Interleukin-37 and Procalcitonin Role in Disease Severity of Covid-19 Iraqi Patients

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ABSTRACT:

Corona-Virus Disease 2019 or what is today known as (COVID 19) is caused by a novel virulent virus belong to coroniviridae and leads to potentially fatal respiratory symptoms that are very similar to the severe respiratory distress syndrome. Different types of cytokines are showed to play an important role in the pathogenesis of this disease. Interleukin-37, often known as IL-37, is an anti-inflammatory cytokine that has been implicated in the modulation of immune responses in a variety of inflammatory illnesses. On the other hand, one of the most important complications of covid-19 was the secondary bacterial infections (SBIs). Procalcitonin (PCT) has been widely investigated as a biomarker for the secondary bacterial infection, especially its unique ability for distinguishing viral from bacterial infection. Hence, the present study was performed to examine the levels of IL-37 and the inflammatory marker procalcitonin (PCT) in serum of 207 patients with COVID-19 who were classified according to their disease severity into asymptomatic, mild and sever patients. Serum levels of IL-37 were significantly (Probability =0.01) decreased while serum levels of PCT were significantly (Probability=0.01) increased in sever patients as compared to other subgroups. In conclusion, the down-regulated of IL-37 in serum of patients with COVID-19 may be related to the elevated PCT levels among the same patients and this in turn may be related to an increase of disease severity.

Key words: COVID-19, IL-37, PCT, SBIs, anti-inflammatory.

INTRODUCTION:

Coronavirus disease 19 (COVID-19) is a new respiratory infection caused by the coronavirus 2 that causes severe acute respiratory syndrome (SARS-CoV- 2)[1]. Cytokines are one of the immune-related mediators that has been predicted to have a key role in COVID-19 pathogenesis.[2]. Interleukin (IL)-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, IL-33, IL-25, IL-37, and IL-38 are some of the cytokines that are dysregulated when SARS-CoV-2 infects the respiratory system[3]. Although the evidence is not conclusive, the cytokine storm phenomenon has been proposed as a possible cause of acute respiratory distress syndrome and multiple organ failure in cases of severe COVID-19[4]. One such cytokine, IL-37, has been postulated to play a role in the immunopathogenesis of COVID-19, but the evidence isn't overwhelming. 5. IL-37, formerly known as IL-1F7, is a novel cytokine in the IL-1 family. This family is essential for the activation of both innate and adaptive immune responses[6]. A substantial link between IL-37 plasma levels and clinical outcome has recently been discovered in COVID-19 patients. Furthermore, it has been shown that the absence of an IL-37-mediated response can predict a poor clinical prognosis[7]. Furthermore, one study found that a large proportion of COVID-19 patients have elevated procalcitonin (PCT) levels, suggesting that continuous monitoring of PCT levels could help forecast the disease's progression into more severe forms[8]. PCT levels are often high after bacterial infection and low after viral infection. The earlier finding that PCT level was an independent predictor in predicting COVID-19 severity was incorrect[9]. The goal of this study was to look at the levels of IL-37 and PCT in the blood of three COVID-19 patients, asymptomatic, mild, and severe, to see if there was a link between these parameters and disease severity.

SUBJECTS AND METHODS:

Subjects

The study included 350 subjects who were classified into 300 patients that were diagnosed with Covid-19, their ages ranged between (16) to (67) years, (118) females and (89) males, who attended Al-Imam Ali general hospital and Doctor Saad-Al-Wetrri hospital in Baghdad, Al Russafa, from April 2020 to May 2021. Patients were classified into asymptomatic, mild and sever patients dependent on their disease severity. In addition to 50 uninfected subjects as controls, their ages ranged between (15) to (59) years, (31) females and (19) males. All subjects of the study were exposed to the same laboratory examinations to estimate and distinguish the relative normal ranges of the studied markers among Iraqi population, (Table 1).

Table 1: Demographic characteristic of studied groups

Character	Patient	Control
Total No.	All= 300	Non infected= 50
	Asymptomatic= 100	
	Mild= 100	
	Sever= 100	
Females No.	165	31
Males No.	135	19
Age (mean ± S.E.)	38.5±3.3 Years	34.7±7.1 Years
Pro-calcitonin (mean ± S.E.)	0.5±0.2 ng/ml	0.2±1.1 ng/ml
IL-37 (mean ± S.E.)	0.07±3.4 IU/ml	0.1±4.8 IU/ml

IL-37: Interleukin-37; IU: International unit; SE: Standard error; ng: Nano gram; ml: microliter; mcg: microgram

Inclusion criteria

Covid-19 infection was diagnosed by molecular detection of viral RNA using GeneXpert technique. Severity of disease was identified according to observing the clinical symptoms including coughing, sneezing, chest tightness, productive sputum, fever, muscular pain, sore throat and oxygen saturation decline as sever group while lacking of smell and taste recorded as mild in addition to asymptomatic group which were completely without symptoms. Moreover, another category had been relied for severity via depending on the levels of SARS-cov-2 antibodies, IgM and IgG, thus, according to IgM/IgG ratio there were four subgroups include; (0/ 0-100), (25/ 50-100), (50/ 100) and (100/100) IU/ml subgroups.

Sample collection

Five milliliters of venous blood were withdrawn from each subject under aseptic conditions, the blood samples were dispensed in sterile gel tubes and left to clot for about one hour, then centrifuged at 1000 rpm for five minutes at room temperature to separate the serum, which was then dispensed into sterile plain tubes and stored at -20°C until assayed.

METHODS

All subjects were exposed to the following:

1. Complete medical history.
2. In vitro evaluation of SARS-Cov-2 IgG and IgM antibodies was semi-quantitatively estimated using Western Immuno-blotting assay with Euroimmun kit, Germany, that provides four levels of antibodies ranged as 0, 25, 50 and 100 IU/ml.
3. Measurement of IL-37 as well as procalcitonin was performed by the enzyme-linked immunosorbent assay ELISA using commercial IL-37, kits (Euroimmune, Germany). The results were calculated automatically and printed by ELISA printer.

Statistical analysis:

The Statistical Program for Social Science (SPSS) version 20.0 was used to analyze the data. The mean standard error was used to express quantitative data. Frequency and percentage were used to express qualitative data. The tests below were carried out:

- A two-way analysis of variance (ANOVA) have used for comparing between over than two means in addition to use Less Significant Differences (LSD) to detect the position of differences among the studies groups.
- Probability (P-value):

- P-value ≤ 0.05 reflects the significant differences.
- P-value > 0.05 reflects the non-significant differences.

RESULTS:

Subgroup of (0-100)/(0), (50-100)/(25), (100)/(50) and (100)/(100) in sever patients has significantly lower levels as compared with other subgroups, moreover, subgroup of (100)/(100) in asymptomatic and mild patients as well as subgroup of (0-100)/(0) in sever patients were with significant lower levels of ferritin as compared with other subgroups as shown in (Table 2)**.**

Table 2. IL-37 levels of studied groups according to disease severity (IgG & IgM).

IL-37	IgM = Zero (IU/ml)			
IgG value (IU/ml)	IL-37 (mean ±SE)			
	Asymptomatic	Mild	Severe	P-value
0 – 100	0.09A±0.00b	0.06B±0.01c	0.04C±0.00c	0.01
IgM = 25 (IU/ml)				
	IL-37 (mean ±SE)			
	Asymptomatic	Mild	Severe	P-value
50 – 100	0.09A±0.00b	0.08B±0.01b	0.06C±0.00b	0.01
IgM = 50 (IU/ml)				
	IL-37 (mean ±SE)			
	Asymptomatic	Mild	Severe	P-value
100	0.09A±0.20a	0.10B±0.00a	0.07C±0.01a	0.001
IgM = 100 (IU/ml)				
	IL-37 (mean ±SE)			
	Asymptomatic	Mild	Severe	P-value
100	0.08A±0.01c	0.06B±0.01d	0.05C±0.00a	0.02
P-value	0.01	0.01	0.01	

IL-37: Interleukin-37; IgG: Immunoglobulin G; IgM: Immunoglobulin M; IU: International unit:, P: Probability. LSD test was used to calculate the significant differences between tested mean, letters (A, B and C for rows and a, b and c column) represented the levels of significant, highly significant start from letter (A or a) and decreasing with the last one. Similar letters mean there are no significant differences between tested mean.

Moreover, subgroup of (0-100)/(0), (50-100)/(25) and (100)/(50) in sever patients has significantly higher levels as compared with other subgroups, moreover, subgroup of (100)/(100) in asymptomatic and mild patients was with significant higher levels of procalcitonin as compared with other subgroups as shown in (Table 3).

Table 3. Procalcitonin levels of tested groups according to clinical symptoms severity (IgG & IgM).

Procalcitonin	IgM = Zero (IU/ml)			
IgG value (IU/ml)	Procalcitonin (mean ±SE)			
	Asymptomatic	Mild	Severe	P-value
0 – 100	0.53C±0.02b	0.67B±0.03b	0.76A±0.02	0.001
IgM = 25 (IU/ml)				

	Procalcitonin (mean ±SE)			
	Asymptomatic	Mild	Severe	P-value
50 – 100	0.60B±0.01a	0.63B±0.02c	0.76A±0.02	0.01
IgM = 50 (IU/ml)				
	Procalcitonin (mean ±SE)			
	Asymptomatic	Mild	Severe	P-value
100	0.56C±0.03c	0.63B±0.03c	0.77A±0.04	0.001
IgM = 100 (IU/ml)				
	Procalcitonin (mean ±SE)			
	Asymptomatic	Mild	Severe	P-value
100	0.62B±0.01a	0.73A±0.02a	0.71A±0.02	0.01
P-value	0.05	0.05	NS	

IgG: Immunoglobulin-G; IgM: Immunoglobulin-M; IU: International unit:,P: Probability. LSD test was used to calculate the significant differences between tested mean, the letters (A, B and C for rows and a, b and c column) represented the levels of significant, highly significant start from the letter (A or a) and decreasing with the last one. Similar letters mean there are no significant differences between tested mean.

Furthermore, as shown in (Table 4), Pearson's correlation clears that IL-37 and PCT have significant negative correlation at the subgroups of 0-100/0, 50-100/25 and 100/100 among mild patients.

Table 4. Correlation between Procalcitonin and IL-37 levels of tested groups according to clinical symptoms severity (IgG & IgM)

Asymptomatic patients Pearson's correlation		
IgM/IgG level	Positive correlation	Negative correlation
0-100/0	NS	NS
50-100/25	NS	NS
100/50	NS	NS
100/100	NS	NS
Mild patients Pearson's correlation		
IgM/IgG level	Positive correlation	Negative correlation
0-100/0	NS	IL-37/PCT -0.95*
50-100/25	NS	PCT/IL-37 -0.98**
100/50	NS	NS
100/100	NS	PCT/IL-37 -0.95**
Sever patients Pearson's correlation		
IgM/IgG level	Positive correlation	Negative correlation
0-100/0	NS	NS
50-100/25	NS	NS
100/50	NS	NS
100/100	NS	NS

*. Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level .IgG: Immunoglobulin-G; IgM: Immunoglobulin-M; IU: International unit:, P: Probability; NS: non-significant; PCT: Procalcitonin; IL-37: Interleukin-37

DISCUSSION:

IL-37, formerly known as IL-1F7, is a novel cytokine in the IL-1 family. This family is important in the activation of both innate and adaptive immune responses. Eleven members of this family (IL-1, IL-1Ra, IL-

18, IL-33, IL-36, IL-36, IL-36Ra, IL-37, and IL-38) have been identified, and their involvement in modulating inflammatory responses has been acknowledged[10]. IL-37 is expressed by a variety of immunological and non-immune cells and organs, including activated B cells, natural killer cells (NKCs), monocytes, epithelial cells, keratinocytes, thymus, lymph nodes, lung, and bone marrow[11]. IL-37 is a well-known anti-inflammatory cytokine that works by inhibiting the release of pro-inflammatory cytokines, therefore reducing inflammatory reactions [12]. IL-37 has been implicated in a number of inflammatory and autoimmune diseases, including inflammatory respiratory diseases, inflammatory bowel diseases (IBD), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), ankylosing spondylitis, psoriasis, and multiple sclerosis, and studies have revealed that IL-37 expression is dysregulated in these conditions.[13]. IL-37 has also been linked to immunization against a variety of pathogenic pathogens, including viruses, bacteria, and fungus[14].

IL-37 is an anti-inflammatory cytokine that inhibits the synthesis of various constitutive or induced pro-inflammatory cytokines including as IL-1a, IL-1b, IL-6, IL-17, IL-23, TNF-a, and IFN-Y by downregulating their production. Furthermore, IL-37 can boost the synthesis of transforming growth factor (TGF)-B, an immunosuppressive cytokine. Covid-19 has been linked to cytokine release syndrome in people with severe disease, and high levels of pro-inflammatory cytokines (IL-1a, IL-1b, and TNFa) have been linked to pulmonary inflammation and substantial lung damage in patients with covid-19. Low levels of IL-37 may lead to an up-regulation of pro-inflammatory cytokines, which can contribute to COVID-19 immunopathogenesis. Furthermore, the findings of this study show that serial procalcitonin measurement may be useful in forecasting disease progression to a more severe stage[7]. There is an explanation for this evidence: during bacterial infections, the generation and release of procalcitonin from extrathyroidal sources is greatly increased, and this is actively sustained by increased amounts of IL-1B, TNF, and IL-6. Nonetheless, INFY, whose concentration rises during viral infections, inhibits the creation of this biomarker [8]. It is thus not surprising that the procalcitonin value would remain within the reference range in several patients with non-severe covid-19 infection, whereas its substantial increase in those developing a severe form of disease would reflect bacterial co-infection, thus complicating the clinical picture, as recently demonstrated in children with viral lower respiratory tract infections[9]. The immune-regulatory role of IL-37 in the inhibition of release most of the common inflammatory mediators such as CRP, pro-inflammatory cytokines, neutrophils, eosinophils, and other granulocytes is a vital and significant role, but the most important point in this regard is the role of this anti-inflammatory interleukin in the regulation of non-common or non-classical inflammatory mediators just like procalcitonin which is considered not only as a chief sign for the inflammation, but also to indicate that the inflammation is bacterial rather than viral, that is dangerous alarm for disease state shift from mild into sever case, the case that makes physicians in continuous worry because of the terror of bacterial antibiotics resistance. For this reason, we can say that, the significant negative and strong correlation between IL-37 and procalcitonin (Table 4) may be great futuristic tool for detection the severity and the case of the disease in addition to the fact that IL-37 may be ideal candidate for COVID-19 treatment especially with its multifunctional role in the immune response regulation.

CONCLUSION:

Individuals with severe COVID-19 had lower levels of IL-37 in their blood than other patients. This downregulation could be linked to a worsening of the condition. Furthermore, as compared to other COVID-19 groupings, PCT was shown to be higher in the serum of individuals with the severe subtype. This rise, combined with the strong and negative significant correlation between IL-37 and PCT, confirms their link to increasing disease severity.

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