42.9%)	
SpO2%		85.3±11.0	73.8±8.4	70.0±4.3	<0.001**
Respiratory Rate (BPM)		25.5±3.7	27.0±3.6	28.6±2.7	<0.001**
Radiological Finding	Normal	10(25.0%)	0(0.0%)	0(0.0%)	<0.001**
	Pneumonia	30(75.0%)	30(100.0%)	70(100.0%)	
Hemoglobin		12.5±1.3	11.6±1.7	12.3±1.9	0.097
Platelet		363.6±93.1	214.0±54.7	121.8±26.5	<0.001**
Total Leukocyte Count		4.7±0.9	5.6±0.6	8.4±3.7	<0.001**
Lymph %		21.5±15.6	9.0±2.2	27.0±11.5	<0.001**
Alanine Transaminase		37.0±4.9	36.3±1.9	49.7±18.4	<0.001**
Creatinine		0.8±0.1	0.9±0.1	1.1±0.2	<0.001**
Lactate dehydrogenase		307.5±124.0	209.7±68.7	556.9±184.6	<0.001**
Symptoms	No symptoms	10(25.0%)	0(0.0%)	0(0.0%)	<0.001**
	Bonyaches, headache	20(50.0%)	30(100.0%)	10(14.3%)	
	Cough, dyspnea, fever	10(25.0%)	0(0.0%)	60(85.7%)	
ICU	No	30(75.0%)	20(66.7%)	10(14.3%)	<0.001**
	Yes	10(25.0%)	10(33.3%)	60(85.7%)	
Outcome	Living	30(75.0%)	30(100.0%)	10(14.3%)	<0.001**
	Dead	10(25.0%)	0(0.0%)	60(85.7%)	
IL-8 in Serum $x10^{-4}$		2.3(1.2- 2.4)	2.6(1.4- 2.1)	3.8(1.4- 7.3)	<0.001**
IL-8 in Plasma $x10^{-4}$		2.2(1.1- 12.7)	2.6(1.6- 3.0)	3.1(3.0- 6.3)	<0.001**
IL-8 Protein ng/µl		14.3±3.7	14.5±4.3	17.0±5.2	0.014*

Age, SpO2%, Respiratory Rate (BPM), Hemoglobin, Platelet, Total Leukocyte Count, LYMPH%, Alanine Transaminase, Creatinine, Lactate dehydrogenase, and Protein of IL-8 ng/µl are represented as Mean±SD; the data were analyzed by ANOVA test. While Sex, C-reactive protein, Diabetes, Hypertension, Radiological Finding, Symptoms, ICU, and Outcome are represented as frequency F and percent (%); the data were analyzed by paired X^2 test through Linear-by-Linear Association. IL-8 in Serum, and Plasma are represented as Median and interquartile range (IQR); the data were analyzed by Kruskal Wallis test.
* P value <0.05 is significant, ** P value <0.01 is highly significant.

Association between IL-8 levels and patients admission to the ICU

Eighty patients were admitted to the ICU, a significant difference was observed between patients who admitted to the ICU and those who were not regarding the expression level of IL-8 in serum and plasma which represented as medians and IQR (25-75%), OR (95% C.I) =1.07(0.57- 3.08), 1.942 (0.773 – 2.993), P calculated depend on log linear regression analysis 0.03 for serum and 1.77(0.51- 3.19) with OR (95% C.I)=1.843 (0.621 – 2.929), and P<0.001 for plasma. Moreover, for the association between the IL-8 protein level and patients who admitted to the ICU, a significant difference was detected between patients who admitted and those who were not admitted with Mean± SD =16.9±5.2, OR (95% C.I) = 1.863(0.797- 2.934) and P<0.001, the previous results revealed that the IL-8 levels could be used as a prognostic marker for prediction of ICU patients admission (Table 5).

Table 5: The association between the IL-8 expression levels with ICU admission in COVID-19 patients.

	ICU admission			Risk assessment	
	No	Yes	P. value	OR (95% C.I)	P. value
IL8 in Serum $x10^{-4}$	0.86(0.52- 2.96)	1.07(0.57- 3.08)	0.035*	1.942 (0.773 – 2.993)	0.03*
IL8 in Plasma $x10^{-4}$	0.64(0.43- 1.09)	1.77(0.51- 3.19)	<0.001**	1.843 (0.621 – 2.929)	<0.001**
IL-8 Protein ng/µl	13.9±3.7	16.9±5.2	<0.001**	1.863(0.797- 2.934)	<0.001**

IL-8 in Serum and plasma are represented as Median and interquartile range (IQR); the data were analyzed by Kruskal Wallis test. While IL-8 Protein is represented as Mean±SD; the data were analyzed by t test.
OR; Odd Ratio, C.I; Confidence Interval, P value calculated depend on log linear regression analysis.
* P value <0.05 is significant, ** P value <0.01 is highly significant.

Association between IL-8 levels and risk of death.

Regarding patients outcome, statistical analysis revealed that no significant difference was observed between living and dead patients concerning the expression levels of IL-8 in serum and plasma and also, the IL-8 protein level, P value was calculated depends on log linear regression analysis, these results reveal that, IL-8 is a risk factor for COVID-19 patients mortality but does not reach the significance level, this means that it could not predict the patient survival (Table 6).

Table 6: The association between the IL-8 expression with Outcome in COVID-19 patients.

	Outcome			Risk assessment	
	Living	Dead	P. value	OR (95% C.I)	P. value
IL8 Serum $x10^{-4}$	1.13(0.52- 3.13)	0.79(0.55- 1.38)	0.5	0.98(0.83 – 1.021)	0.7
IL8 Plasma $x10^{-4}$	0.68(0.43- 2.58)	0.98(0.46- 3.27)	0.1	0.78(0.67 - 0.98)	0.3
Protein of IL-8 ng/µl	15.7±5.2	16.0±5.5	0.3	0.89(0.83 - 1.21)	0.5

IL-8 in Serum and plasma are represented as Median and interquartile range (IQR); the data were analyzed by Kruskal Wallis test. While IL-8 Protein is represented as Mean±SD; the data were analyzed by t test.
OR; Odd Ratio, C.I; Confidence Interval, P value calculated depend on log linear regression analysis.
* P value <0.05 is significant, ** P value <0.01 is highly significant.

DISCUSSION

We followed 140 patients hospitalized for confirmed COVID-19 at NHMRI from the day of hospitalization to the day of discharge or death.
We evaluated the expression and protein levels of of the inflammatory cytokine IL-8 in serum and plasma of COVID-19 patients upon hospital admission and correlated these results with clinical and laboratory markers of disease severity to understand its role on COVID-19 disease course and outcome.
COVID-19 was diagnosed based on the presence of clinical symptoms and SARS-CoV-2 nucleic acid using a real-time reverse transcription polymerase chain reaction assay with nasal and pharyngeal swab specimens, blood samples were collected at different time points when laboratory biomarkers were requested by physicians to monitor disease progression.
Hematological parameters were determined for all patients within the first 2 days of hospital admission using SYSMEX XT-1800i automated hematology analyzers (Sysmex, Kobe, Japan).

Our results revealed an increased expression of the IL-8 in serum and plasma of COVID-19 patients compared to healthy volunteers with highly significant level rises to the use of IL-8 as a powerful diagnostic marker. These findings are compatible with those published by **Tiwari et al, 2023,** who reported that the baseline levels of IL-8 significantly increased in COVID-19 patients ($p < 0.05$). **Li et al, 2021** supported our results and reported that serum IL-8 showed a gradual increase in infected patients. Our results go parallel with **Gomes et al, 2023** 's results who stated that COVID-19 patients had significantly higher levels of IL-8 than the unexposed group.
The AUC for ROC curve was used for early prediction of the severity of COVID-19. It shows an excellent predictability of coronavirus progression, the cutoff point of >5.85, with sensitivity of 100.0%, specificity of 100.0%, with an area under curve (AUC)= 1, and 95% C. I (1.0-1.0) (P <0.0001). These results are slightly different from the results reported by **Hassan et al, 2023,** who stated that IL-8 showed a good predictability for disease severity with AUC of 0.655 ± 0.077 (95% CI)= 0.583–0.884, $P < 0.044$. The cut-off value for IL-8 was 17 pg/ml with an accuracy of 74.5%, sensitivity of 70%, and specificity of 79%.

The pathogenesis of human coronavirus is still not completely understood, to clarify this issue, We

assessed whether coronavirus was associated with comorbidities or not. We found that coronavirus infection was significantly associated with increased C-reactive protein values with highest levels in patients with severe infection, increased glucose levels and blood pressure but this increase does not reach significance level. A significant association was found between coronavirus and decreased saturation of peripheral oxygen, increased respiratory rate, increased pneumonia that causes the lungs' air sacs (alveoli) to become inflamed and fill up with fluid or pus, and so one or both lungs affected. It was observed that the lowest level of SpO2, increased BPM and pneumonia were associated with severely infected patients. **In 2020, Mejía et al.,** announced that low blood oxygen level and fast, shallow breathing were associated with significantly elevated death rates in a study of hospitalized COVID-19 patients.

Total leukocyte and lymphocyte count were significantly associated with coronavirus, a gradual increase was observed reaches highest count in patients with severe infection, this means that WBC variations could contribute in increasing clinical severity of the disease, while hemoglobin level was not significantly different between the groups. Our results of hematological parameters are similarto those of **Mahmood et al., 2023** who reported that total leukocyte and lymphocyte count were significantly more in the severe group. As for the blood platelet count, a gradual significant decrease associated with disease severity was detected, this decrease in platelet count can be attributed to platelet apoptosis and the incorporation of platelets into microthrombi (peripheral consumption) and severe thrombotic events, **Rohlfing et al., 2021** published the same findings as they stated that platelet count differs between mild and serious infections, and he added that patients with mild symptoms have a slightly increased platelet count, whereas thrombocytopenia is a hallmark of severe COVID-19 infections.

In line with other studies, our study showed that blood levels of ALT, creatinine and LDH increase significantly with infection severity. The ALT level in severely infected patients was greater than in mild and moderate patients, greater ALT level may be an indicator of liver injury. In addition, creatinine was significantly associated with severe COVID-19, we therefore assumed that altered serum creatinine levels with kidney dysfunction were significantly associated with acute kidney injury.

LDH is a hydrogen transfer enzyme present in the cytoplasm of almost all cells. Upon tissue damage, LDH is released into the bloodstream. Thus, serum LDH elevation generally indicates tissue damage and helps to predict the severity of COVID-19 **(Ergenc et al., 2023).**

Kojima et al., 2023 had a similar results to ours, they reported that laboratory data at admission as ALT, creatinine and LDH were included as covariates in the logistic regression model with the outcome of severe COVID-19.

Respiratory symptoms on admission were more frequently higher in the severe group, whereas Bonyaches and headache were more common in the mild and moderate groups.

Paired X^2 test through Linear-by-Linear association was used to analyze the association between the ICU admitted patients and the mortality rate with the disease severity, the results revealed a significant association with $P<0.001$ refers to a high frequency of the admitted ICU patients and died patients were of severely infected category. Our results are consistent with those of **Alammari et al., 2023** who reported that the mortality rate increased among patients with severe infection.

We found that IL-8 level was significantly increases gradually until it reaches the highest value in patients with severe coronavirus, this result of IL-8 level is supported by **Abou Hassan et al., 2023** who stated that Il-8 levels were significantly increased in severe compared to nonsevere cases and also by **Alabdullatif etal., 2023** who reported that IL-8 were significantly higher in critical than non-critical patients.

Our findings of the association between the increased IL-8 levels with patients admission to the ICU provide an important information to predict the disease exacerbation and the high level of IL-8 considered a risk factor for ICU admission. These findings are not compatible with those of **Samsami et al., 2022** who statistically proved that no significant difference in the expression level of IL-8 between ICU admitted and non-ICU admitted patients. Our finding of IL-8 as a correlating factor for inhospital death in COVID-19 patients may provide a new way to evaluate and treat COVID-19 pneumonia. IL-8 was exactly a risk factor for death for COVID-19 patients, but with non-significant association. These findings are not consistent with those published by **Li et al., 2021** who concluded that IL-8 levels were associated with in-hospital death in severe/critical COVID-19 patients.

These results show a similar trend to previous studies and indicate that the population we used for our study is not unique.

In a conclusion, we indicated that IL-8 exhibited an early elevation response to coronavirus infection supporting its role as an early predictor of the disease and that it might be a sensitive and specific prognostic indicator for infection and disease severity and clinical outcomes such as respiratory failure and ICU dmission.

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