Prognostic Value of TLR7, and Serum level of IL-17 and IL-23 in COVID-19 Infection

Hayder A. Ali[1], Ibrahim A. Altamemi[2]
[1,2]Department of Microbiology, College of Medicine, University of Al-Qadisyiah/ Iraq
haiderabbasali18@gmail.com

Abstract

Background: In December 2019, Wuhan, China, had seen an uncommon outbreak of coronavirus disease 2019 (COVID-19), a form of viral infection with pneumonia that is brought on by coronavirus 2 causing severe acute respiratory syndrome (SARS-CoV2) due to Severe acute respiratory CoV-2 level of contagiousness and COVID-19 disease pandemic. Objective: The aim of the present study is to find out the role of of TLR7 gene expression in COVID- 19 patients and disease progression to use them as a prognostic biomarker, also evaluation serum levels of IL-17 and IL-23 in disease course of patients. Materials and Methods: a case control study have been conducted, blood Samples has been collected from hospitals in Dhi-Qar Health Department, COVID-19 isolation centers for those infected with COVID-19 disease. The patients were sub-divided into three groups: mild cases group (N: 50), sever cases group (N: 50), and third control group (N: 50). genomic RNA was extracted from blood for molecular assay to study the role of TLR7 and Enzyme linked immunosorbent assay(ELIZA)technique was used to detect serum level of IL-17 and IL-23 in COVID -19 patients. Results: higher significantly variation on median TLR7 fold change, the median TLR7 fold change in the severe group which was 69.04 (39.81), in the mild group which was 28.38 (38.62) and in the control group which was 1.84 (2.84) which were significant ($p < 0.05$). There was higher significantly variation on IL-17 and IL-23 level, change severity group was higher comparison in both the control with mild groups ($p < 0.05$). Conclusion: All these studded parameters show high sensitivity and specificity, thus, we can using them as a combination for prognostic value in COVID-19 patients.

Keywords: Biomarker, TLR7, IL-17 and IL-23 ELISA, COVID-19 patients

1. Introduction

Coronavirus disease 2019 (COVID-19) is a contagious disease that causes severe acute respiratory syndrome coronavirus 2 is the source of the contagious sickness known as coronavirus disease 2019 (COVID-19) (SARS-CoV-2). Wuhan, China, recorded the discovery of the initial case in December 2019. the illness became widespread, causing the COVID-19 pandemic [1]. Although COVID-19 symptoms might vary, they frequently include fever [2] coughing, headaches [3] exhaustion, breathing issues, loss of smell, and loss of taste [4, 5]. One to (14) days after the viral exposure, the symptoms appear. TLR7 is expected to be involved in the clearance of SARS-CoV-2 since it can identify viral single-stranded RNA. The transcription factors NF-B, IRF-3, and IRF-7 are nuclear translocate as a result of TLR activation via MyD88- and TRIF-dependent pathways, and innate pro-inflammatory cytokines (IL-1, IL-6, TNF-) and type [6].

IL-17 level was also found to be increased in COVID-19 patients, particularly in those with a severe and critical disease, according to evidence [7] As a result of the cytokine storm, elevated IL-17 levels have been observed in SARS-CoV-2 patients, and they have been linked to the viral load and disease severity [8] and IL-23 is an inflammatory cytokine generated by monocytes, macrophages, and dendritic cells that also expresses through the transcriptional factors IL-12-R and IL-23R [9].

2. Material and Methods

A case control study had been conducted and based on three groups, blood Samples had been collected from hospitals in Dhi-Qar Health Department ,COVID-19 isolation centers for those infected with COVID-19 disease after they are diagnosed by(RT-PCR and CT-Scanning) in dependent Central public health laboratories in the period from January to June 2021. In addition to that the patients were sub-divided into three groups, According to degree of lung infection (CT scan and clinical symptoms) patients had been classified into mild cases group (N:50), sever cases group (N:50)and third control group (N:50) control volunteers considered (According to PCR, Rapid tests and CT scan (Negative) tests .1ml of blood collected directly in a sterile tube containing EDTA, with trizol tube for total RNA extraction to be use for TLR7 qPCR and 2ml of blood were collected in gel tubes isolation of serum by Centrifuge to isolated in pandrof tube to kept in the refrigerator for the purpose of performing ELISA tests for evaluate the serum levels of IL-17 and IL-23.

Total RNA extraction

The TRIzol® reagent kit (Bioneer, Korea) was used to extract the total RNA as per the instruction of the company. The concentration and purity of the extracted genomic RNA were determined using a Nanodrop by measuring absorbance at (260/280) nm.

Real Time -qPCR

In blood patients and normal samples, Real-Time PCR was used to quantify the levels of the housekeeping gene (GAPDH) and the TLR7 gene. This procedure was carried out in accordance with the instructions provided by Varkonyi-Gasic et al. [10].Primers

The qPCR Primers for TLR7 was created in this work by selecting miRNA sequences from (The Sanger Center miRNA database Registry) and utilizing the miRNA Primer Design Tool and Housekeeping gene (GAPDH) were design in this study by using NCBI

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TLR7 qPCR primer	F	AATGCCCCATTTCCTTGTGC	80bp
	R	TCTGTCAGCGCATCAAAAGC	
GAPDH qPCR primer	F	AATTCCATGGCACCGTCAAG	104bp
	R	ATCGCCCCACTTGATTTTGG	

Data analysis of RT- PCR

The impact on actual transcriptomic levels (fold change) (The CT Technique Using a reference gene) was used to assess the data from q RT-PCR for the target and housekeeping genes:
Ratio (reference/target) = 2CT (reference)–CT (target)

Serological test

ELISA Kit for IL-17 and IL-23
Human Interleukin 17 and Human Interleukin 23 ELISA Kit were utilized in this investigation for quantitative determination of IL-17 and IL-23 from serum samples from the patients and the healthy controls, and it was had been done according to the manufacturer's instructions. The Optical Density (OD) was measured using a spectrophotometer at 450 nm, from which the quantity of IL-17and IL-23 could be estimated. This sandwich kit is used to detect IL-17 and IL-23 in serum in an accurate quantitative manner.

3. Results

The Expression Level of TLR7 in COVID-19 Patients and Control Groups

There was high significant variation in median TLR7 fold change among the study groups ($p < 0.001$), the median TLR7 fold change in the severe group which was 69.04 (39.81) higher, than in the mild group which was 28.38 (38.62) and in the control group which was 1.84 (2.84) were significantly ($p < 0.05$), in the mild group which was significantly higher than in the control group ($p < 0.05$) table (1) Figure1.

Table 1: Comparison of TLR7 Fold change between patients with COVID-19 and control group

TLR7 Fold change	Control N:50	Mild N:50	Sever N:50	
Median (IQR)	1.84 (2.84) C	28.38 (38.62) B	69.04 (39.81) A	< 0.001 HS
Range	0.01 - 10.67	0.05 - 85.91	14.78 - 373.99	

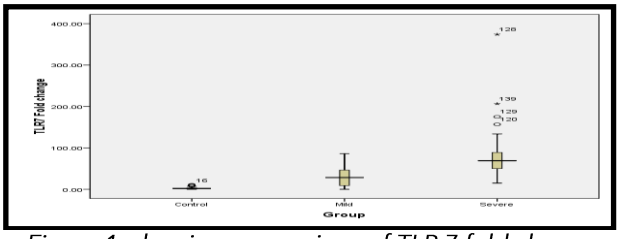

Figure 1: showing comparison of TLR-7 fold change among study groups

The comparison of IL-17 and IL-23 between the Patient and Control Group in COVID- 19 Patients

The comparison of IL-17 and IL-23 in COVID- 19 is show in table (2) figure (2, 3). There was higher significantly variation on IL-17 level, the median IL-17 level change severe group which was higher compared in both the control with the mild which significantly ($p < 0.05$), the mild which was significant more compared that of the control which significant ($p < 0.05$). Also, higher was significantly variation IL-23 level among the study groups which ($p < 0.001$), the median IL-23 level change in severe was more compared in both control and mild significantly.

Diagnostic Role of TLR7 Fold Change and Serum IL-17 and IL-23 in the Detection of COVID-19 Patients

Receiver operator characteristics (ROC) curve analysis was carried out in order to evaluate the diagnostic role of TLR7 which was >10.67 fold Change was 87.0 % sensitivity, 100.0 % specificity and of 95.3 % accuracy %, that of IL-17 was >63.14 fold change with 97.0 % sensitivity, 100.0 % specificity and 99.8 % accuracy and that of IL-23 was >74.06 fold Change was 88.0 % sensitivity, 88.0 % specificity and 91.6 % accuracy table (3) figure (4, 5, 6)

Table 2: A Relationship IL-17 and IL-23 in patient and control group in COVID-19.

Characteries	Control N:50	Mild N:50	Severe N:50	p
IL-17				
Median (IQR)	39.95 (17.14) C	82.52 (20.55) B	92.14 (104.96) A	< 0.001 HS
Range	19.83 - 63.14	58.98 - 372.29	62.07 - 341.28	
IL-23				
Median (IQR)	50.55 (28.03) C	83.60 (23.36) B	116.64 (133.46) A	< 0.001 HS
Range	18.50 - 130.62	49.79 - 389.34	63.35 - 288.00	

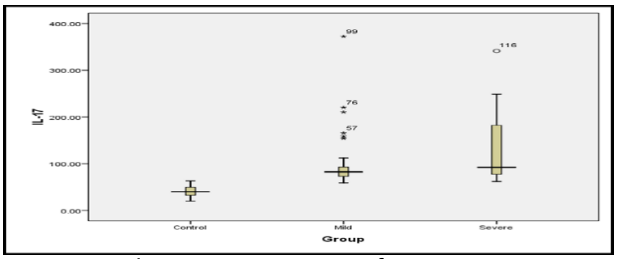

Figure 2: showing comparison of serum IL-17 among study groups

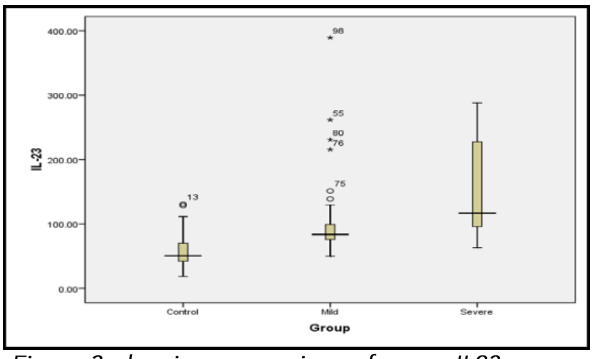

Figure 3: showing comparison of serum IL23 among study groups

Table (3). Diagnostic role of TLR7 Fold change and serum IL-17 and IL-23 in the detection of COVID-19.

Characteristic	TLR-7	IL-17	IL-23
Cutoff	>10.67	>63.14	>74.06
AUC	0.953	0.998	0.916
95 % CI	0.906 to 0.981	0.972 to 1.000	0.859 to 0.955
p	< 0.001 HS	< 0.001 HS	< 0.001 HS
Sensitivity %	87.0	97.0	88.0
Specificity %	100.0	100.0	88.0
Accuracy %	95.3	99.8	91.6

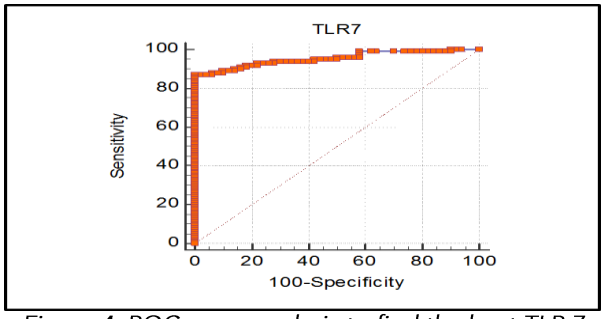

Figure 4: ROC curve analysis to find the best TLR-7 cutoff value

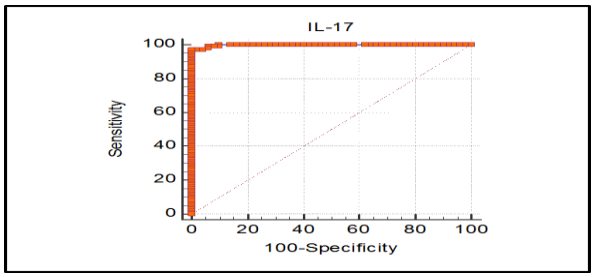

Figure 5: ROC curve analysis to find the best IL-17 cutoff value

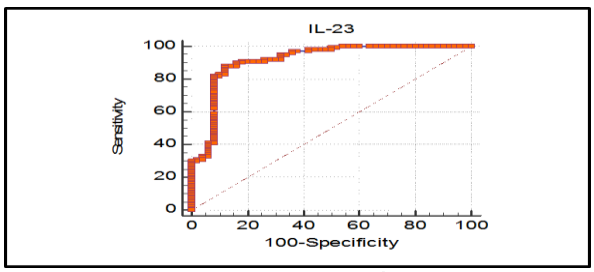

Figure 6: ROC curve analysis to find the best IL-23 cutoff value

4. Discussion

According to above data, TLR7 expression seem to have a role in patients COVID-19 fold change was higher in sever (69.04) while (28.38) in mild compared with that of control (1.84). In previously conducted study there was noticeable increase gene expression of TLR2, TLR7, IRF3 were noted in COVID-19 patients of moderate severity as compared with controls group [11].this came in consistence with the current results, the relative level of TLR7 expression, as well as the generation of proinflammatory and type I IFNs, were shown to be higher in SARS-CoV-2 infected, and both IRF3 and NF-B appeared to be involved in the TLR7 signal transduction pathway [12].Thus ,So any impairment mutations in the TLR7 receptor on the X-chromosome are linked to poor outcomes in COVID-19 hospitalized patients, notably lower than normal levels of type I and type II cytokines, including IFN-, the major macrophage activator [13].

Moreover, The TLR7 response, as demonstrated by numerous investigations, is essential for a successful COVID-19 outcome [14], underscoring the clinical significance of the innate immune system in SARS-CoV-2 infection. It has been demonstrated that SARS-CoV-2 causes peripheral blood mononuclear cells to respond with type I and type III IFNs in a TLR7/8-dependent manner. This response may either be protective or it may contribute to the cytokine storm seen in COVID-19 [15].

The first round of TLR7 activation eventually causes transcription factors to be released from the nucleus, promoting the production of types I and III IFNs as well as a number of other significant pro-inflammatory cytokines. The second phase of signaling makes sure that all nearby, uninfected cells as well as any infected cells are beginning to express a large number of interferon-stimulated genes that further develop the so-called antiviral state [16].

In agreement with the current results, the patients of COVID 19 positivity, TLR7 expression was comparatively higher in the symptomatic patients than in the asymptomatic patients, suggesting its involvement in immunopathogenesis. Since it is already known that the JAK/STAT signaling pathway is activated as part of the TLR7-mediated recognition process. This can also activate NF-, IRF3, and IRF7, which in activated the production of pro-inflammatory cytokines such IL-1, IL-6, IL-8, TNF-, and Type-1 IFN responses, this study indicated that a boosted pro-inflammatory response via TLR7/8 recognition could mediate the lung injury in patients of COVID 19 in the sever group in compared with the healthy.

On the other hand, the current study came in agreement with many previous studies that noticeable an increase in the level of IL-17 in the patients with severe and mild COVID-19 than the healthy peoples, it had been shown that lung tissue destruction can be explained by recruitment of neutrophils mediated by Th17 cells [17].

IL-17 can promote pulmonary inflammation, following the infection by neutrophil and monocyte migration to the lungs, and by activating other cytokine cascades (G-CSF, TNF, IL-1). This cytokine was produced in the lung by TH17 cells in response to viruses and leads to the induction of several chemokines that recruit immune cells to the inflammation site, besides these local effects, IL-17 also had systemic effects. Indeed, the combination of IL-17

with TNF-α induced the expression of pro-coagulation factors, which promote thrombosis and inhibit endothelial anti-coagulator pathway [18].

In consistent, the present study, also demonstrated that the occurrence of high level of IL-23 in the sever and the mild groups compared with the control group was ($p < 0.001$), such increases in IL-23 facilitates lung inflammation by inducing the production of various innate proinflammatory cytokines (IL-1b, IL-6, TNF-a, IL-12, IL-33) in several target cells leading to the most severe forms of the severe patients COVID-19 [19].

Other studies, came in consistence with the present study, among which study based on 40 severe COVID-19 ICU-admitted patients and 40 patients in mild cases, IL-23 cytokine is measured in both mild and severe COVID-19 patient and healthy control, such study showed an increased in the IL-23 in severe patients (354.2 ± 185.1), mild patients (222.8 ± 87.20) with high significant ($p<.0001$) compared with healthy controls (141.7 ± 65.92) [20].

The results of the present I study indicated an importance value measuring serum IL-17 and IL-23 levels in COVID-19 patients with severe and mild which could provide a biomarker for the evolution of lung involvement in COVID 19. The serum levels of, IL-17, and IL-23 are also, risk factors that associated with COVID-19 severity and suggesting the important role of this cytokine in the progression of COVID-19.

On the other hand, ROC of TLR7 variants and association with COVID-19 of accuracy 60% average value, ROC-AUC score (68%), sensitivity (75%) and specificity (43%), [14].

The previous study that had been done by Ghazavi, A et al to evaluate the value of increased IL-17 concentration in serum as a screening test for severity COVID -19 disease. The ROC curve analysis of IL-17 of severe COVID-19 reviled that the optimum cut-off point for IL-17 (>224.1), (AUC. 0.642), Sensitivity 93.55 % Specificity 38.24 %; CI. (0.507–0.777), ($p = 0.049$) [21].

Moreover, the studied showed the ROC curve analysis of IL-17 (AUC 0.800, CI. (0.718–0.882), ($P<0.001$) [22] and study by (25) had been mentioned that AUC (0.539), (CI 0.451–0.627), with Sensitivity 69.8% and Specificity 39.8% with $P= 0.393$. These studies agree with the present study. The presence of innate pro-inflammatory mediators is supported by the elevated systemic levels of IL-17 and IL-23 in the severe type of COVID-19. The findings are consistent with other research showing a substantial relationship between illness severity and elevated levels of a few innate immunity-related proinflammatory cytokines [22].

5. Conclusion

All these studded parameters show high sensitivity and specificity, thus, we can using them as a combination for prognostic value in COVID-19 patients.

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