Evaluating the Effectiveness of the Covid-19 Vaccine and the Cytokine Storm.

Jihan Qais Dakhal AL-lamy[1], Ahmed D. Jabbar[2]
[1,2] Department of Biology, College of Science, Wasit University, Iraq
Correspondence author: Email: jihan.Qais1986@gmail.com

Abstract
Corona viruses belong in the corona family and are part of the nidovirales order. The family of closed-cell, positive-sense, single-stranded RNA viruses known as coronaviruses (CoVs) possesses a diverse variety of traits. The respiratory, digestive, hepatic, and neurological systems are all affected, and they cause a wide range of health issues in both people and animals. In this study, individuals who received the Pfizer vaccine had their IL6 and IL10 levels measured. The study includes 167 cases, they were distributed on the basis of age and sex into four groups, The first group included 47 patients, which is the control group, the second group included 42 patients' diseases only, the third group included 39 patients with Covid-19 only, and the fourth group included 39 patients Covid-19 and diseases. The study indicated a significant increase in (p <0.01) in IL6 for patients with Covid and chronic diseases with chronic diseases compared to other groups. The study we obtained indicated a significant increase in (P< 0.01) in IL10 ratio in relation to gender and age groups. According to these findings, the Corona virus may have an impact on and pose a threat to everything that results in the body's critical organs failing.
Keywords: Covid 19, IL6 Level Measurement, IL10 Level Measurement, Cytokine storm.

1. Introduction

In late December 2019, Wuhan, Hubei Province, China, saw an outbreak of an unknown disease identified as pneumonia of unclear etiology. [1]. the outbreak had infected 9720 people in China, resulting in 213 deaths, and 106 persons in 19 other countries. [2]. China alerted the World Health Organization of the outbreak on December 31st. The virus was recognized as a coronavirus on January 7th, having >95% homology with the bat coronavirus and >70% resemblance to the SARS-CoV. The virus was also found in environmental samples from the Huanan Sea food market, indicating that it originated there, [3]. Several independent laboratories identified the primary agent of this unusual pneumonia as a new coronavirus (nCoV) a few days later [4]. The World Health Organization has temporarily designated the causal virus as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the corresponding infected disease as coronavirus disease 2019 (COVID-19) [5]. Coronaviruses (CoVs) infect a wide spectrum of vertebrate species, causing respiratory, gastrointestinal, hepatic, and neurological illnesses [6]. Coronaviruses are members of the Coronavirinae subfamilyde. Coronaviridae, coupled with Torovirinae, make up the order Nidovirales' Coronaviridae family. Alphacoronavirus, Betacoronavirus, Deltacoronavirus, and Gammacoronavirus are the four genera of Coronavirinae. Alphacoronavirus, Betacoronavirus, Deltacoronavirus, and Gammacoronavirus are the four types of coronaviruses. [7]. Coronaviruses are encased positive sense RNA viruses with spike-like projections on their surface that give them a crown-like appearance under the electron microscope, hence the name coronavirus. Coronaviruses have a spherical shape with a ring of huge, bulbous projections on the surface. Coronaviruses infect cells largely by connecting their spike protein to the cell receptors of their host (fig 1). [8].

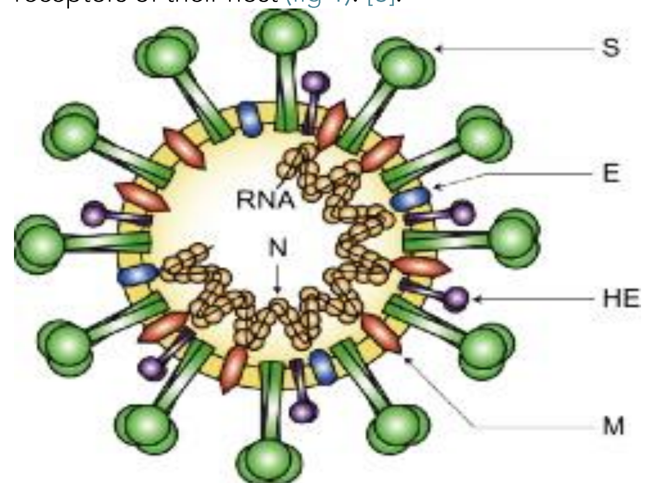

Figure 1. Coronavirus virion structure. The genome RNA is complexed with the N protein to form a helical cased within the viral membrane, HE, hemagglutinin-esterase; S, spike; E, small membrane envelope; M, membrane are all transmembrane proteins.

multiple studies have revealed that N protein interacts with nsp3, a protein that plays a vital role in virus replication early in infection. Because it initiates the infection process, the communication between viral S protein and Angiotensin-converting enzyme 2 (ACE2) on the host cell surface is of great interest. The binding affinity of SARSCoV-2 S protein to ACE2 is approximately 1020 times higher than that of SARS-CoV S protein binding to ACE2, according to cryoelectron microscopy (Cryo-EM) structural study

doi.org/10.31838/hiv22.02.544 Received: 03.07.22, Revised: 15.08.22, Accepted: 28.08.22

[9]. The COVID-19 vaccine candidate mRNA-1273 from Moderna is an LNP-encapsulated mRNA vaccine that encodes the S protein and is administered in two doses through intramuscular (IM) injection. [10]. The US Food and Drug Administration (USFDA) has approved the use of the Pfizer-BioNTech (BNT162b2 vaccine) and the Moderna vaccine in the present pandemic emergency (mRNA-1273 vaccine), Both are mRNA vaccines (in which the SARS-CoV-2 spike glycoprotein (S) antigen is encoded by mRNA and then created in lipid nanoparticles or LNPs) with over 90% efficiency in candidates 16 years of age and over with 2 days within 21 days.[11].

2. Materials and Methods

Study design and samples collection

This a case- control study from patients from November 2021 to February 2022, at Al¬-Kindi Teaching Hospital in Baghdad. After obtaining the approval of the Ministry of Health for the study.As well as the consent of the patients who are unable to give blood,as well as for the lack of sufficient information for collection the sample.We have collacted 167 samples from patients who underwent the pfazer vaccine after confirming them through the vaccination card,a blood sample was taken 21 days after receiving the vaccine or more.They were divided into four groups

Group1: included 47 people who had received the Pfizer vaccine, without previously infected with corona and without chronic diseases.

Group 2: included 42 peoples who had received the pfazer vaccine, without previously infected with corona and had chronic diseases.

Group 3; included 39 peoples who had received the pfazer vaccine,who were previously infected with corona and without chronic diseases.

Group 4: included 39 people who received the pfazer vaccine, who were previously infected with corona and chronic diseases

Samples collection

Venus blood of 10 ml was drawn using a sterile syrings, which can be disposed of after use.We distributed the blood into two tubes, I put 3ml of blood into EDTA tube for RT PCR genetic testing, the sample was left for 15 minutes at a temperatureof (20-25 C) after which the sample was placed in the freezer at a freezing point -20 C. The second tube was placed 7 ml of blood in a tube gel for the purpose of the immunological study (ELISA) enzyme-linked immune-sorbent assay and left the sample for 15 minutes at room temperature (20-25 C) after that we put the samples in a centrifuge approximately 2500-3000 cycles per minute and for 5 minutes to obtain the serum, We distribute the serum into three eppendorf tubes, after which the tubes were placed in the freezer at a freezing degree of -20C.

Human Interleuken 6, IL6

proceduer

1. Dilution of Standards

Dilute the standard by small tubes first, then pipette the volume of 50ul from each tube to microplate well,each tube use two wells, total ten wells		
180 pg/ml	**Standard No.1**	**300ul Original Standard +150ul Standard diluents**
120 pg /ml	Standard No.2	300ul Standard No.1+150ul Standaed diluents
60 pg/ ml	Standard No.3	150ul Standard No.2 +150ul Standard diluents
30 pg/ml	Standard No.4	150ul Standard No.3 +150ul Standard diluation
15 pg/ ml	Standard No.5	150ul Standard No.4+150ul Standard diluation

2. In the Microelisa stripplate, leave a well empty as blank control. In sample wells, 40μl Sample dilution buffer and 10μl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
3. Incubation: incubate 30 min at 37°C after sealed with Closure plate membrane.
4. Dilution: dilute the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T).
5. Washing: carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.
6. Add 50 μl HRP-Conjugate reagent to each well except the blank control well.
7. Incubation as described in Step 3.
8. Washing as described in Step 5.
9. Coloring: Add 50 μl Chromogen Solution A and 50 μl Chromogen Solution B to each well, mix with gently shaking and incubate at 37°C for 15 minutes. Please avoid light during coloring.
10. Termination: add 50 μl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

Human Interluken 10, IL10

Proceduer

Dilution of StandardsDilute the standard by small tubes first, then pipette the volume of 120ul from each tube to microplate well,each tube use two wells, total ten wells .		
320pg/ml	**Standard No.5**	**120ul Original Standard+120ul Standard diluents**
160pg/ml	Standard No.4	120ul Standard No.5+120ul Standard diluents
80pg/ml	Standard No.3	120ul Standard No.4+120ul Standard diluents
40pg/ml	Standard No.2	120ul Standard No.3+120ul Standard diluents
20pg/ml	Standard No.1	120ul Standard No.2+120ul Standard diluents

2. In the Microelisa stripplate, leave a well empty as blank control, Standerd solution well:add 50ul standerd and 50ul HRP,Sample well:add 40ul sample and 10ul IL-10 Ab, 50ul HRP.
3. Incubation: incubate 60 min at 37°C after sealed with Closure plate membrane.
4. Dilution: dilute the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T).
5. Washing: carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.
6. Coloring: Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37°C for 10 minutes. Please avoid light during coloring. (Fig 2).
7. Termination: add 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow. (fig 3)
8. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution

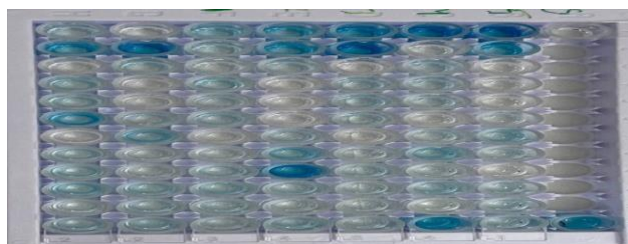

Figure 2: IL-10 assay by ELISA method, where the blue color shows positive IL-10

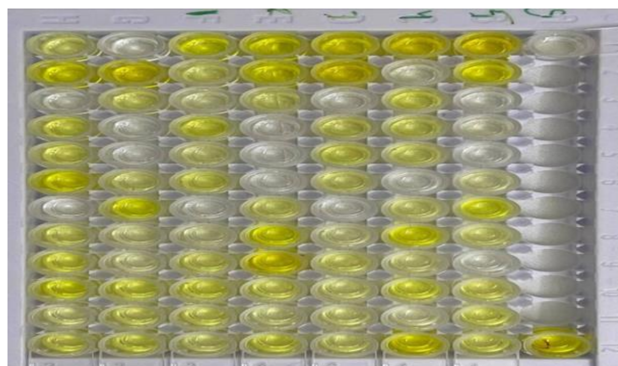

Figure 3: IL-10 assay by ELISA method, where the yellow color shows positive IL-10 after adding a stop solution.

3. Statistical Analysis

Data were analyzed using SPSS statistical software, version 23. ANOVA test was performed for independent samples between patients, recovery and control groups, and the resulting values were expressed as mean and standard deviation (SD). Statistical tests were significant at $p < 0.05$ and highly significant at $p < 0.01$ with 95% confidence interval. ROC CURVE was carried out for the studied parameters and the area under the curve (AUC) was determined. The sensitivity and specificity of the above-mentioned analyzes were also determined, and the cut-off value was determined.

4. Results and Discussion

The level of cytokines was measured, and the results were compared for gender and age groups.

Table 1: Measuring the level of IL-6 and comparing the results between gender and the groups participating in the research.

Age (Year) Group	Female Mean+SD	Male Mean+SD	P-value	Sign.
Control	D,a 35.159±4.242	D,a 34.088±4.476	0.416	Non-Sign.
isease only	C,a 50.93±9.29	C,b 43.84±5.46	0.005	Sign.
With COVID only	B,a 64.58±5.02	B,a 64.29±5.66	0.868	Non-Sign.
COVID+D.	A,b 78.358±4.213	A,a 82.93±5.63	0.007	Sign.
P-Value	0.0002	0.00005		
Sign.	Sign.	Sign.		

Table 2: Measuring the level of IL-10 and comparing the results between gender and the groups participating in the research.

Age (Year) Group	Female Mean+SD	Male Mean+SD	P-value	Sign.
Control	D,a 58.29±8.48	D,a 56.75±8.81	0.554	Non-Sign
Disease only	C,a 90.76±18.41	C,b 76.89±10.92	0.006	Sign
With COVID only	B,a 118.61±10.3	B,a 117.16±11.32	0.684	Non-Sign
COVID+D.	A,b 145.79±8.42	A,a 154.77±11.25	0.008	Sign
P-Value	0.00043	0.0002		
Sign.	Sign	Sign		

Table 3: Measuring the level of IL-6 and comparing the results in terms of age groups and groups participating in the research

Age (Year)Group	20-30 Mean+SD	31-40 Mean+SD	41-50 Mean+SD	51->60 Mean+SD	P-value	Sign.
Control	D,a 36.09±4.89	D,ab 33.91±3.93	D,ab 33.98±2.92	D,b 32.64±3.73	0.0163	Sign.
Disease only	C,a 48.71±8.41	C,a 53.55±10.71	C,ab 49.54±6.53	C,b 42.81±6.09	0.037	Sign.
With COVID only	B,a 63.56±5.01	B,a 66.02±5.15	B,a 65.58±6.97	B,a 62.61±5.88	0.710	Non-Sign.
COVID+D.	A,a 80.01±5.07	A,a 82.39±6.24	A,a 78.06±4.44	A,a 81.54±5.55	0.395	Non-Sign.
P-Value	0.00011	0.00005	0.00003	0.00016		
Sign.	Sign.	Sign.	Sign.	Sign.		

Table 4: Measuring the level of IL-10 and comparing the results in terms of age groups and groups participating in the research.

Age (Year) Group	20-30 Mean+SD	31-40 Mean+SD	41-50 Mean+SD	51->60 Mean+SD	P-value	Sign.
Control	D,ab 57.59±8.82	D,b 52.09±10.63	C,a 63.06±5.58	D,ab 59.02±5.43	0.045	Sign.
Disease only	C,a 99.55±11.42	C,c 67.3±11.14	C,bc 73.08±14.24	C,b 78.52±9.31	0.0004	Sign.
With COVID only	B,a 117.66±10.22	B,a 116.03±13.85	B,a 121.06±9.39	B,a 119.2±7.39	0.854	Non-Sign.
COVID+D.	A,b 146.85±8.49	A,b 145.91±8.39	A, 146.36±11.35	A,a 160.57±8.64	0.001	Sign.
P-Value	0.00021	0.0006	0.00005	0.00014		
Sign.	Sign.	Sign.	Sign.	Sign.		

Table 5: Comparison of cytokines (IL-6, IL-10) with the proteins participating in the research.

Group Parameters	Control Mean+SD	Disease only Mean+SD	With COVID only Mean+SD	COVID+D. Mean+SD	P-value	Sign.
IL-6	D 34.521±4.369	C 47.55±8.42	B 64.412±5.323	A 80.702±5.44	0.0004	Sign.
IL-10	D 57.37±8.62	C 84.15±16.67	B 117.79±10.77	A 150.39±10.84	0.00051	Sign.

By using an ELISA, we measured the serum levels of these cytokines in COVID-19 patients and non-COVID-19 patients. The results revealed that IL-10 and IL-6 levels were significantly higher in COVID-19 patients than in non-COVID-19 patients, while IL-6 levels were only significantly higher than in healthy individuals. [12]. There was a cytokine storm in COVID-19 patients, as evidenced by the fact that IP-10 in these individuals was much lower than in patients with other disorders. The potential study value of IL-10 and IL-6 for COVID-19[13]. It should be mentioned that the serum levels of all cytokines in patients were strongly positively connected with age, and that COVID-19 preferred male and older individuals. Furthermore, the serum levels of IL-10 in male COVID-19 patients were significantly greater than those in female patients. According to the cytokine production rule, IL-6 was considerably negatively connected with SARS-CoV-2 IgM while IL-10 was significantly negatively correlated with SARS-CoV-2 IgG.[14]. According to our findings, older adults with COVID-19 have greater serum concentrations of IL-6 and IL-10 than do individuals under the age of 65. Serum levels of IL-6 are not directly correlated with age, whereas IL-10 is independently correlated with age and disease severity. This appears to be mostly due to the comorbidity index, which has a strong correlation with IL-6 levels in people 65 and older as well as the severity of the condition.[15]. Like our findings, other studies have shown that some COVID-19 patients not only have increased levels of IL-6 and IL-10, but also that the severity of the disease is predicted by this imbalanced cytokine production.[15]. As a result, Abers and colleagues examined the levels of 66 soluble biomarkers in 175 Italian patients with various COVID-19 severity levels. [16]. demonstrated that higher levels of IL-6 and IL-10 were connected to mortality on their own. Furthermore, IL-6 and IL-10 levels are higher in individuals with severe illness, according to a recent meta-analysis of 44 publications (50 studies) involving 7865 patients. [17]. The fact that most of these studies did not account for intervening variables like age, disease severity, and comorbidities highlights the significance of our findings and the necessity of taking other factors (like disease severity and comorbidity) into account when assessing the risk of older people with COVID-19. [18]. Innate response causes infected cells to generate several pro-inflammatory mediators during viral illnesses, including COVID-19, resulting in a powerful inflammatory response. [19]. At the locations of tissue inflammation, a variety of cells, including macrophages, lymphocytes, fibroblasts, endothelium and epithelial cells, generate and release IL-6 and IL-10 into the bloodstream. [20]. Once created, these cytokines stimulate macrophages and other phagocytic cells to eliminate the infection-causing virus as well as infected cells from the area.[21]. IL-6 is a crucial pleiotropic mediator involved in a few inflammatory processes, including infection and tissue damage. Contrarily, IL-10 is well known for its anti-inflammatory capabilities, which start innate and adaptive immune responses while restricting pro-inflammatory responses to minimize tissue damage.[22]. As a result, IL-10 suppresses the activity of T cells, NK cells, and macrophages during the acute phase of an infection. These cells, while essential for the removal of viruses, are also major contributors to tissue damage. Therefore, IL-10 may hinder effective viral eradication while reducing collateral tissue harm.[23]. Therefore, the balance between the serum levels of the cytokines IL-6 and IL-10 may be a valuable indicator of illness severity, and more research should focus on this aspect. [24]. To understand how this cytokine contributes to the severity of disorders, it may be necessary to comprehend the mechanisms that control the synthesis and production of IL-6. We suggest that other independent pathways may be engaged in this regulation and that IL-10 may play a significant role in this process, even if the involvement of TNF- in the synthesis of IL-6 was not proven in this work. As a result, it has recently been proposed that the early production of IL-10 following SARS-CoV-2 infection may in fact constitute a negative feedback mechanism that works to reduce inflammation brought on by other proinflammatory mediators. [24]. Endogenous IL-10 may act as a proinflammatory substance that promotes the synthesis of additional

cytokine storm mediators when it is produced more frequently. The first data are insufficient, and additional research is necessary to confirm the function of IL-10 in COVID-19.[25].

5. Conclusion

This study evaluates some important vital signs of Covid-19 patients. The results of this study showed an increase in the indices of IL6, IL10 and cytokine storm in Covid-19 patients and the efficacy of the vaccine.

Conflict of Interest

None

Funding

Self

Ethical Clearance

Not required

References

1. Li, Q. (2020). An outbreak of NCIP (2019-nCoV) infection in China—wuhan, Hubei province, 2019– 2020. China CDC Weekly, 2(5), 79.
2. He, F., Deng, Y., & Li, W. (2020). Coronavirus disease 2019: What we know? Journal of medical virology, 92(7), 719-725.
3. Singhal, T. (2020). A review of coronavirus disease-2019 (COVID-19). The indian journal of pediatrics, 87(4), 281-286.
4. Singhal, T. (2020). A review of coronavirus disease-2019 (COVID-19). The indian journal of pediatrics, 87(4), 281-286.
5. Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., ... & Tan, W. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The lancet, 395(10224), 565-574.
6. Grabherr, S., Ludewig, B., & Pikor, N. B. (2021). Insights into coronavirus immunity taught by the murine coronavirus. European Journal of Immunology, 51(5), 1062-1070.
7. Roy, A., Datta, S., Roy, M., Alghamdi, S., Rajab, B. S., Babalghith, A. O., & Islam, M. (2022). Nanomaterials and bioactive compounds against SARS-CoV-2. Journal of Nanomaterials, 2022.
8. Khedekar, N., Shiragave, S., More, P., Jagtap, V., Ingalwad, P., & Mohite, S. (2020). COVID–19: A REVIEW OF CLINICAL FEATURES, DIAGNOSIS AND TREATMENT.
9. Yin, C. (2020). Genotyping coronavirus SARS-CoV-2: methods and implications. Genomics, 112(5), 3588-3596.
10. Arashkia, A., Jalilvand, S., Mohajel, N., Afchangi, A., Azadmanesh, K., Salehi-Vaziri, M., ... & SARS CoV-2 Rapid Response Team of Pasteur Institute of Iran (PII). (2021). Severe acute respiratory art and future prospects. Reviews in medical virology, 31(3), e2183.
11. Gargano, J. W., Wallace, M., Hadler, S. C., Langley, G., Su, J. R., Oster, M. E., ... & Oliver, S. E. (2021). Use of mRNA COVID-19 vaccine after reports of myocarditis among vaccine recipients: update from the Advisory Committee on Immunization Practices—United States, June 2021. Morbidity and Mortality Weekly Report, 70(27), 977.
12. McElvaney, O. J., McEvoy, N. L., McElvaney, O. F., Carroll, T. P., Murphy, M. P., Dunlea, D. M., ... & McElvaney, N. G. (2020). Characterization of the inflammatory response to severe COVID-19 illness. American journal of respiratory and critical care medicine, 202(6), 812-821.
13. Zhao, Y., Qin, L., Zhang, P., Li, K., Liang, L., Sun, J., ... & Zhang, Y. (2020). Longitudinal COVID-19 profiling associates IL-1RA and IL-10 with disease severity and RANTES with mild disease. JCI insight, 5(13).
14. Grau-Expósito, J., Sánchez-Gaona, N., Massana, N., Suppi, M., Astorga-Gamaza, A., Perea, D., ... & Genescà, M. (2021). Peripheral and lung resident memory T cell responses against SARS-CoV-2. Nature communications, 12(1), 1-17.
15. Luporini, R. L., Joice, M. D. A., Kubota, L. T., Martin, A. C. B. M., Cominetti, M. R., de Freitas Anibal, F., & Pott-Junior, H. (2021). IL-6 and IL-10 are associated with disease severity and higher comorbidity in adults with COVID-19. Cytokine, 143, 155507.
16. Abers, M. S., Delmonte, O. M., Ricotta, E. E., Fintzi, J., Fink, D. L., de Jesus, A. A. A., ... & NIAID COVID-19 Consortium. (2021). An immune-based biomarker signature is associated with mortality in COVID-19 patients. JCI insight, 6(1).
17. Zhang, Y., Wang, M., Zhang, X., Liu, T., Libby, P., & Shi, G. P. (2021). COVID-19, the pandemic of the century and its impact on cardiovascular diseases. Cardiology discovery, 1(04), 233-258.
18. Deserno, M. K., Borsboom, D., Begeer, S., Agelink Van Rentergem, J. A., Mataw, K., & Geurts, H. M. (2019). Sleep determines quality of life in autistic adults: A longitudinal study. Autism Research, 12(5), 794-801.
19. Conti, P., Caraffa, A., Gallenga, C. E., Ross, R., Kritas, S. K., Frydas, I., ... & Ronconi, G. (2020). Coronavirus-19 (SARS-CoV-2) induces acute severe lung inflammation via IL-1 causing cytokine storm in COVID-19: a promising inhibitory strategy. J Biol Regul Homeost Agents, 34(6), 1971-1975.
20. Tatiya-Aphiradee, N., Chatuphonprasert, W., & Jarukamjorn, K. (2019). Immune response and inflammatory pathway of ulcerative colitis. Journal of basic and clinical physiology and pharmacology, 30(1), 1-10.
21. Pasrija, R., & Naime, M. (2021). The deregulated immune reaction and cytokines release storm (CRS) in COVID-19 disease. International Immunopharmacology, 90, 107225.
22. Bazlini Baharun, B. N., & Safuan, S. (2021). Macrophages Polarization and Tumor Immunity in Metastatic Cancer: A Review. International Medical Journal, 28(2).
23. Jubel, J. M., Barbati, Z. R., Burger, C., Wirtz, D. C., & Schildberg, F. A. (2020). The role of PD-1 in acute and chronic infection. Frontiers in immunology, 11, 487.
24. Luporini, R. L., Joice, M. D. A., Kubota, L. T., Martin, A. C. B. M., Cominetti, M. R., de Freitas Anibal, F., & Pott-Junior, H. (2021). IL-6 and IL-10 are associated with disease severity and higher comorbidity in adults with COVID-19. Cytokine, 143, 155507.
25. Yang, L., Xie, X., Tu, Z., Fu, J., Xu, D., & Zhou, Y. (2021). The signal pathways and treatment of cytokine storm in COVID-19. Signal transduction and targeted therapy, 6(1), 1-20.