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Evaluation of Interleukin -29 Serum Levels and Their Gene Expression in Iraqi COVID-19 Patients

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Abstract: Inflammation plays a significant part in the pathophysiology of COVID-19 which is caused by SARS-CoV-2. Severity of this infection is linked with dysregulation of inflammatory immune responses, which sequentially inhibit the development of protective immunity to the infection. The aim of the study to evaluate serum cytokine levels throughout the acute phase are linked also to a higher risk of disease severity and death rate. Interlukin-29 (IL-29) also known as Interferon λI, as an inflammatory cytokine, is an early line of defence in upper respiratory tract infections like SARS-CoV-2. It's of critical importance in innate immune regulation and modulation of immune responses during acute viral infection and tissue inflammation. Therefore, this study evaluated IL-29 serum levels in 80 COVID-19 Iraqi patients and 50 apparently healthy controls. ELISA technique was used for the quantitative detection of human IL-29. Findings indicated that median IL-29 serum concentrations differed insignificantly between moderate cases, severe/critical cases and control groups. It was concluded these differences in IL-29 serum levels were not affected by sex, age, or underlying disease. Il-29 mRNA showed up-regulated in PBMC in COVID-19 Patients.

Keywords: IL-29, COVID-19, ELISA, Iraqi patients, viral infection.

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Introduction

The worldwide spread of coronavirus disease 2019 (COVID-19) is attributed to the transmission of its causative agent; severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (1). This disease was announced as a pandemic by the World Health Organization (WHO); furthermore, since May 2, 2023, it reached every country all over the world (2,3 and 4). SARS-CoV-2 infected more than 687 million individuals globally with 6.87 million deaths. From 3 January 2020 to 19 April 2023, there were 2,465,545 confirmed cases with 25.375 fatalities in Iraq, according to WHO (5).

A profound comprehension of the immune reactions against SARS-CoV-2 is essential for the development of efficacious treatments. Interferons (IFNs), which are powerful and versatile cytokines released by different types of cells, are of particular interes (6). Among them, type III IFNs have significant functions in innate immune responses when confronted with viral infections. Type III IFN comprised four IFN-λ molecules known as IFN-λ1 (IL29), IFN-λ2 (IL28A), IFN-λ3 (IL28B), and IFN-λ4 (7) that have been reported for the first time (8). They share similarities with type I interferons in terms of function. Nevertheless, their impacts are milder and primarily act as

an initial defence mechanism against viral infection within the epithelial layer (9). Mature dendritic cells and macrophages are the primary producers of IL-29 which plays a vital role in different immune reactions in addition to its association with antiviral action. In preclinical studies using different experimental animals, the pegylated form of IFN-λ1 revealed a reduction in the SARS-CoV-2 severity as well as transmission (10).

Type III IFNλs have significant consequences regarding the severity of respiratory infections like COVID-19. They play an important role in keeping a stable antiviral activity, particularly in the respiratory tract. In contrast to type I IFNs, type III IFNλs are stimulated at a previous stage of infection, even at lower viral levels, to restrict the primary infection. They achieve this by inducing viral resistance in cells, helping them cope with the viral load and preventing disease progression (11). While type III IFNλs are well-known for their antiviral effects, they also contribute to the immune response against bacterial pathogens (12, 13). The exclusive feature of IFN-λ is its capability to stimulate a narrower variety of genes in precise target cells expressing IFNλR. Such specificity renders type III IFNλs a promising therapeutic choice (14). The effects of IFN-λ principally emphasise viruses that target cells in the liver, urogenital tract, gastrointestinal tract, and respiratory tract (15,16).

Considering SARS-CoV-2 infections, the defensive impacts of IFN signalling are demonstrated through studies showing the severity of COVID-19 depends on the lowered IFN signalling, the existence of autoantibodies hindering the effects of specific IFNs, and genetic variants that impair IFN signalling (17, 18). IFN- λ reduced the viral load of SARS-CoV-2 and inflammatory responses but did not affect mortality rates (8).

Given the limited existing investigations on the prognostic impact of IL-29 in COVID-19, the objective of the current study was to establish the part of IL-29 played in the context of COVID-19 and to evaluate its levels as a predictor for the severe condition in this disease.

Materials and methods

Subjects

A total of 140 unvaccinated COVID-19 patients participated in the present case-control study; including 45 severe/critical cases, 45 moderate cases and 50 apperantly healthy volunteers. The patients were admitted for diagnosis and treatment to the Dar Al-Salam Field Hospital in Baghdad during the period from November 2021 to May 2022. The diagnosis of COVID-19 patients was confirmed by positive nasopharyngeal swabs using PCR technique on the first day of admission and by a chest computerized tomography scan. The World Health Organization (WHO) Interim Guidance for determining disease severity were adopted to enrol the patients. Information regarding age, sex and underlying diseases (diabetes and cardiovascular) were documented for all participants. The control group (HC) consisted of individuals who had not experienced any respiratory infections in the previous four months and did not have any underlying diseases. Only those who gave negative results for the CRP test and normal ESR value (< 20 mm/h) alongside a negative nasopharyngeal swab were included as a control group.

Nasopharyngeal swabs and 10 millilitres of blood samples were collected from COVID-19 patients and HC. However, for severe and critical patients the blood was collected after 7

days of hospitalization. Afterwards, sera were collected from 2 ml of blood and stored at – 20°C to be used later. However, the rest 8 ml was used to PBMCs isolated by Ficoll-Hypaque density centrifugation.

Determination of COVID-19 positive by PCR

Bio-Rad Reliance SARS-CoV-2 RT-PCR Assay Kit (BIO-RAD/ USA) was used by qualitative determination of SARS-COV2 in nasopharyngeal swab.

Evaluation of IFN-λ (IL-29) serum levels

The Human IL-29 ELISA Kit (Invitrogen / Austria) was used for the quantitative detection of human IL-29 by ELISA technique.

Detremination of of IFN-λ (IL-29) gene expression

Reverse transcription-quantitative PCR (RT-qPCR) method was used to evaluate of the gene expression of IFN- III in PBMC (Saha et al., (2022).

Total RNA was extracted from PBMC using TRIzol (Invitrogen, Carlsbad, CA, USA). Reverse transcription reaction was conducted to obtan cDNA at 42ºC for 60 min, 80ºC for 5 sec in a 20 µL mixture containing 5µl of total RNA, and ProtoScript® First Strand cDNA Synthesis Kit (NEB, UK). Each real-time PCR was prepared in a 20 µL reaction mixture containing 10 µL Luna Universal qPCR Master Mix, 5µL cDNA, 1 µL primers (200 nM each of forward (GCCCCCAAAAAGGAGTCCG) and reverse primers (AGGTTCCCATCGGCCACATA), , then the program for Real-Time PCR was setup with indicated thermocycling protocol, Cycling conditions consisted of initial denaturation 60 sec at 95ºC, followed by 42 cycles of 15 sec at 95ºC, and 30 sec at 60ºC. 18S rRNA using as housekeeping genewith forward primer (GTAACCCGTTGAACCCCATT) and reverse primer (CCATCCAATCGGTAGTAGCG). All samples of patients and controls were assayed in triplicate. Relative gene expression was determined by the 2-ΔΔct method.

Statistical analysis

Categorical variables were expressed as numbers (percentage) and Pearson Chi-square test was performed to assess the significant differences. Also, variables were presented as median and range and significant differences were assessed using Kruskal-Wallis test. P values of ≤ 0.05 or ≤ 0.001 were considered to indicate statistical significance. The statistical analysis was performed using IBM SPSS Statistics 25.0 (Armonk, NY: IBM Corp.).

Results and discussion

Demographic characteristics of subjects

The results of RT-PCR of Positive COVID-19 and demographic characteristics of those patients included in this study were age, sex and underlying diseases, as shown in Table 1. age was divided into 3 age groups (≤ 29, 30-60 and >60 years old) and the disease was more frequent significantly ($p < 0.001$) (57, 63.3%) in >60 years old group, followed by 30-40 years old group (20, 22.2%) and 13 cases (14.5%) in ≤ 29 years old group. However, the distribution of healthy controls (HC) according to age group was 20 (40%), 16 (32%), and 14 (28%) in the three age groups, respectively. Of note, the patients significantly outnumbered the controls ($p < 0.05$). The COVID-19 disease was more frequent in males than females (65.6% vs. 34.4%, $p < 0.05$) in patients, while in HC (64% vs. 36%), the difference between them was

attended a significant level. 58.9% of patients had chronic diseases while others didn't and the results showed significant differences <0.01.

Table (1): Demographic variables of COVID-19 patients and controls.

Characteristics		COVID-19 patients N=90(%)	Control N=50(%)	*P*- value
SARS-CoV-2 RT-PCR Assay		90(100%)	0(0%)	
Age groups/years	≤ 29,	13 (14.5)[a]	14 (28)	<0.05
	30-60	20 (22.2)[b]	16 (32)	
	> 60	57 (63.3)[c]	20 (40)	
	P-value	<0.001		
Sex	males	59 (65.6)[a]	32 (64)	
	females	31 (34.4)[b]	18 (36)	
	P-value	<0.05		
Underlying diseases	have	53 (58.9)	0 (0)	<0.001
	haven't	37 (41.1)	100(100)	
	P-value	NS		

NS: non-significant, Different latters represented significant difference between parameters.

Determination of IL-29 serum levels

The serum levels of IL-29 were assayed in COVID-19 patients (moderate and severe/critical) and HC groups using the ELISA technique. The serum level was tested for normality, and it was found that they distributed not normally (Kolmogorov-Smirnov test P value< 0.01, and by Shapiro – Wilk test P value< 0.001), Accordingly, they were given as median and range. The present findings revealed that the IL-29 median serum level was 10.5 pg/ml (3.7- 62.0) in moderate cases, 8.2 pg/ml (1.2-29.9) in severe cases and 9.9 pg/ml (2.6-78.1) in healthy control. However, the differences between the three IL-29 serum levels did not reach significant (P > 0.05) levels between the two types of infection (Figure 1).

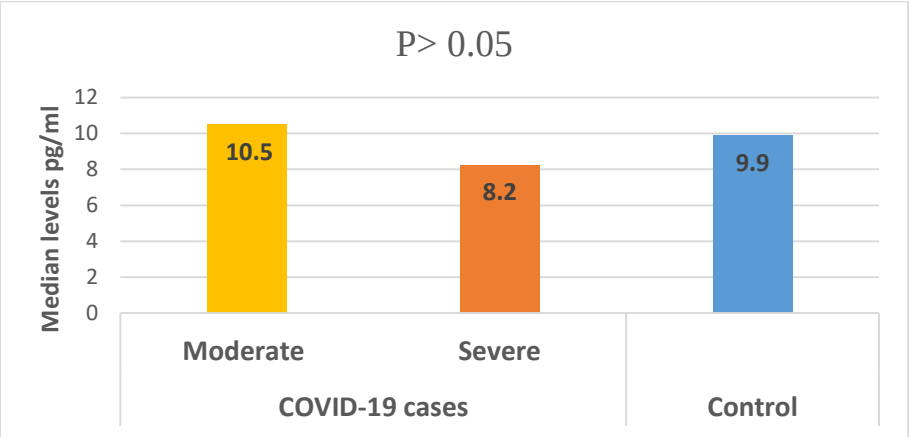

Figure (1): Serum levels of IL-29 in COVID-19 patients and controls.

Serum levels of IL-29 grouped based on the patient variables

To assess the influence of some of the characteristics of COVID-19 cases on serum levels of IL-29, the patients were stratified according to age, sex, and underlying diseases. The current findings showed that these parameters did not influence the IL-29 concentrations (Table 2).

Table (2): Serum levels of IL-29 in COVID-19 patients grouped based on characteristics of patients.

Characteristic		Median Serum levels of IL-29 (pg/ml0
Age (year)	<29	11.7
	30-60	9.3
	>60	9.7
	P value	>0.05
Sex	male	10.9
	Female	8.7
	P value	>0.05
Underlying diseases	yes	9.9
	No	10.2
	P value	>0.05

ROC curve analysis

Upon the analysis of the ROC curve (Figure 2), the IL-29 levels cannot serve as a predictor of COVID-19. Given that AUC was 0.5031 (95% CI = 0.3341 to 0.6721; p <0.971). According to the Youden index the cut-off value was calculated to be 11.1 pg/mL, and the sensitivity and specificity of IL-29 serum levels were 40.0 and 65.1%, respectively.

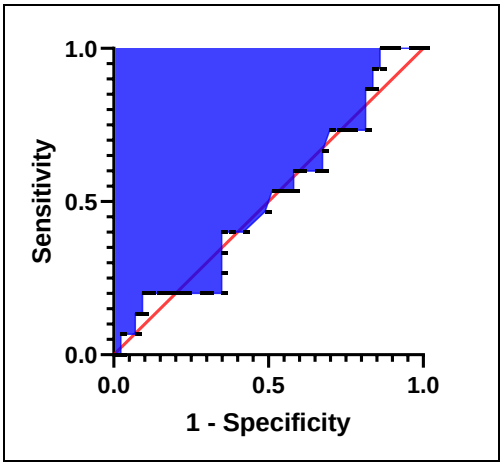

Figure (2): Receiver operating characteristic (ROC) curve plot of IL29 for predicting COVID-19.

Interferon gamma-I (IL-29) gene

Mean of the relative expression ($2^{-\Delta\Delta Ct}$) of IL-29 mRNA was increased by 1.85± 0.25-fold in Total COVID-19 patients than control. In sever COVID-19 patients the relative expression increased (2.30 ± 0.62) folds than in moderate (1.65 ± 0.33) and critical COVID-19 patients (1.78± 0.499). Although the increased was not reach to significant levels P > 0.05 (Table 3).

Table (3): Expression of INFλ-I mRNA in COVID-19 patients and controls.

Studied groups	Number	Folding $2^{-\Delta\Delta Ct}$ (Mean ± SEM)
Total COVID-19 Patients	90	1.83 ± 0.25
Moderate COVID-19 patients	45	1.60 ± 0.33
Sever / Cretical COVID-19 patients	45	2.33 ± 0.62
Controls	50	1

ROC curve analysis revealed the predictive insignificance of IL-29 (AUC= 0.511; 95%CI= 0.354 - 0.669; P = 0.88; Youden index= 0.109; cut-off value = 1.2881fold; sensitivity = 48.4%; specificity = 62.5%) was not discriminating between COVID-19 cases and control (Figure 3).

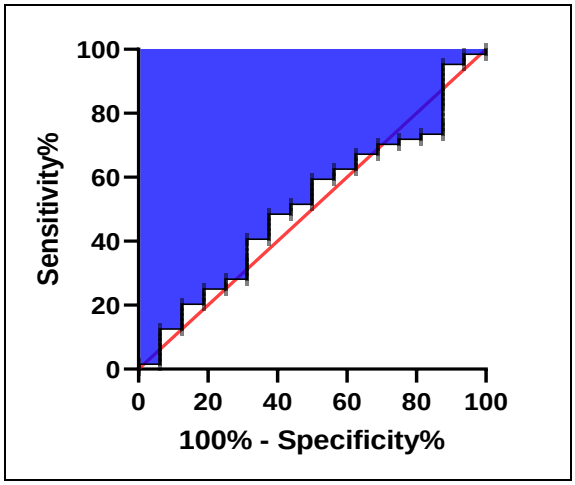

Figure (3): ROC curve analysis of INFλ- I in COVID-19 cases verses HC.

The immune system has a crucial participation in defence against viral infections. One of these infections is COVID-19. Therefore, the present work concentrated on the role of one component of the immune system; IL-29 in COVID-19 disease. Small proteins known as interferons are produced and released by virally infected host cells that have a vital contribution in protection against viral infection, from them IL-29 (Interferon lambda-I) (19,20). The present results revealed that IL-29 was elevated in the sera of moderate COVID-19 patients and reduced in the sera of severe/critical cases than HC. However, the upregulated and downregulated do not reach significant levels. few studies have evaluated IL-29 in COVID-19 patients; Sarrafi *et al*. (21) found no significant difference in IL-29 serum levels between patients with SARS-CoV-2 infection and healthy controls. Despite the presence of pro-inflammatory reactions in hospitalized patients (22), the researchers concluded that IL-29 does not contribute to the inflammation observed in these patients. The IL-29 is mainly produced by epithelium, and since SARS-CoV-2 infects these cells, it is possible to hypothesize that IL-29 plays a role in defending against the virus, independent of its elevated serum levels. Therefore, assessing the concentrations of IL-29 could provide valuable insights into the functions of IL-29 in combating SARS-CoV-2 and its impact on the development of the disease (23).

In contrast, the study by Vastani *et al*. (10) found no significant difference in serum concentrations of IL-29 between patients presented with onset of mild or severe manifestation, indicating that IL-29 may not contribute to the inflammatory response in these patients. Yet, their results did show that recovered patients had significantly elevated concentrations of IL-29 compared to those who died, suggesting a potential protective role of this cytokine in COVID-19 patients. Furthermore, low levels of IL-29 may serve as an indicator of severe COVID-19 infection (24).

The present results revealed that the relative expression of IFNλ-I mRNA was upregulated in PBMC of total and in two types of COVID-19 (moderate, severe / critical) more than healthy control. Type III interferons (IFNλs) may play a more important role than type I interferons in providing first-line defence at barrier surfaces like the respiratory, gastrointestinal, and urogenital tracts (25). Injection of recombinant or pegylated forms of IFNλ can suppress viral replication while preventing the development of a damaging "cytokine storm." (26,27) Furthermore, IFNs have little effect on the adaptive immunity. Rather, they stimulate T helper 1 cells, cytotoxic T cells, and antibody responses; which are crucial for long-term immunity (28,29). As such, IFNλs work together with type I interferons to optimize antiviral protection while lessening accidental unwanted effects (12).

Interferons -λ have important consequences for respiratory infection pathogenesis like COVID-19. They are critical for keeping a harmonized antiviral response in the infection of the respiratory tract (30). IFNλs are stimulated at lower viral loads ahead of type I interferons to restrict the early infection. This is done by inducing viral resistance in cells and assisting them to cope with the viral burden (31). However, the serum concentration of IL-29 in COVID-19 patients did not reveal age, sex and chronic disease-associated variables, and this could suggest that age, sex or chronic diseases did not affect IL-29 serum levels.

Conclusion

In conclusion, this study proposed that IL-29 serum levels showed no significant differences between moderate and severe/critical patients infected with COVID-19. The IL-29 serum levels were not affected by age, sex, or underlying diseases.

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Declaration of competing interest

No competing interests to declare.

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Ethical Clearance

After receiving the permission of the Ethics Committee of the College of Science, University of Baghdad (CSEC/0122/0157), all participants provided written consen.

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