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Original article

Evaluation of Interleukin-4 in Corona Virus Disease-19 Patients

Samir M. Kabil[a], Naglaa El-Toukhy[a], Amal A. Mohamed[b], Saad E. Mehawesh[a]

[a] Department of Hepatology, Gastroenterology and Infectious Diseases, Faculty of Medicine Benha University, Egypt.

[b] Department of Biochemistry, National Hepatology and Tropical Medicine Research Institute – Cairo, Egypt.

Corresponding to: Saad E. Mehawesh, Department of Hepatology, Gastroenterology and Infectious Diseases, Faculty of Medicine Benha University, Egypt.

Email:

Saadelbelasy9199@gmail.com

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Abstract

Background: Cytokines are a group of polypeptide signaling molecules responsible for regulating a large number of biological processes via cell surface receptors. **Aim and objectives:** To study the interleukin-4 levels in patients with Coronavirus-19 Disease and its possible relations with the disease severity. **Patients and Methods:** This cross-sectional study was conducted on 200 subjects in National Hepatology and Tropical Medicine Research Institute. Patients were divided into: Cases (150 subdivided into group A: mild group, B: moderate group, C: severe group) and control (50 group). Full medical history, physical examination, laboratory tests (IL-4, CRP, Procalcitonin, Serum ferritin, D-Dimer, LDH, CBC, renal and liver function tests), CT chest and COVID-19 PCR were done for all groups. **Results:** There was high statistically significant increase in IL4 in group C when compared with those of group A, Group B and group D. Serum IL-4 is good to discriminate between group B and group C with AUC of 0.706, sensitivity of 58%, specificity of 84%, PPV of 78.4%, NPV of 66.7% and p-value < 0.001. By multivariate analysis, IL-4 is a predictor for COVID-19 severity. Also, there was high statistically significant positive correlation between IL-4 and severity score. **Conclusion:** Interleukin-4 (IL-4) levels may be linked with the disease severity in patients with Coronavirus disease-19 (COVID-19) and can be used as an effective indicator to predict the severity of COVID-19 disease.

Key words: Interleukin-4; Corona Virus; COVID-19.

Introduction

The coronavirus disease 2019 (COVID-19) causes a large number of fatal cases worldwide. It is developed as a result of infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) **(1)**. This virus is a novel strain related to a bat coronavirus (RaTG13) that has never been isolated in humans **(2)**.

It first appeared in Wuhan city, China in early December 2019, and owing to its

tremendous rapid expansion and fatal cases, the World Health Organization (WHO) considered it 'Public Health Emergency of International Concern' **(3, 4)**.

On February 14, 2020, Egypt announced its first COVID-19 case **(5)**.

Cytokines are a group of polypeptide signaling molecules responsible for regulating a large number of biological processes via cell surface receptors. Key cytokines include those involved in adaptive immunity (e.g., IL-2 and IL-4), pro-inflammatory cytokines and interleukins (ILs) (e.g., interferon (IFN)-I, -II, and -III; IL-1, IL-6, and IL-17; and TNF-α); and anti-inflammatory cytokines (e.g., IL-10). In response to stress-generating internal processes (e.g., cancer or microbial infection), host cells secrete cytokines with a highly important role in cell metabolism reprogramming as a defensive response **(6)**.

The aim of the work was to study the interleukin-4 levels in patients with Coronavirus-19 Disease and its possible relations with the disease severity.

Patients and methods

This case/control cross-sectional study was conducted in National Hepatology and Tropical Medicine Research Institute (NHTMRI). This study was conducted on 200 subjects.

Type of the study: cross sectional study

Sample collection: This study was conducted on 150 cases attending the outpatient clinic and/or inpatients of Benha University Hospital and Hepatology and Tropical Medicine Research Institute (NHTMRI) in a period from August 2022 till March 2023. They were matched with 50 apparently healthy subjects served as a control group.

Inclusion criteria: Patient acceptance, Age ≥ 18 years old and Confirmed patient with COVID-19 by deep nasopharyngeal swab PCR and Chest computed tomography (CT).

Exclusion criteria: patients were not diagnosed as COVID-19 cases or pediatric and pregnant COVID-19 cases, Patients with malignancy, HIV-Abs positive patients, Patients with allergic diseases as acute bronchial asthma, atopic dermatitis and systemic anaphylaxis and Patients who had other comorbidities in the form of chronic liver diseases, cardiac diseases, chronic kidney diseases and chest diseases.

After obtaining their informed consent, enrolled subjects were categorized into four groups according to MOHP protocol 2021: Group A: 50 patients whom are classified as mild, Group B: 50 patients whom are classified as moderate cases, Group C: 50 patients whom are classified as severe and critical ill cases and Group D (Control group): 50 control apparently healthy individuals.

Ethical approval: All enrolled participants gave their agreement in a written consent as well as Benha Faculty of Medicine's research ethics committee authorized the project {M.S.23.9.2022}.

Methods

Both patients and controls were subjected to: Full medical history, General examination, Local chest examination and Laboratory investigations. Complete blood count (CBC), Kidney function tests, Liver profile, Inflammation biomarkers, D-dimer, Lactate dehydrogenase (LDH),

Serum procalcitonin, Human Interleukin-4 (IL-4) ELISA Kit and COVID-19 PCR swabs.

The Method of Patients selection: Random

Scoring of the Covid-19 infection:

Scoring of the Covid-19 severity according to protocol for COVID-19 patients issued by Egyptian Ministry of Health and Population (MOHP) (2021): Mild Case, Moderate Case, Severe Case and Critically-ill Case.

Statistical analysis

Data were analyzed using Statistics Package for Social Sciences (SPSS) version 25. Normality tests (Kolmogorov-Smirnov & Shapiro-Wilk test) were performed and data of (age, gender, smoking, body mass index, systolic blood pressure, diastolic blood pressure, oxygen saturation, hemoglobin, white blood cells, hematocrit, platelets, neutrophils, lymphocytes, ferritin, D dimer, C-reactive protein, ferritin, lactate dehydrogenase, 1st hour erythrocyte sedimentation rate, 2nd hour erythrocyte sedimentation rate, aspartate aminotransferase, alanine aminotransferase, urea, creatinine, international normalized ratio, procalcitonin and Human Interleukin-4) were abnormally distributed. Qualitative data were expressed as frequency and percentage. Continuous quantitative data were expressed median and Interquartile range (Median with IQR). The following tests were done: Chi-square test, Pearson's correlation coefficient (r), Post Hoc test, ROC curve which was used to used to detect cutoff value, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) and probability (P-value) when < 0.05 was considered significant, < 0.001 was considered as highly significant and > 0.05 was considered insignificant.

Results

There was no statistically significant difference between the studied groups as regard gender and age p= 0.67, 0.2 respectively (Table1). Smoking was higher in group A and group C than group B and group D with statistically significant difference p < 0.001 (Table 1). There was high statistically significant increase in serum ferritin and CRP in group C, when compared with those of group a, group B and group D p < 0.001 (Table 2). High statistically significant increase in D-Dimer and in group B and group C when compared with those of group A and group D p < 0.001 (Table 2). High statistically significant increase in serum Procalcitonin in group C when compared with those of group A, B and D (Table 2). High statistically significant increase in serum IL4 in group C when compared with those of group A, B and D (Table 2). Using roc curve, it was shown that: Serum IL-4 is good to discriminate between moderate covid group (group B) and severe COVID-19 group (group C) p < 0.001 (Table 3) (Figure 1). There was High Statistically significant positive correlation between IL-4 and severity score p < 0.001 (Table 4) (Figure 2). Using Multivariate logistic regression analysis, the following factors were predictive for severe COVID-19: Headache, Abdominal Pain, vomiting, dry cough, oxygen saturation, white blood cells count, Neutrophils, Lymphocytes, Ferritin, D Dimer, Procalcitonin, C-reactive protein, Glutamic pyruvic transaminase, Glutamic oxaloacetic transaminase, Creatinine and Interleukin-4 (Table 5).

Table (1): Comparison of all studied groups as regard demographic data.

Variables		Group A (n = 50)	Group B (n = 50)	Group C (n = 50)	Group D (n = 50)	P-value
Gender	**Female**	**21 42%**	**17 34%**	**20 40%**	**23 46%**	**0.67 NS**
	Male	**29 58%**	**33 66%**	**30 60%**	**27 54%**	
Age (years)	**Median**	**49.5**	**50**	**50**	**43**	**0.2 NS**
	IQR	**40 – 55**	**37 – 59**	**40 – 58**	**38 – 5**	
Smoking		**19 38%**	**3 6%**	**18 36%**	**7 14%**	**P < 0.001HS** **P1<0.001HS** **P2 = 0.8 NS** **P3= 0.006 S** **P4<0.001HS** **P5= 0.18 NS** **P6= 0.01 S**

P = statistically difference between all studied groups. HS: Highly significant. P1= statistically difference between group A and group B. NS: Non-significant. P2= statistically difference between group A and group C. S: Significant. P3= statistically difference between group A and group D. BMI: Body mass index. P4= statistically difference between group B and group C. P5= statistically difference between group B and group D. P6= statistically difference between group C and group D. BMI: Body mass index.

Table (2): Comparison of all studied groups as regard inflammatory markers.

Laboratory Test		Group A (n = 50)	Group B (n = 50)	Group C (n = 50)	Group D (n = 50)	P-value
Ferritin (Normal: 13-150 ng/ml)	**Median**	**123**	**85**	**300**	**125**	**P < 0.001 HS P1= 0.77 NS P2 < 0.001 HS P3= 0.78 NS P4 < 0.001 HS P5= 0.9 NS P < 0.001 HS**
	IQR	**103 - 137**	**60 – 193**	**170 - 500**	**88 - 145**	
D-Dimer (Normal: < 0.5 mg/l)	**Median**	**0.3**	**1.1**	**0.9**	**0.3**	**P < 0.001 HS P1 < 0.001 HS P2 < 0.001 HS P3= 0.08 NS P4= 0.005 S P5 < 0.001 HS P6 < 0.001 HS**
	IQR	**0.2 - 0.5**	**0.98 - 1.65**	**0.7 - 1.1**	**0.2 - 0.4**	
CRP (Normal: < 6 mg/dl)	**Median**	**5**	**12**	**15**	**5**	**P < 0.001 HS P1= 0.03 S P2 < 0.001 HS P3= 0.8 NS P4 < 0.001 HS P5= 0.01 S P6 < 0.001 HS**
	IQR	**4 - 7**	**10 – 13**	**9 - 36**	**4 - 6**	
Procalcitonin (Normal < 0.05 ng/ml)	**Median**	**0.2**	**0.5**	**4.6**	**0.3**	**P < 0.001 HS P1 < 0.001 HS P2 < 0.001 HS P3= 0.96 NS P4 < 0.001 HS P5 < 0.001 HS P6 < 0.001 HS**
	IQR	**0.2 - 0.3**	**0.2 - 2.8**	**0.4 - 5.7**	**0.2 - 0.3**	
IL-4 (Normal 0 – 2.2 pg/ml)	**Median**	**55**	**74**	**136**	**50**	**P < 0.001 HS P1= 0.8 NS P2 < 0.001 HS P3= 0.07 NS P4 < 0.001 HS P5 < 0.001 HS P6 < 0.001 HS**
	IQR	**45 - 80**	**45 – 90**	**67 – 167**	**43 – 64**	

HS: Highly significant. NS: Non-significant. S: Significant. CRP: C-reactive protein.

Table (3): Diagnostic performance of serum IL-4 in discrimination between patients with moderate covid (group B) and patients with severe covid (group C).

	Cut off	AUC	Sensitivity	Specificity	PPV	NPV	p-value
IL-4 (pg/ml)	**> 114**	**0.706**	**58%**	**84%**	**78.4%**	**66.7%**	**<0.001 HS**

PPV: Positive predictive value. AUC: Area under curve. NPV: Negative predictive value.

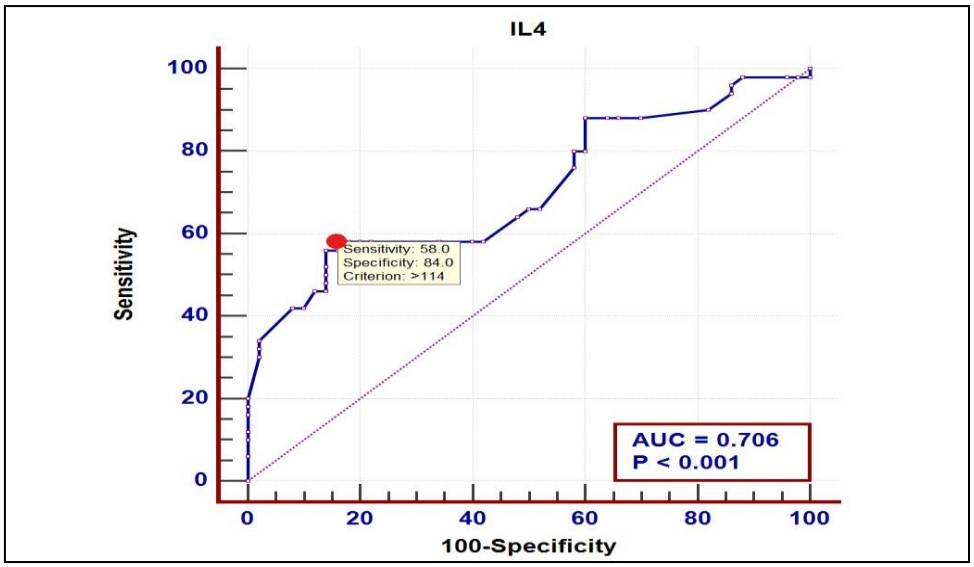

Figure (1): ROC curve between patients with moderate covid (group B) patients with severe covid (group B) as regard IL-4.

Table (4): Correlation study between serum IL-4 and severity score.

	IL4	
	R	**P-value**
severity score	**0.684**	**<0.001**

(r): Pearson correlation coefficient. P-value < 0.001: Highly significant.

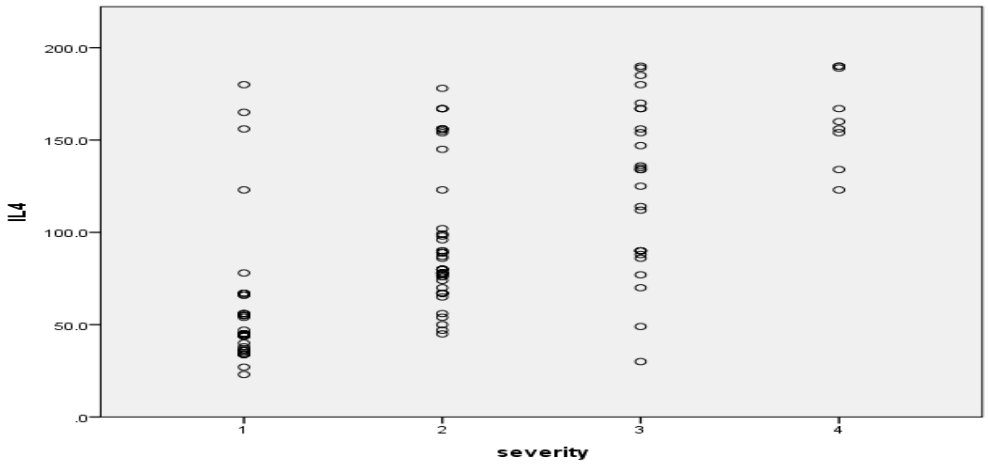

Figure (2): Description of correlation between IL4 and severity score.

Table (5): Multivariate logistic regression analysis for factors predictive of covid severity.

	B	SE	p-value	Odds		95% CI
Headache	**1.321**	**0.52**	**0.011**	**3.74**	**1.35**	**10.38**
Abdominal Pain	**3.748**	**1.051**	**0.000**	**42.43**	**5.4**	**333.1**
Vomiting	**2.937**	**1.077**	**0.006**	**18.86**	**2.29**	**155.53**
Dry cough	**-2.157**	**1.048**	**0.04**	**0.116**	**0.02**	**0.9**
Oxygen saturation	**-0.512**	**0.087**	**< 0.001**	**0.599**	**0.51**	**0.71**
WBC	**-0.19**	**0.09**	**0.032**	**0.825**	**0.69**	**0.98**
Neutrophil	**0.135**	**0.036**	**< 0.001**	**1.144**	**1.066**	**1.228**
Lymphocyte	**-0.114**	**0.031**	**< 0.001**	**0.89**	**0.84**	**0.95**
Ferritin	**0.012**	**0.002**	**< 0.001**	**1.012**	**1.007**	**1.016**
D Dimer	**0.596**	**0.285**	**0.037**	**1.814**	**1.037**	**3.174**
Procalcitonin	**0.517**	**0.091**	**< 0.001**	**1.677**	**1.403**	**2.005**
CRP	**0.137**	**0.029**	**< 0.001**	**1.146**	**1.08**	**1.21**
SGPT	**0.087**	**0.026**	**0.001**	**1.09**	**1.036**	**1.15**
SGOT	**0.061**	**0.021**	**0.003**	**1.063**	**1.020**	**1.11**
Creatinine	**12.57**	**2.155**	**< 0.001**	**288933**	**4229**	**19741101**
Interleukin-4	**0.023**	**0.004**	**< 0.001**	**1.023**	**1.014**	**1.031**

B: Regression coefficient. SE: Standard error.
CI: Confidence interval. SGPT: Glutamic pyruvic transaminase.
CRP: C-reactive protein. SGOT: Glutamic oxaloacetic transaminase
WBC: White blood cells count.

Discussion

As regard inflammatory markers in this study, there was high statistically significant increase in serum ferritin in group C, when compared with those of group A, group B and group D. Along with this result, it was reported that patients diagnosed with severe COVID-19 had higher serum ferritin levels than in other groups (p<0.001) **(7)** **a**lso, it was reported that serum ferritin level was significantly higher in the severe to critical COVID patients compared to mild to moderate covid patients **(8).**

As regard D-dimer, in this study, there was high statistically significant increase in D Dimer in group B and group C when compared with those of group A and group D. Along this result, scientists reported that D-dimer was increased and significantly higher in COVID-19 patients compared with healthy controls **(9).**

As regard CRP, in this study there was high statistically significant increase in CRP in group C and group B when compared with those of group A and group D. In the same line, researchers reported that overall, COVID-19 patients had significantly elevated CRP at baseline when compared to PCR-negative controls p=0.0004 **(10).**

In this study, there was high statistically significant increase in serum procalcitonin in group C when compared with those of group A, Group B and group D.

There was high statistically significant increase in serum IL4 in group C when compared with those of group A, Group B and group D. In the same line, reported that the COVID-19 group presents statistically significant higher tissue expression of IL-4 compared to the control group **(11).**

In this study, serum IL4 was good to discriminate between group B and group C, with sensitivity of 58%, specificity of 84%, PPV of 78.4% and NPV of 66.7%. Similarly, characterized the kinetic increase of inflammatory cytokine levels, including IL-2, IL-4, IL-6, IL-10, IFN-γ, and TNF-a, at 16 days after disease onset, in 13 patients with severe COVID-19 **(12).**

In this study, there was a high statistically significant positive correlation between IL-4 and severity score. Compared to current findings, it was demonstrated that patients with comorbidities had greater disease severity compared with those without **(13).**

Using Multivariate logistic regression analysis demonstrated that the following factors were predictive for severe COVID: Headache, Abdominal Pain, vomiting, dry cough, oxygen saturation, white blood cells count, Neutrophils, Lymphocytes, Ferritin, D Dimer, Procalcitonin, C-reactive protein, Glutamic pyruvic transaminase, Glutamic oxaloacetic transaminase, Creatinine and Interleukin-4. Similarly, reported that increased IL-4 level can be a predictive factor for COVID-19 severity **(14,15)**.

Conclusion

Interleukin-4 (IL-4) levels may be linked with the disease severity in patients with Coronavirus disease-19 (COVID-19) and can be used as an effective indicator to predict the severity of COVID-19 disease.

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