Section A -Research paper

Interleukin 6 Detection in COVID-19 Infected Patients

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Abstract:

Background: Study of interleukin 6 could help to understand the pathophysiology of COVID-19 infection, development of novel treatment protocols and reducing rate of mortality among COVID-19 infected patients. **Aim:** To correlate interleukin 6 with the inflammatory markers and clinical status of COVID-19 infected patients. **Patients and methods:** This prospective study was performed at Zagazig University Isolation Hospitals and clinical pathology department, Zagazig, Egypt on 80 persons who were enrolled in this study; they were divided into group I: 40 COVID-19 patients with pneumonia and group II: 40 COVID-19 patients without pneumonia. Interleukin 6 was measured in both groups. **Results:** There was significant increase in interleukin 6 in patients with pneumonia than those without pneumonia. **Conclusion:** High levels of IL-6 are associated with a greater risk of pneumonia in COVID-19 patients.

Keywords: IL-6, Cytokines, COVID-19.

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Introduction:

Coronavirus disease 2019 (COVID-19) is a viral infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel β-coronavirus firstly identified during an outbreak of respiratory illness cases in Wuhan City, China(**1).**

Some subjects developed various fatal complications, including organ failure, septic shock, severe pneumonia, and acute respiratory distress syndrome (ARDS), which suggested that dysregulated immune responses involved in the development of SARS-CoV-2 infection (**2).**

Cytokine release syndrome are also essential features in patients with severe SARS-CoV-2 infection**.** Previous studies have shown that elevated levels of proinflammatory cytokines, such as TNF-α, IL-6, and IL-8, are associated with severe pulmonary injury and adverse outcomes of SARS and MERS infections (**3).**

Patients and Methods:

This prospective study was performed at Zagazig University Isolation Hospitals and clinical pathology department, Zagazig, Egypt during the period from July 2021 to July 2022. 80 persons were enrolled in this study; they were divided into two groups:

- **Group I**: **40 COVID-19 patients with pneumonia**; having pneumonia manifestation through image results **(4).**
- **Group II**: **40 COVID-19 patients without pneumonia**; did not have pneumonia manifestation through image results

Patients with clinical presentations and radiological findings suggesting COVID-19 and laboratory confirmed by positive SARS-CoV-2 RT-PCR testing of respiratory sample (nasopharyngeal swab) were included in the study. Patients suffering from autoimmune diseases, cancers or end stage diseases and patients having

any other non-COVID-19 respiratory symptoms were excluded from the study.

All patients were subjected to full history taking, clinical examination and laboratory investigations: including complete blood count (CBC), infection related biomarker C-reactive protein (CRP), LDH, ESR, D dimer, ferritin, IL-6 and computed tomography (CT) of chest.

Blood sample collection:

Under complete standard aseptic technique, 10 ml blood were withdrawn from each participant at time of admission and before receiving any treatment in 2 different tubes; 3ml blood in EDTA tube for CBC and flowcytometric analysis, 2 ml for ESR , 5 ml blood in serum separator tube (SST) for liver enzymes, CRP, LDH, ferritin.

The two blood sample tubes were transferred immediately to Zagazig University Hospitals laboratory for further steps.

1. CBC was analyzed by Sysmex-XN-330.
2. CRP was done by Cobas 6000, c501 module by turbidimetry, Roche Diagnostic, Germany.
3. ESR (wester green method): **(5).**
4. LDH and ferritin was done by Roche Integra 400 plus.

Detection of human IL-6 by ELISA Technique:

Procedure: Five (5) ml blood samples were collected then centrifuged and serum samples were stored at -80°C. Stored serum was centrifuged for 20 minutes at the speed of 2000- 3000 r.p.m. and supernatant was removed. Serum samples were tested for IL-6 using human IL-6 ELISA kit (bioassay technology lab, Cat. No: E0090Hu, China).

Results:

Table (1): Complete blood picture of the studied patients

Findings	Covid 19 Patients (n=80)		t	P value
	Pneumonic	Non Pneumonic		
Hb	11.5 ± 2.4 (5.8-15.6)	10.9 ± 2.5 (6.8-15.9)	0.9	0.3
WBCS	10.8 ± 6.2 (2.9-26.6)	8.7 ± 4.6 (2.9-19.3)	1.6	0.1
Platelets	214.5±105.7 (72-490)	77.5 ± 14.8 (71-413)	0.73	0.46
Lymphocytes	1.8 ± 0.4 (0.4-4.5)	1.9 ± 0.5 (0.7-4.4)	7.3	<0.001*
Neutrophils	8.3 ± 1.6 (4.6-10.4)	6.7 ± 1.2 (3.7-8.4)	0.19	0.84

There was significant decrease in lymphocytes in pneumonic patients than non pneumonic patients while there was no significant difference between both groups regarding to other CBC data (table 1).

Table (2): LDH, CRP, ESR, D Dimer and ferritin of the studied patients

Findings	Covid 19 Patients (n=80)		t	P value
	Pneumonic	Non Pneumonic		
LDH	450.9±110.3 (271-803)	363.1 ±90.2 (240-530)	3.9	<0.001*
CRP	31.9 ± 21.8 (11-96)	16.8 ± 6.7 (5-34)	4.1	<0.001*
ESR	34.1 ± 19.5 (8-85)	19.3 ± 11.2 (5-46)	3.7	<0.001*
Dimer	755 ± 460 (399-2450)	514.1±75.2 (383-618)	3.2	0.002*
Ferritin	939 ± 590.2 (89-2420)	459.9±199.9 (88-752)	4.8	<0.001*

There was significant increase in LDH, CRP, ESR, D-Dimer and ferritin in pneumonic group than non-pneumonic group (Table 2).

Table (3): Interleukin 6 among patients with and without pneumonia.

Findings	Covid 19 Patients (n=80)	t	P value

	Pneumonic	Non Pneumonic		
IL 6	37.08±24.8	21.2 ± 7.3	3.8	<0.001*

There was significant increase in IL-6 in patients with pneumonia than those without pneumonia (Table 3).

Table (4): Pearson correlation between IL6 and other parameters among the studied patients

Findings	Covid 19 Patients (n=80)	
	r	P
Age	0.1	0.35
Oxygen Saturation	-0.78	<0.001*
Respiratory Rate	0.52	<0.001*
Temperature	0.25	0.02*
Hb	0.2	0.06
WBCS	0.17	0.12
Lymphocytes	-0.27	0.01*
LDH	0.79	<0.001*
CRP	0.94	<0.001*
ESR	0.75	<0.001*
Dimer	0.95	<0.001*
Ferritin	0.91	<0.001*

There was positive correlation between IL6 and respiratory rate, temperature, LDH, CRP, ESR, D dimer, ferritin and a negative correlation with oxygen saturation and lymphocytes while there was no significant difference regarding the other parameters (Table 4).

Table (5): Validity of IL6 as diagnostic markers to predict pneumonia.

Variable	AUC	Std. Error	P	95% Confidence Interval		Cutoff	Sensitivity	Specificity
				Lower Bound	Upper Bound			
IL6	0.782	0.052	<0.001*	0.679	0.884	>27.9	70%	84%

IL-6 was valid as a diagnostic marker in prediction of pneumonia (Table 5).

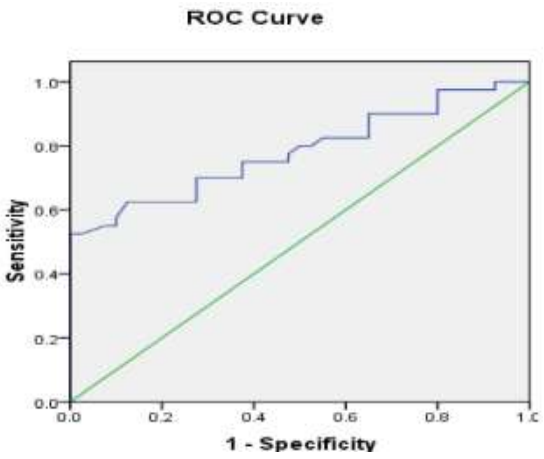

Figure (2): ROC curve of IL-6 to predict pneumonia.

Discussion:

In agreement with the present study, **Wan et al. (6)** demonstrated that a total of 102 patients with COVID-19 with a mean (SD; range) age of 43.05 (13.12; 15–82) years in the no pneumonic group and 21 patients with a mean (SD; range) age of 61.29 (15.55; 34–79) years in the pneumonic group were enrolled in the study with significant difference regarding to age. Morbidities were more common in the pneumonic group than in the non-pneumonic group.

Also, consistent with a previous report they also found that older patients, particularly those with underlying comorbidities, are more likely to develop severity of the disease and occurrence of pneumonia, which suggested that SARS-CoV-2 is more likely to infect elderly individuals with chronic comorbidities due to weaker immune functions **(7). Qian et al. (8)** who conducted a study on 99 patients that showed a median of age in patients with non-severe and severe (complicated with pneumonia) COVID-19 were 37 (30–49) years and 67 (53–74) years, respectively.

The current findings were also concordant with **Zhang and colleagues, (9)** who conducted a study on 90 COVID-19 patients and found the proportion of men in the pneumonia patients' group (65% men) were not significantly different from the non-pneumonia patients' group (51.4%).

In the current study, there was significant increase in respiratory rate, heart rate, SBP, DBP and temperature in pneumonic group than non-pneumonic group while there was significant decrease in oxygen saturation in pneumonic group than non-pneumonic group.

This came in agreement with **Du et al. (10)** who found that the increase in heart rate and respiratory rate, SBP, DBP as well as the decrease in SpO2 are all independent risk factors for pneumonia and death in patients with COVID-19.

In the present study, there was significant decrease in lymphocytes in pneumonic patients than non-pneumonic patients while there was no significant difference between both groups regarding to other CBC data.

This came in agreement with **Wan et al. (6)** who found that there was no significant difference between both groups regarding to Hb and WBCs.

In addition, **Qin et al. (11)** assessed the absolute lymphocytic count among 452 COVID-19 cases and found that severe cases with pneumonia had lower absolute lymphocytes count than non-severe cases (median 0.8 vs 1.0 × 103 cells/uL).

In the present study, there was significant increase in LDH, CRP and ESR in pneumonic group than non-pneumonic group.

In agreement with the present study, **de Sanctis et al. (12)** reported that increased C-reactive protein, high serum lactate dehydrogenase and erythrocyte sedimentation rate were the most common laboratory findings in the majority of COVID-19 patients with pneumonia.

D-Dimer and ferritin were significantly higher in pneumonic group than non-pneumonic group.

In agreement with the current study, **Myronenko et al. (13)** found that the level of ferritin was higher in COVID-19 pneumonia patients than those without.

In the current study, there was significant increase in IL-6 in patients with pneumonia than those without pneumonia.

In agreement with the present study, **Wan et al. (6)** demonstrated that 57 (55.88%) patients had IL-6 values of zero and 14 (13.73%) within normal values, 31 (30.39%) were higher than normal. Significant differences were observed in IL-6, between the two groups ($P < 0.05$). Also, **Han et al. (14)** reported that IL-6 level was statistically different among the pneumonia and non-pneumonia patients**.**

In addition, **Aziz et al. (15)** conducted a meta-analysis of a total of nine studies with laboratory-confirmed 1426 patients. A comparison of mean serum IL-6 for severe COVID-19 with pneumonia and non-severe COVID-19 patients revealed a significantly higher IL-6 levels in severe patients [mean 56.8 (41.4-72.3 pg/mL)] as compared with the non-severe patients [mean 17.3 pg/mL (13.5-21.1 pg/mL)].

Recent studies indicated that cytokine storm was implicated in SARS-CoV-2 infection and served as a cause for deleterious consequence and occurrence of pneumonia (**16, 17).**

This suggests that IL-6 can be used to predict the transition from no pneumonia to pneumonia in COVID 19 patients. If so, this would be consistent with the concept of 'cytokine storm' presented by **Liu et al (18).**

In the present study, there was positive correlation between IL6 , LDH and D dimer .

In agreement with the current study, **Liao et al. (19)** demonstrated that IL-6 was positively correlated with LDH. Also observed strong correlation between IL-6 and D-dimer ($P < 0.001$).

In the present study, regarding validity of IL6 as diagnostic markers to predict pneumonia, IL-6 was valid as a diagnostic marker in prediction of pneumonia. IL-6 showed sensitivity 70%, specificity 84% and AUC 0.78.

Liao et al. (19) demonstrated that the ROC curve was 0.774 for IL-6 ($P < 0.001$) and the sensitivity and specificity to predict the pneumonia in COVID-19 were 76.7% and 76.6%, respectively.

Conclusion:

Our study demonstrates that high levels of IL-6 are associated with a greater risk of pneumonia in COVID-19 patients. Therefore, surveillance of IL-6 is helpful in the early screening and timely intervention for severe COVID-19 patients with pneumonia.

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