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Assessing of Interleukin-6 Gene Expression and Some Biomarkers in COVID-19 Patients

[1]Halah J. Kadhim, [1]Abdulameer M. Ghareeb, [2]Mohammed G. Mahdi

[1]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad
[2] Cancer Genetic Department of Educational Laboratories, Medical City

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Abstract: The elevation in inflammatory cytokines suggests a cytokine storm could significantly contribute to COVID-19 pathogenesis. It was concluded that Interleukin-6 playing a crucial role in the immune response with virus. Specimens were collected from 70 patients admitted to the ICUs of Medical City Hospital in Baghdad suspected of being infected with COVID-19, in addition to 35 healthy controls. Specimens were collected from both genders during the period from June 2022 to April 2023, with an age range of 15 to 80 years. The diagnosis of patients was so severe that 35 patients out of 70 showed severe infection, while the other 35 patients showed moderate infection. The IL-6 serum level was measured using the ELIZA assay, and the expression level of the IL-6 genes was measured using RT-PCR. Among 105 samples, the results of the IL-6 level showed a significant difference between the severe and moderate COVID-19 and control groups with IL-6 in a higher-level severe infection followed by moderate infection to control (263.71± 87.63a and 92.54± 27.47b vs. 27.16± 3.78c, respectively). The IL-6 gene expression level in the COVID-19 patients showed a significant difference between the COVID-19 with severe and moderate compared to healthy control IL-6 gene expression relations upon moderate and severe infection of SARS-CoV2 (10.66±6.12 and 5.19±2.72, respectively). A significant difference was also obtained for each IL-6, CRP, and D. dimer among the study groups. The patients with severe disease were high in all three biomarkers, and neutrophilia with lymphopenia, where it found the mean concentrations of them were 15.03 ± 9.61, 100.335±22.379 vs. 551.49±270.53, respectively, were higher in the patient's group than in controls. The study found a significant positive correlation between IL-6, CRP, and D-dimer, while it showed a significant negative correlation between lymphocytes.

Keywords: COVID-19, Gene expression; IL-6, cytokine storm, Laboratory biomarkers

Corresponding author: (Email: ameermgh@ige.uobaghdad.edu.iq)

Introduction

COVID-19 has emerged as a global pandemic; A pandemic Severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) has been found as the causative agent for the COVID-19 outbreak (19) , ACE2 is a physiologically related receptor during the COVID-19 disease (5) Angiotensin-converting enzyme-2 (ACE2) is an enzyme and functional receptor that allows SARS-CoV-2 to enter host cells. It is abundantly expressed in the kidneys, lungs, and heart (1) Moreover, SARS-CoV-2 uses ACE2 for entry and TMPRSS2 for S protein priming (7), releasing the viral RNA genome into the cytoplasm, forming virion-containing vesicles, envelope glycoproteins, and nucleocapsid

proteins, and then fusing them with the plasma membrane to release the virus (15). Many researches are being conducted to understand the genetic nature of a recently found coronavirus with distinct clinical characteristics. Many genetic changes in SARS-CoV-2 genomes have been discovered, indicating that mutations are being produced and the virus is constantly changing (2) Interleukin-6 is a multifunctional cytokine that is produced in response to tissue damage caused by viral infections, also a reliable predictor of illness prognosis and clinical profile worsening. The function of IL-6 as a pleiotropic cytokine with pro- and anti-inflammatory activity, the major hallmark of the disease is the systemic inflammatory immune response characterized by Cytokine Storm (17), In order to better understand COVID-19 immunology, the study investigates the possibility of these pro-inflammatory and anti-inflammatory responses as diagnostic and prognostic tools(30)

Due to the cytokine storm, lung-barrier integrity and vascular permeability are reduced in COVID-19, allowing cytokine loads to enter the systemic circulation. As a result, whenever it travels from the lung to other organs such as the kidney, heart, and pancreas, it may produce an increased inflammation and damage to that organ (12).Although most cases were moderate to moderate, some patients developed severe symptoms characterized by respiratory dysfunction and/or multiple organ failure (20).Cytokines in severe COVID-19 cause lymphocyte and NK cell reductions, affecting D-dimer, ferritin, procalcitonin, and C-reactive protein levels (3), making lymphocyte count a crucial marker for disease severity prediction (18). This study aimed to find the level differences between of CRP, D.dimer and some other laboratory biomarkers with Interleukin - 6 in orders find the correlation between each of those parameters among patients suffering from and severity related COVID19 and comparison with healthy control.

Materials and methods

Clinical samples collection

During the period from July 2022 to April 2023, seventy specimen collected from patients infected with SARS-COV2 from both gender with age ranging from (15-80) year. Patients who reported positive cases of SARS-CoV2 using nasopharyngeal swabs were diagnosed using a molecular test. An information sheet was filled out for each patient and written consent was obtained. The information included gender, age, body mass index, and the presence and absence of chronic diseases. The following lab tests were also included: complete blood count, HGB (Hb), HCT (Pcv) Lymphocyte, platelet count and total white blood cell count (WBC), C-reactive protein (CRP), and (D. dimer). Also 3 ml collect of blood were collected from both patients and control 100µl of the blood had mixed with EDTA then transferred into a new sterile test tube and 200 µl of DNA/ RNA shield was added to the blood and kept in the freezer until the working day. The rest of the blood have been separated by centrifugation for 20 min. and then the upper layer of serum has been transferred to a plane tube and then kept until the working day of ELISA.

Estimation of IL-6 using ELISA method

Prior to performing the procedure, serum samples, standards and required solutions were brought to room temperature, gently mixed, and left to equilibrate under room temperature for 30 minutes. The assay procedure was carried out according to the manufacturer's instructions, Company protocol was used BT- LAB (Bioassay Technology Laboratory), human IL-6 ELIZA Kit, Code (E0090Hu).Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2–8 °C. Add 50 ul of standard to the standard well. Note: Don't add antibodies to the standard solution because the standard solution contains biotinylated antibodies. Add 40 ul of sample to sample wells, and then add 10 ul of human IL-6 antibody to sample wells, and then add 50 ul of streptavidin-HRP to sample wells and standard wells (not blank control wells). Mix well. Cover the plate with a sealer. Incubate for 60 minutes at 37°C. Remove the sealer and wash the plate five times with wash buffer. Soak wells with 300 ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash five times with wash buffer. Blot the plate onto paper towels or other absorbent material. Add 50 ul of substrate solution A to each well, and then add 50 ul of substrate solution B to each well. Incubate the plate covered with a new sealer for 10 minutes at 37°C in the dark. Add 50-ul Stop Solution to each well; the blue colour will change to yellow immediately. Determine the optical density (OD value) of each well immediately using a micro plate reader set to 450 nm within 10 minutes after adding the stop solution. Calculations: A standard curve was plotted against the concentrations of the standard by tabulating in EXCEL sheet the absorbance of the pre-determined standards for each serum marker. Then an equation that fits the curve was created and used to obtain the level of the unknown samples (Figure 1).

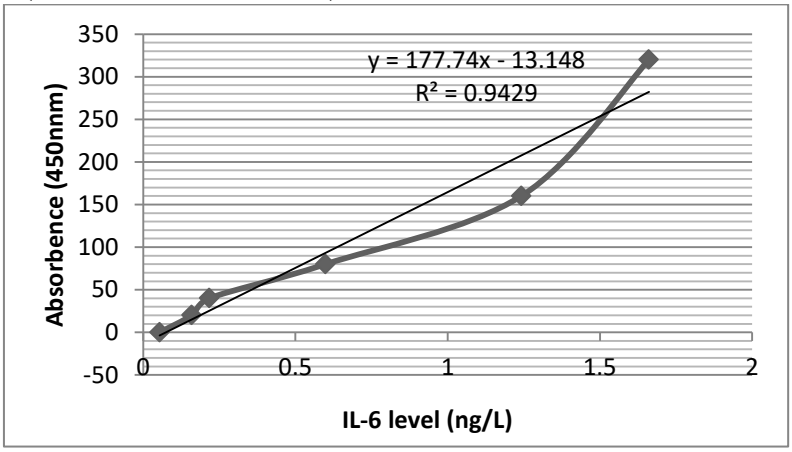


Figure (1): Standard curves of IL-6.

Complete blood count (CBC)

The blood specimen in EDTA tube was shaken up then was examined as soon as possible in CELL-DYN Ruby automated haematology analyzers to count white blood cells, lymphocyte, HCT, HGB and platelet.

C-reactive protein (CRP) and D.dimer

The gel tube containing serum was entered to the equipment and analyzed automatically The Roche Cobas C111 Chemistry Analyzer from Roche Diagnostics is used to detect C-reactive protein and D-dimer. CRP Normal Range: Less than 13 mg/l; High: greater than 13 mg/l and D-dimer Normal Range: Less than 255 ng/L; High: greater than 255 ng/L

Molecular diagnosis of COVID-19

Testing was performed on samples obtained from the patient through a nasopharyngeal swab and preserved in a VTM viral sampling tube. RNA was manually extracted according to the manufacturer's protocol (Add Bio, Germany). Then, COVID-19 RNA was qualitatively detected in the sample by a one-step RT-PCR method using the COVID-19 nucleic acid detection kit (*Maccura Biotechnology* Co. Ltd., 16#. China) by reverse transcription to complementary DNA (cDNA) into the RT-PCR reaction system and combined with specific primers and probes for PCR amplification (FAM, HEX, ROX, and CY5). PCR amplification parameters were prepared as follows: reverse transcriptase: 55 °C, 2 min, 1 cycle; Taq polymerase activation; pre-denaturing: 95°C, 10 sec, 1 cycle; denaturation: 95 °C, 1 sec,40 cycle; and annealing, extension, florescence acquisition: 58 °C, 15 sec, 40 cycle. When the reaction was finished, the results were analyzed and described as positive when the ct of three targets (FAM, ROX, and CY5) was < 38. The interpretation and annotation of the results were according to the criteria provided by the groups.

RNA isolation by TRIzol

RNA was extracted from serum samples according to the protocol of TRIzol Reagent. The serum (0.4ul) was mixed with 0.5 mL of TRIzol reagent in each tube, and the lysate was homogenized by pipetting up and down multiple times. For each tube, 0.2 mL of chloroform was added to the lysate before securing the tube top. All mixtures were incubated for 3 minutes before being centrifuged at 10,000 rpm for 10 minutes to separate the mixture into a lower organic phase, interphase, and a colourless upper aqueous phase. The aqueous phase containing the RNA was transferred to a new tube. 0.5mL of Isopropanol was added to the aqueous phase and incubated for 10 minutes then centrifuged for 10 minutes at 12,000 rpm, total RNA was precipitated and formed a white gel-like pellet at the bottom of the tube, and Supernatant was then discarded. 0.5mL of 70% ethanol was added to each tube and vortexes briefly then centrifuged for 5 minutes at 10000 rpm. Ethanol then aspirated and air-dried the pellet. Pellet was rehydrated in 50µl of Nuclease Free Water then incubated in a water bath or heat block set at 60°C for 15 minutes.

IL-6 gene expression detection

The required volume of each component was calculated. The IL-6 gene of interest was quantified using qRT-PCR SYBR. Green, synthesized cDNA was immediately used as a

template for PCR. Thermal cycler steps and conditions cDNA Reverse Transcription: Step 1, 42°C in 30 min; Step 2, 85 °C in 5 min; and Step 3, 4 °C. Macrogen Company provided these primers in lyophilized form. The detection of IL-6 expression was done using the RT-qPCR Biolab kit primer sequences used in this study.

Primer sequence: GABDH F-(5'ACAACTTTGGTATCGTGGAAG G3′) and GAPDH R (5'GCCATCACGCCACAGTTTC3′),

Primer sequence: IL-6 F-(5'AGGAGACTTGCCTGGTGAAA3′) and IL-6 R-(5'ACACACCCACCTTTTTCTGC3′), qPCR master mix (SYBR): 10 µl, forward and reverse Primer: 0.5 µl, cDNA Template: 4 µl, and Nuclease-Free Water up to 20 µl, The qPCR reaction was run, and the cycling protocol was programmed according to the thermal profile. The mixtures were subjected to the following thermal profile of IL-6 and GAPDH gene expression cycling parameters in an RT-PCR (Router gene): Initial denaturation at 95°C for 1 min, followed by 1 cycle of amplification at 60°C for 30 sec, followed by 40 cycles The samples were analyzed in triplicate, and GAPDH was used as an endogenous control for normalization. The normalized target amount in the sample is then equal to 2-ΔΔCt and this value can be used to compare expression levels in samples , The relative changes in mRNA expression levels were determined using comparative threshold cycle (CT) method (2-ΔΔCt).

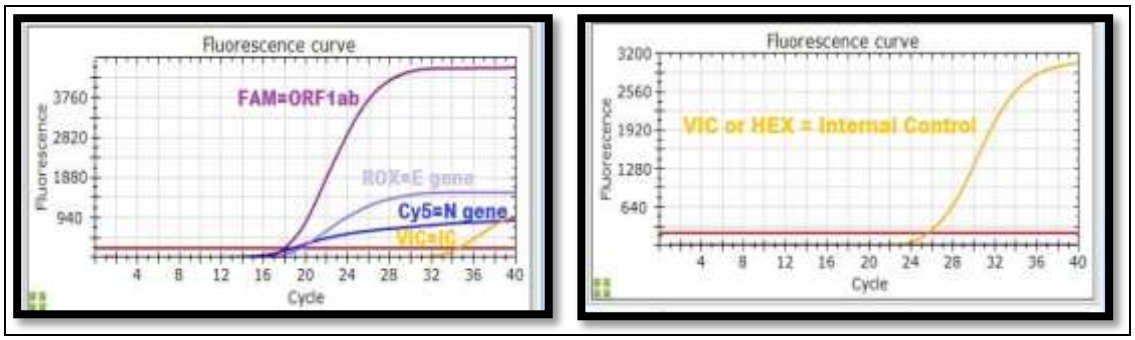
Statistical analysis

The Statistical Analysis System SAS (2012) program was used to detect the effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study (14). And use GraphPad Prism to plot the data.

Results and discussion

Isolation and detection of COVID-19

The sample population consisted of one hundred and five cases. Specimens were collected from 70 patients admitted to the ICUs of Medical City Hospital in Baghdad suspected of being infected with COVID-19, in addition to 35 healthy controls. All of them underwent a swab from the nasopharyngeal cavity by means of a real-time PCR test to confirm their health status and to distinguish between those infected with SARS-CoV2 and a control sample. Infection was detected by a multiplex real-time reverse transcription PCR system containing specific primers and a fluorescent probe targeting the ORF 1ab, E, and N genes of the SARS-CoV2 virus. Virus RNA load is detected by monitoring the fluorescence intensity of ORF1ab, E. and Gene N in real time. In addition, the endogenous control (GAPDH gene) can monitor the process of sample collection, preservation, transmission, nucleic acid extraction and amplification, which can effectively prevent false negative test results, as shown in Figure 2 (A,B).



(A) **(B)**

Figure (2): Covid-19 detection by a multiplex real-time reverse transcription PCR.(A): Positive results [FAM channel (ORF 1ab gene), ROX (E gene). Cy5 channel (N gene) and HEX or VIC channel (Internal Control)] ;(B): Negative results [VIC or HEX channel (Internal Control)]

Tahamtan and Ardebili's (24) study highlights the importance of nucleic acid detection in medical diagnosis, with PCR being the gold standard. Real-time reverse transcriptase-PCR (RT-PCR) is particularly relevant for early SARS-CoV-2 detection due to its specificity and sensitivity. A study conducted by Shen *et al.* (25) found that virus RNA sequence variation can affect real-time RT-PCR results, emphasizing the novel Corona virus's genetic diversity and quick evolution. For confirmation of COVID-19, an RT-PCR and chest CT scan are required(29).

Distribution of COVID-19 according to age and gender

The distribution of SARS-CoV2 infection according to the gender shod those 26 (37.1%) males and 44 (62.85%) females. According to age factor, the patients were then divided into three age groups: (15-45; 46-65 and 66-80) years and their numbers were 20 (28.5%), 35(50%), and 15(21.42%) respectively.

It was noted that the incidence rate was (0.0037 **) high significantly (50%) among the ages that ranged between 46-65 yrs, followed by the ages between 15-45 yrs. (28.5%) then 66-80 yrs (21.42%), In a study similar to our research by Ghazzi *et al.* (27) older individuals, particularly those with co-morbidities like hypertension and diabetes, etc., are more susceptible to SARS-CoV-2 infection and have higher mortality rates. In a study for Ad'hiah and Al-Bayatee (28) research, critical conditions were identified (mean age = 60.9 ± 12.0 years; age range = 32–84 years; 69.0% males).

Research shows that individuals aged 45-80 with chronic conditions are more likely to die and are more likely to be hospitalized. (22, 32).The effect of age on the SARS-CoV2 score has also been attributed to immune and inflammatory mechanisms. A pro-inflammatory state has been observed in the lungs of chronically ill elderly patients, and this may lead to abnormalities in immune responses in patients with SARS-CoV2 (23), this result is in agreement with Khudhr and Shehab (16), the most severity patients were the oldest that had further systemic diseases in comparison with other patients , Numerous studies have investigated the impact of SARS CoV-2 on the neurological system, particularly sensory nerves, where the virus causes loss of taste and smell(31).

The information of clinical features of patients were collected and the results showed that 39(78%) of them were suffering from fever,43(86%) headache, 47(94 %) loss of smell and tasting and 42(84%) cough

(82.05%), while 19(38%) of patients had a shortness of breath and 4(8%) diarrhea The usual symptoms of COVID-19 include fever , cough , shortness of breath , and muscle ache, some patients may have sore throat, rhino rhea, headache and confusion a few days before the onset of fever, indicating that fever is a critical symptom, but not the only initial manifestation of infection (10). The statistical analysis found highly significant differences among all parameters. Regarding severity of infection, 35 of positive SARS-CoV2 was considered as moderate level of infection while 35 was considered as severe infection.

A control sample of 35 apparently healthy individuals were included, and their serum profile for infectious pathogens antibodies tested and results of presence SARS-CoV2 were negative. Their ESR was below 20 mm/hour, and the CRP serum status was negative.

Expression level of IL-6 gene according to the severity of COVID-19 infection

In this study, IL-6 gene expression was examined in the innate immune system of 70 patients with SARS-CoV2 (35% moderate and 35% severe infection) using RT-PCR analysis to determine gene expression in innate immune cells. The results showed an elevated level of gene expression in SARS-CoV2 patients in general from IL-6, but patients with severe symptoms had higher gene expression in moderate cases compared to gene expression in the healthy control samples. Figure 3 illustrates the distribution sample fold of IL-6 gene expression and relations between moderate and severe SARS-CoV2. The study findings revealed a significant variation in IL-6 levels among individuals with severe and moderate SARS-CoV2 infections, as well as the control group. The severe infection group exhibited the highest IL-6 level (10.66±6.12), followed by the moderate infection group (5.19±2.72), while the control group had the lowest level (1.12±0.15). This difference in IL-6 levels was found to be statistically significant (P-value = 0.001), indicating that the severity of SARS-CoV2 is associated with higher IL-6 levels.

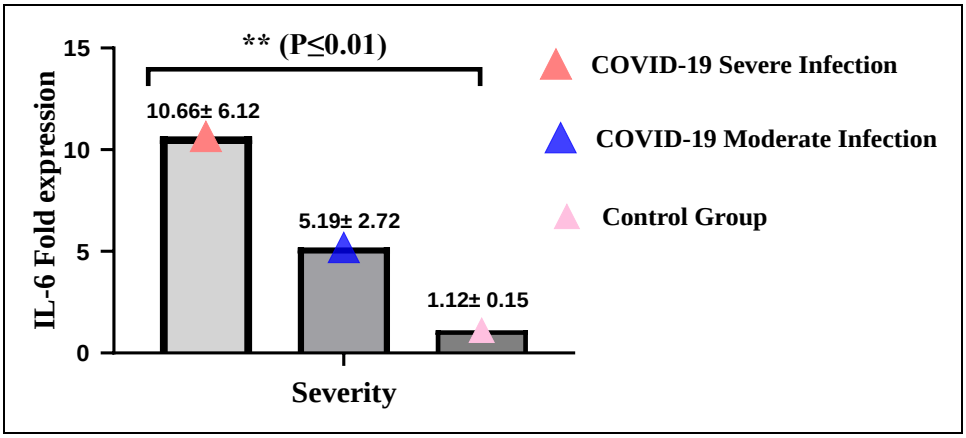


Figure (3): IL-6 gene expression upon Moderate and Severe infection of COVID-19.

The dye was specifically bound to the genes under study via the Syper Green chemistry. Figure (4) shows the amplification plots for the IL-6 genes.

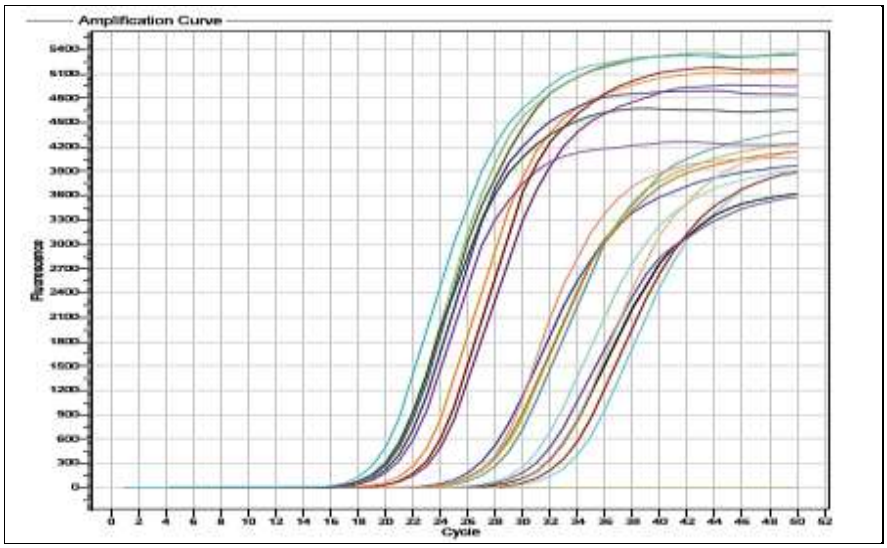


Figure (4): Amplification plots for IL-6 and housekeeping gene on RT-qPCR

Initially, the results of the qRT-PCR experiment showed that the IL-6 pathway genes were elevated compared to the healthy control group in SARS-CoV2 patients, whether these cases were severe or moderate, males or females. Severe cases of patients infected with SARS-CoV2 were more likely to express IL-6 genes than patients who were diagnosed with moderate symptoms. For the severity-symptomatic group of patients, the means of expression for the IL-6 gene was the melting curve, as illustrated in (Figure 5).

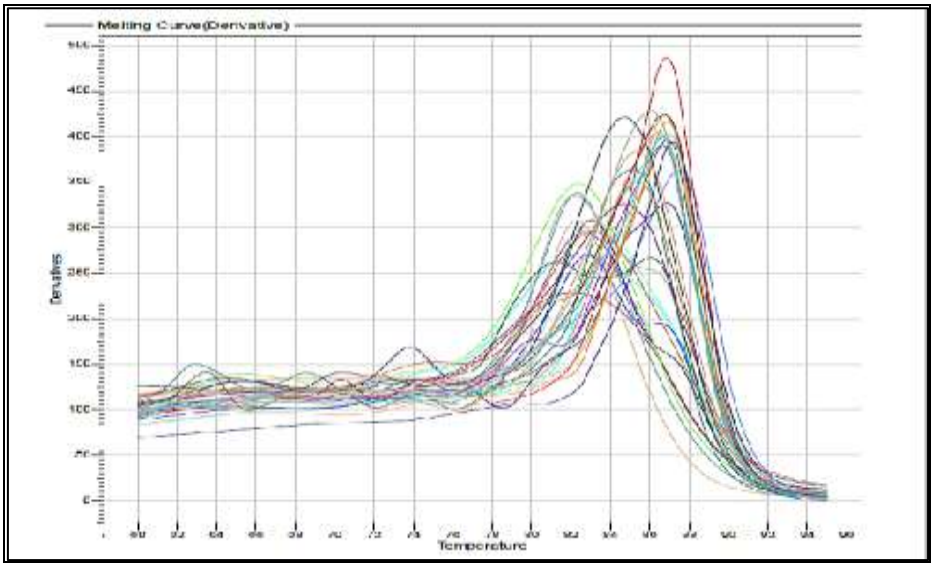


Figure (5): Melting curve for IL-6 and housekeeping gene in RT-qPCR.

The results of the IL-6 level showed a significant difference between the severe and moderate COVID-19 and control groups with IL-6 in a higher level of severe infection followed by moderate infection to control 10.66±6.12 and 5.19±2.72, respectively). P-value = 0.001), and the level of IL-6 in the COVID-19 patients with severe infection (10.66±6.12)

showed high significance with both COVID-19 patients with moderate infection and patients, A study of ICU patients indicated considerably higher levels of IL-6 in the non-survivor group(6). Also, another analysis that some hospitals found also revealed higher baseline IL-6 levels in severe cases. Baseline IL-6 levels appear to be a reliable predictor of severe COVID-19 risk (21).

Correlation of serum levels and fold of IL-6 genes expression

The IL-6 serum level was measured using an enzyme-linked immunosorbent assay, and the expression level of the IL-6 gene was measured using RT-PCR. The IL-6 gene expression relations upon moderate and severe infection of SARS-CoV2 are illustrated in this figure (6) and table (1).

The relationship between serum levels and gene expression fold of IL-6 demonstrated a strong positive correlation, indicating the effectiveness of both techniques. The serum levels refer to the amount of IL-6 present in the blood, while gene expression fold represents the increase or decrease in the expression of the IL-6 gene. The strong positive correlation suggests that higher levels of IL-6 in the blood are associated with increased expression of the IL-6 gene. This finding highlights the efficiency and reliability of these techniques in assessing the relationship between serum levels and gene expression fold of IL-6.

Overall, studying the correlation between serum levels and fold expression of IL-6 genes can provide valuable insights into the regulation and activity of this pro-inflammatory cytokine. This information can contribute to our understanding of various diseases and potentially aid in the development of therapeutic interventions targeting IL-6 signaling pathways.

Table (1): IL-6 measured using ELIZA and RT-PCR

Cytokine	Severe infection (Mean± Std. Deviation)	Moderate infection (Mean± Std. Deviation)	Healthy control (Mean± Std. Deviation)
IL-6 (Con. By ELIZA)	263.71± 87.63a	92.54± 27.47b	27.16± 3.78c
IL-6 (Fold expression)	10.66±6.12a	5.19±2.72 b	1.00 ±0.00 c
Means having with the different letters in same column differed significantly, ** (P≤0.01).			

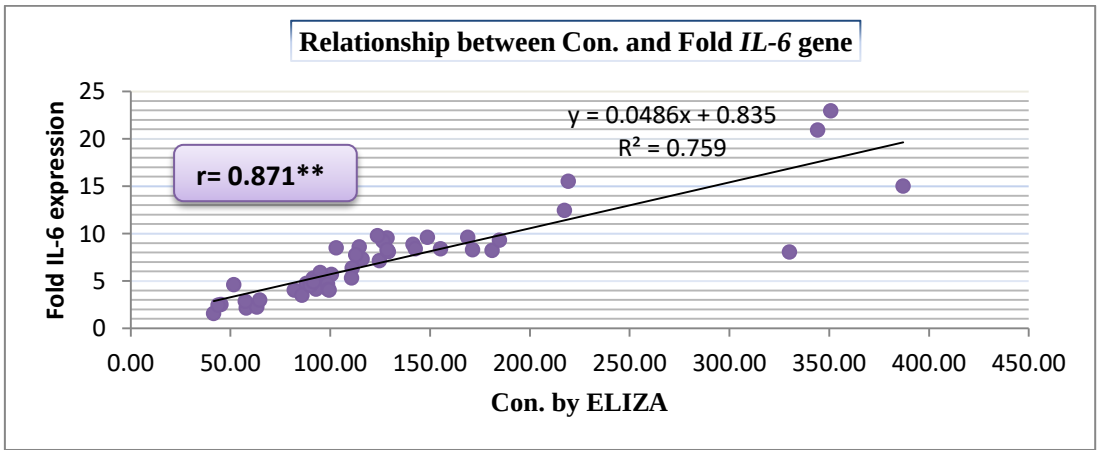


Figure (6): IL-6 Correlation Coefficient of Serum Level and expression Fold

The results present the mean mRNA gene expression for the studied cytokine (IL-6) has significant positive correlation with serum level among

patients group with respect to the level of infection and healthy group. This finding in agreement with study conducted by Noh *et al*. (26) who indicated that the serum IL-6 concentration was increased by viral-induced inflammation prior to treatment and In response to tissue damage and infection, IL-6 is immediately and transiently produced by various cells, including fibroblasts, vascular endothelial cells, mast cells, macrophages.

Laboratory biomarkers Characteristics among COVID-19 patients

The patients with severe disease were older and had high D-dimer, C-reactive protein, IL-6, and with decrease lymphocyte. This result is in agreement with (13), there was a significant difference between moderate and severe cases for CRP and D. dimer. There was elevated CRP in both moderate and severe cases; the median value in moderate cases was 35 mg/L compared with 150 mg/L in severe cases; the relationship was significant (P≤0.005); D-dimer values were elevated in both groups, ranging from 229–425 ng/ml in moderate cases, while in severe cases they ranged from 358–700 ng/ml; there was a significant relationship (P≤0.005)The present study showed that severe patients have low rates of lymphocytes; values were few in both groups, ranging from 6.2–7.3 10^9/L in moderate cases while in severe cases, values ranged from 2.3–4.3 10^9/L. The results of the comparison of CBC, CRP and D-dimer among the studied groups are summarized in Table 2. The samples of COVID-19, severe and moderate infection.

Table (2): Comparison of laboratory biomarkers according to moderate and severe level of patient COVID-19and healthy control

Parameters	SARS-CoV2 Severe (mean±SD.)	SARS-CoV2 moderate (mean±SD.)	Healthy Controls (mean±SD.)
D.dimer (ng/L)	742.79 ± 317.55a	360.20±139.55b	227.86 ± 66.40c
CRP (mg/l)	116.16±27.07a	84.51±18.55b	12.34±0.378c
WBC (10^9/L)	15.90 ± 3.34a	14.11 ± 2.48b	7.99 ± 2.09c
Lymphocyte (10^9/L)	3.78 ± 1.63a	5.79 ± 1.7b	22.65 ± 2.66ab
Platelets (10^9/L)	312.75± 82.49a	265±58.21b	223.75±15.84c
HGB	12.62 ± 1.74ab	12.11 ± 2.80ac	11.94 ± 1.77cd
HCT	43.0±1.953ab	43.2±1.70bc	38.3±1.701cd
Means having with the different letters in same column differed significantly (P≤0.01).**			

The severity of adult COVID-19 severity was found to be positively correlated with CRP, ESR, and ferritin levels, indicating a significant difference between severely ill and non-severe groups(33)

The results of the present study showed that patients with severe COVID-19 were more likely to develop lymphocyte decrease than moderate patients. This result is in agreement with (9). The present study showed that the mean levels of lymphocyte count were lower in the patient group when compared with those of the control group. This result is in agreement with(8). The present study showed that the mean levels of HGB were higher in the patient group when compared with those of the control group. This result is in agreement with (11).

The present study showed that D-dimer levels were higher in severe patients than moderate patients; D-

dimer levels have been linked to the severity of COVID-19. High D-dimer levels are common in acute-illness individuals with a number of infectious and inflammatory diseases, and the present study found that the D-dimer level in severe COVID-19 patients was higher than control, with values greater than 0.22μg/ml (4). Further research is necessary due to the considerable relationship between the COVID-19 severity, progression, and susceptibility and the D-dimer, CRP, and ferritin levels (32).

Correlation of biomarkers and interlukin-6 genes

The correlation coefficients of hemoglobin (HGB) in severe patients with COVID-19 were (r = 0.115). There was a non-significant association (P≤0.005) between HGB and study markers IL-6 and the WBC correlation coefficient (r = 0.566) in IL-6. Concerning lymphocytes, a correlation coefficient of (r = -0.627) among study markers IL-6 the correlation coefficient of platelets in a severe patient with COVID-19 was 0.184 in IL-6. The C-reactive-reactive (CRP) correlation coefficient was (r = 0.789) in IL-6. the studied groups in Table 3 .Regarding the correlation coefficient of D. dimer in severe patients with COVID-19, it was (r = 0.666) in IL-6, which is a transmembrane receptor that plays a crucial role in the innate immune response to viral pathogens. The dysregulation of IL-6 has been implicated in the pathogenesis of several diseases, including COVID-19; these findings support the idea that IL-6 is involved in a positive feedback loop in COVID-19; both severe and moderate are illustrated in this (Figure 7).

Table (3): Correlation between IL-6 and some biomarkers in moderate and severe COVID-19 cases

Laboratory data		IL-6 gene
HCT	Correlation Coefficient	0.145
	P-value	0.313
HGB	Correlation Coefficient	0.115
	P-value	0.423
WBC(10^9/L)	Correlation Coefficient	0.566
	P-value	0.000018
lymphocyte(10^9/L)	Correlation Coefficient	-0.627
	P-value	0.0000011
Platelets (10^9/L)	Correlation Coefficient	0.164
	P-value	0.253
CRP mg/l	Correlation Coefficient	0.789
	P-value	0.00000000001
D.dimer ng/L	Correlation Coefficient	0.666
	P-value	0.00000012
**** Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level**		

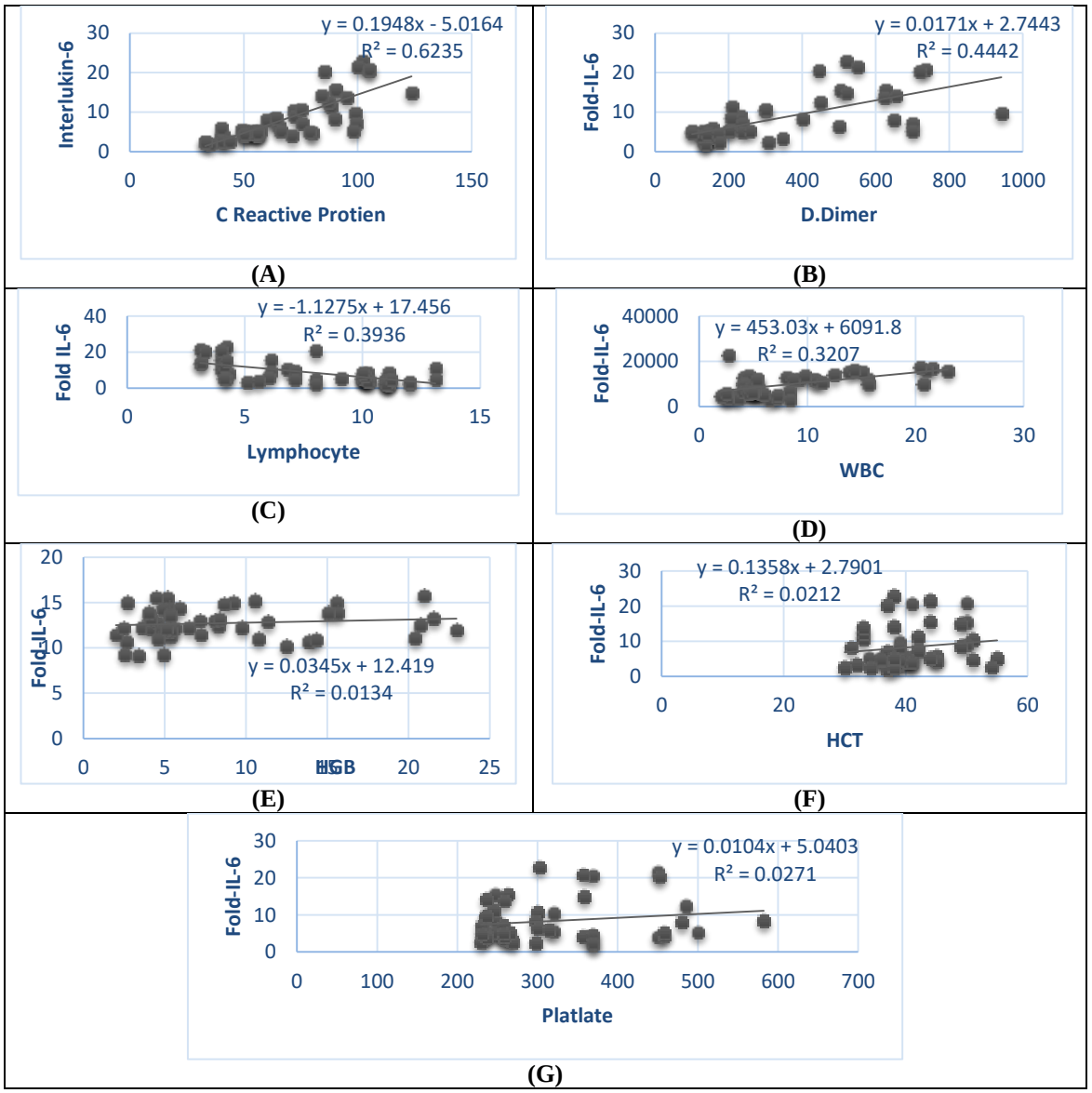


Figure (7): Covid -19 correlation coefficient between IL-6 and laboratory biomarkers: (A): Correlation IL-6 and C. Reactive Protein, (B): Correlation IL-6 and D.dimer, (C): Correlation IL-6 and Lymphocyte, (D): Correlation IL-6 and WBC, (E): Correlation IL-6 and HGB, (F): Correlation IL-6 and HCT and (G): Correlation IL-6 and Platelets.

Conclusion

Innate immune parameters may serve as potential predictive biomarkers for the prognosis of COVID-19 disease. These findings support the idea that IL-6 are involved in a positive feedback loop in the pathogenesis of COVID19, However, over activation IL-6 can also contribute to the cytokine storm and excessive inflammation in severe COVID-19 cases. The levels of IL-6 and C-reactive protein correlate significantly with the severity of COVID-19.

COVID-19 severe infection patients were older, had high D. dimer, C-reactive protein, IL-6, WBC, and low lymphocytes at admission.The results of both IL-6 levels showed a significant difference between the patients with COVID19 compared to control. The relationship between IL-6 expressions is represented in showed, IL-6 level showed a strong positive relationship

with C Reactive Protein, and weak positive with WBC, D.dimer, HGB, HCT and Platelets.

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