Immune checkpoint inhibitors PD-1, PDL-1 With IL-6 and TNF-alpha in SARS-COV-2 patients: single center study in Salahddin province

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Abstract

Background: The coronavirus disease 2019(COVID-19) was identified in Wuhan, China, in December 2019. And on February 24, 2020, Iraq received a report of its first COVID-19 patient. A student from Iran who traveled there. The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a zoonotic virus that causes this disease. They are contagious from humans to humans, from humans to animals, and from animals to animals. Although most COVID-19 individuals experience a mild sickness similar to the flu or show no symptoms, a minority number of patients experience severe pneumonia, ARDS, or multi-organ failure. Additionally, it appears to have a mortality rate of about 6.4%. Methodology: A total of (90) samples included in this study, which will be divided into three groups. The Case group will consist of 60 patients (30 sever and 30 moderate cases based on the disease severity as defined by the WHO guidelines) with positive PCR COVID-19 test results, and the Control group will be composed of 30 individuals with asymptomatic and PCR negative for SARS-cov-2. who were attended to the COVID-19 specialized ward in the salahddin province between December 2022 and February 2023, oropharyngeal swabs, SARS-CoV-2 nucleic acid (RT-PCR) was used to confirm the positive status, an ELISA test to detect concentration cytokines (IL-6, TNF-alpha, and immune checkpoint inhibitors including PD-1 and PDL-1). Results: The case group patients' blood levels of IL6, TNF-alpha, PD-1, and PDL-1 were considerably greater than those of the healthy control group. The level of IL6 being mean SD (58.71±39.52 vs 10.45±10.95) with P-value of 0.001) pg/ml. for TNF-alpha, (126.3±570.70 vs 73.1±413.48 with P-value of 0.001) pg/ml, PD-1level were (2.14± 91.833 vs 0.64± 30.284) with P-value of 0.001) ng/ml, and (5.977±4.449vs 0.837±0.348with P-value of 0.001) ng/ml for PDL-1. There was a significant variation in serum level of IL6 between severe and non-severe patients and control groups; for IL6 higher mean ± SD for the severing group. Increased level of IL6 inpatients showed significant correlation with PD-1 and PDL-1. Conclusion: The increased serum levels of IL6 and increased level of PD-1,PDL-1compared to the control group, indicate the host's immune responses against the coronavirus infection and the "cytokine storm "that caused by a sudden acute rise in circulating levels of different pro-inflammatory cytokines like IL-6 and may lead to acute lung injury and ARDS with death As a result, the serum concentration of these biomarkers could be considered a reflection of COVID-19 severity, and these findings point to different immuno-regulatory events during SARS-CoV-2 infection, which could be useful in early detection of severe COVID-19 infected patients, as well as early recognition of Cytokine Strom, which could lead to a better outcome.

Keywords: COVID-19, IL-6, PD-1, PDL-1, TNF-alpha

1. Introduction

Coronaviruses (CoV) are viruses that can cause a variety of respiratory diseases in humans, ranging from a simple cold to severe respiratory distress syndrome. In Latin, the word "corona" signifies "crown." SARS-CoV-2, also known as COVID-19 (coronavirus disease 2019), is a new CoV illness that is posing an increasing global health hazard. (1) Coronaviruses (CoVs) are a genus of enclosed, positive-sense, single-stranded RNA viruses belonging to the Coronaviridae family with a varied variety of features. (2). Viruses with pin-like projections on their surface, which look as a crown under an electron microscope, with diameters ranging from sixty to one hundred and forty nanometers. Coronaviruses cause a variety of respiratory problems, ranging from the flu to pneumonia (3) .Two novel CoVs that have developed and cause fatal human diseases are the Middle East respiratory syndrome CoV (MERS-CoV) and the severe acute respiratory syndrome CoV (SARS-CoV) (4).

SARS-CoV-2, the new virus shared high similarity with the coronavirus that caused SARS in 2002-2004, (5). Human-to-human transmission of SARS-CoV-2 was quickly established, as it was with the coronaviruses that cause SARS and MERS, but the virus displayed far more infectivity than the other two coronaviruses. (6).

The cluster of differentiation279 (CD279) also known as Programmed cell death protein -1 (PD-1), is a

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protein on the surface of cells that regulates the immune system's response to human body cells by down-regulating the immune system and promoting self-tolerance by suppressing T cell inflammatory activity. (7).

The PD-1/PD-L1 signaling pathway is responsible for balancing the stimulatory and inhibitory signals necessary for successful immune responses to microbes as well as has been linked to a variety of diseases and self-tolerance (8). PD-1's ligand is expressed on macrophages and DCs and is regulated when they are activated (9). Some immune cells, such as T cells, NK cells, B cells, and monocytes, express PD-1, DCs, macrophages, vascular endothelial cells, and other cells express PD-L1, which is the ligand for PD-1 (9).

Interleukin-6 is a glycoprotein that excreted by several cell types, including macrophages, monocytes, eosinophils, hepatocytes, and glial cells. The interleukin-6, a pro-inflammatory cytokine, encourages the development of neutrophils, cytotoxic T-lymphocytes, and natural killer cells (10). When cells are stimulated by IL-1 or tumor necrosis factor (TNF)-a, or when toll-like receptors (TLR)-4 are activated, IL-6 production and release are induced (11).

Tumor Necrosis Factor-alpha is a pro-inflammatory cytokine that regulates cell proliferation, differentiation, and apoptosis. It is located on chromosome 6p21.33 inside the HLA III region. Macrophages are one of the main sources of TNF-a. It is mostly produced by active macrophages and monocytes, numerous cells, including T and B cells, osteoblasts, smooth muscle cells, endothelium, epithelial, and tumor cells, can also release it. (12).

This study aimed to evaluate the IL-6 and TNF alpha levels in COVID-19 patients and to have an insight if there is a connection between the expression of programmed death-1 (PD-1) and its ligand programmed death-ligand (PD-L1).in Saladin Governorate Baghdad/Iraq

Methodology: A total of (90) samples included in this study, which will be divided into three groups. The Case group will consist of 60 patients, (54 male and 6 female). (30 sever and 30 moderate cases based on the disease severity as defined by the WHO guidelines) with positive PCR COVID-19 test results, and the Control group will be composed of 30 individuals with asymptomatic and PCR negative for SARS-cov-2. Who were attended to the COVID-19 specialized ward in the Saladin province between December 2021 and February 2022, the participants were all older than 18 years old. Oropharyngeal swabs, SARS-CoV-2 nucleic acid RT-PCR (BIO-RAD CFX96/ USA) was used to confirm the positive status, an ELISA test (Mindray, China) to detect concentration cytokines (IL-6, TNF-alpha, and immune checkpoint inhibitors including PD-1 and PDL-1).

2. Results

The current study revealed that 66% (n=60/90) of patients and 33 %(n=30/90) of control group, all of whom underwent a swab of the nasopharyngeal cavity by a Real-time PCR test to detect positive cases with SARS-CoV-2. According to the gender 54 (90%) were males and 6 (10%) females in patients' group while there were the same proportion in the control group, 15(50%) of male and female. The results of this study indicated that males are more frequently than females (90% verse 10%) in patients group, while it was the same proportion in control group (50% verse 50%). According to the age, we founded that the age group 70-79 years was more frequently (33.3%). there was a significant correlation between patients and control in age group, The Mean±SD of patients was (65.7±13.7 in severe group, (68.0±14.8) in moderate group and for (61.7 ±20.1) in control group.and the range of severe group was (38-85), (22-85) years in moderate groups,and (15-84) years in control group. Concerning the association between COVID-19 severities, there was a statistically significant relationship among the severity of COVID-19 with patients' age. The proportion of severe COVID-19 was significantly higher among the patients aged > 50 years (P= 0.894), as show in table (1).

The serum concentration of the IL-6 was measured in different groups and the results showed a statistically significant higher means in patient group (p<0.001). (78.59±38.15and 38.83±30.07) in severe and moderate group verse (10.45±10.95) in control group with a P-value of (0.001) (Table 2 &Figure 1).

Table (1): age and gender distribution in COVID-19 positive patients and control group

		Severe COVID-19		Moderate COVID-19		Control		P value
		No	%	No	%	No	%	
Age (years)	<40years	2	6.7	2	6.7	5	16.7	0.894
	40---49	4	13.3	2	6.7	2	6.7	
	50---59	1	3.3	2	6.7	1	3.3	
	60---69	9	30.0	7	23.3	8	26.7	
	70---79	10	33.3	10	33.3	10	33.3	
	=>80years	4	13.3	7	23.3	4	13.3	
	Mean±SD (Range)	65.7±13.7 (38-85)		68.0±14.8 (22-85)		61.7±20.1 (15-84)		
Gender	Male	26	86.7	28	93.3	15	50.0	0.001*
	Female	4	13.3	2	6.7	15	50.0	
* Using the Pearson Chi-square test (x2-test) at the 0.05 level, there is a significant difference between the percentages.								
# Using the Students' t-test at the 0.05 level, there is a significant difference between two independent means.								
^ ANOVA-test significant difference between more than two independent means at the 0.05 level.								

The current study of serum levels of PD-1 and PDL-1 showed a significant variation between the studied groups. Patients with severe COVID-19 had a significantly higher means of PD-1, and PDL-1 and TNF-alpha compared to the controls. Mean±SD of PD-1 was (3.138±2.066), (1.16±0.737), and (0.64±0.284) in sever, moderate and control group respectively with P-value (0.001). And the Mean± SD of PDL-1 was (8.539±4.634), (3.415±2.285), (0.837±0.348) in sever, moderate and control group respectively, with p.value (0.001). (Table 3) and figure (2, 3).

The TNF-alpha Mean±SD was (153.63±87.30), (99.06±31.75), (73.14±13.476) in sever, moderate and control group respectively with P-value (0.001) (Table 4 and figure 4).

Table (2): concentration level of IL-6 in COVID-19 patients.

		Severe COVID-19		Moderate COVID-19		Control		P value
		No	%	No	%	No	%	
IL-6 (pg/mL)	<10pg/mL	-	-	-	-	25	83.3	0.001*
	10---	-	-	9	30.0	3	10.0	
	20---	1	3.3	7	23.3	-	-	
	30---	1	3.3	4	13.3	-	-	
	40---	3	10.0	2	6.7	1	3.3	
	50---	7	23.3	1	3.3	1	3.3	
	60---	5	16.7	3	10.0	-	-	
	70---	3	10.0	2	6.7	-	-	
	80---	2	6.7	1	3.3	-	-	
	90---	1	3.3	-	-	-	-	
	=>100pg/mL	7	23.3	1	3.3	-	-	
	Mean±SD (Range)	78.59±38.15 (28.8-183.4)		38.83±30.07 (10.8-151.4)		10.45±10.95 (0.797-53.6)		

* Using the Pearson Chi-square test (x2-test) at the 0.05 level, there is a significant difference between the percentages.

Using the Students' t-test at the 0.05 level, there is a significant difference between two independent means

^ ANOVA-test significant difference between more than two independent means at the 0.05 level.

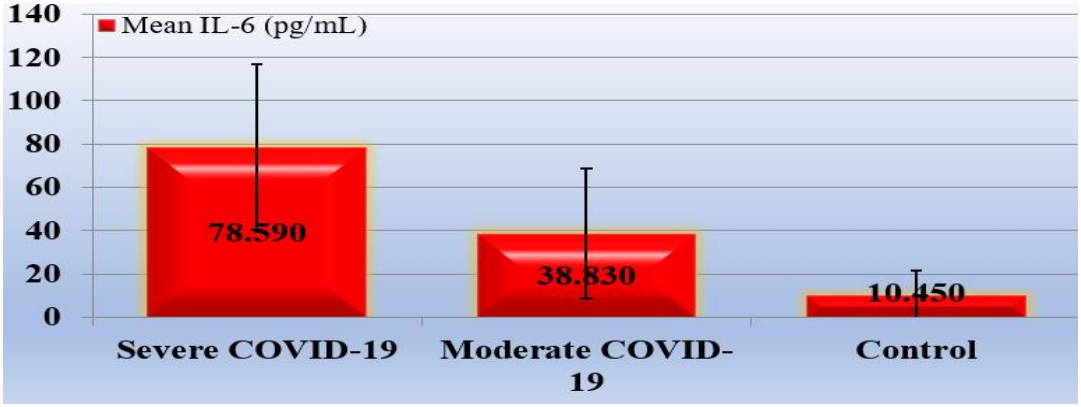

Figure (1): Mean of IL-6 in COVID-19 patients and control groups.

Table (3): the level of PD-1and PDL-1 in COVID-19 patients

		Severe COVID-19		Moderate COVID-19		Control		P value
		No	%	No	%	No	%	
PD-1 (ng/mL)	<1ng/mL	1	3.3	14	46.7	28	93.3	0.001*
	1---	9	30.0	14	46.7	2	6.7	
	2---	8	26.7	1	3.3	-	-	
	3---	6	20.0	1	3.3	-	-	
	4---	2	6.7	-	-	-	-	
	=>5ng/mL	4	13.3	-	-	-	-	
	Mean±SD (Range)	3.138±2.066 (0.36-9.1313)		1.16±0.737 (0.096-3.9)		0.64±0.284 (0.054-1.27)		
PDL-1 (ng/mL)	<1ng/mL	1	3.3	1	3.3	23	76.7	0.001*
	1---	-	-	10	33.3	7	23.3	
	2---	-	-	4	13.3	-	-	
	3---	1	3.3	8	26.7	-	-	
	4---	2	6.7	2	6.7	-	-	
	5---	6	20.0	2	6.7	-	-	
	6---	4	13.3	-	-	-	-	
	7---	5	16.7	2	6.7	-	-	
	8---	2	6.7	-	-	-	-	
	9---	-	-	-	-	-	-	
	=>10ng/mL	9	30.0	1	3.3	-	-	
	Mean±SD (Range)	8.539±4.634 (0.086-18.4)		3.415±2.285 (0.142-11.41)		0.837±0.348 (0.05-1.6403)		
	Mean±SD (Range)	153.63±87.30 (10.62-477.6)		99.06±31.75 (10.47-188.3)		73.14±13.476 (38.48-110.4)		

* Using the Pearson Chi-square test ((x2-test) at the 0.05 level, there is a significant difference between the percentages.

Using the Students' t-test at the 0.05 level, there is a significant difference between two independent means

^ ANOVA-test significant difference between more than two independent means at the 0.05 level.

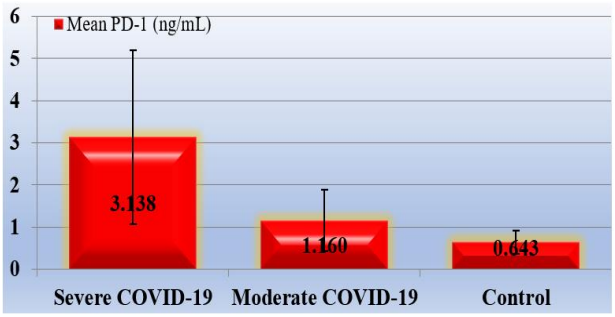

Figure (2): Mean of PD-1 in COVID-19 patients and control groups.

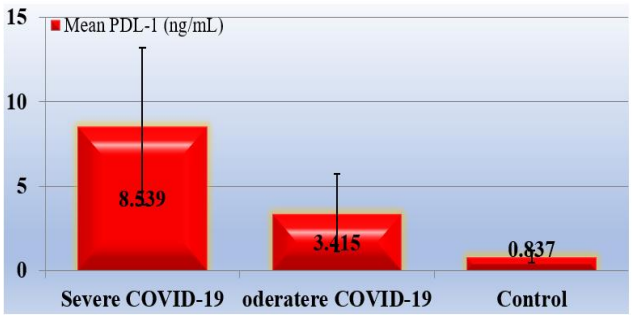

Figure (3): Mean of PDL-1 in COVID-19 patients and control groups.

Table (4): the level of TNF-alpha in COVID-19 patients.

		Severe COVID-19		Moderate COVID-19		Control		P value
		No	%	No	%	No	%	
TNF-alpha (pg/mL)	<50pg/mL	1	3.3	2	6.7	2	6.7	0.001*
	50---	5	16.7	14	46.7	27	90.0	
	100---	12	40.0	12	40.0	1	3.3	
	150---	6	20.0	2	6.7	-	-	
	=>200pg/mL	6	20.0	-	-	-	-	
	Mean±SD (Range)	153.63±87.30 (10.62-477.6)		99.06±31.75 (10.47-188.3)		73.14±13.476 (38.48-110.4)		

* Using the Pearson Chi-square test ((x2-test) at the 0.05 level, there is a significant difference between the percentages.

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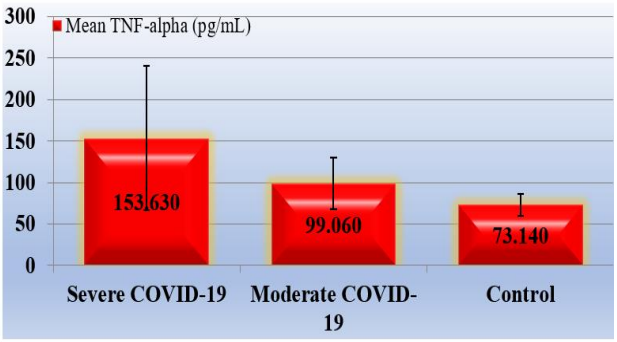

Figure (4): Mean of TNF-alpha in COVID-19 patients and control group.

3. Discussion

This study involved 90 patients in total; 60 of them had COVID-19 (Case group), which was further divided into a mild to moderate group (30 patients) and a severe group (30 patients), while the remaining (30persons) did not have COVID- 19 (Control group). The mean and standard deviation (SD) of age in case group were 66.8± 14.2 years, with a range of 22- 85 years, and had the highest proportion of older participants. Men predominated the case group as well (93.3 percent in the mild, and 86.7 percent in the severe group). When the case and control groups were compared by age and gender, there was no discernible difference (P= 0. 0. 750), however there was a strong association in the gender data (P= 0.001), in Yang et al study 67 percent of cases were male, which indicates a male predominance (13). In the Kong et al study, 2020, which had a mean age of 47.7 years, SD of 13.4 years, a male preponderance of 65 percent, a similar result was attained (14). The mean and SD ages of the patients in research done by Hammadi et al. published in 2021 were 50.4 + 15.1 years, the majority of patients were male, accounting for 59.2 percent of the study groups. A study done in 2021 by Sayah et al. they found that 67 percent of patients were male with a standard deviation of 13.95 years, the average age was 61.95 years. Patients in the severe group were older than patients in the non-severe group (P = 0.0003). (15). Elderly adults have been reported to be more prone to developing severe COVID-19 disease than those under the age of 50, which may be related to their higher prevalence of comorbid conditions and other health issues. Additionally, most studies show a correlation between male predominance and a higher risk of infection, which is related to male employment risk factors in crowded environments, social activity, and markets (16). Accordingly, a study done by Barman et al. in 2021who discovered that severe patients were substantially older than other patient groups (P=0.001). primarily male (P0.001), and had a high number of comorbidities, including hypertension, diabetes, and cardiovascular disease (P0.05) Although people of all ages are susceptible to COVID-19, older people are more likely to experience significant illnesses because to physiological changes brought on by aging as well as other underlying health conditions (17).

Regardless of age. A worse prognosis is typically associated with an earlier transition to intensive, respiratory, or even dialysis care. This brief laboratory report intended to determine whether we could pinpoint any pertinent function for risk prognosis utilizing additional tests that are frequently requested in positive SARS-CoV-2 hospitalized patients. (18).

In this investigation, both the severe and mild COVID-19 had noticeable increased levels of IL-6 (P 0.001). Mean and SD revealed that severe COVID-19

had higher mean IL-6 (P = 0.001). Liu and colleagues approved of his investigation same results in 2020
Other researchers found that 67.9% of patients had higher IL-6 levels upon admission. The percentage of patients in the severe category who had elevated IL-6 levels was significantly higher (P 0.001). (19) and that similar to our study. Additionally, the Coomes et al study from 2020 discovered that individuals with COVID19 had noticeably higher IL6 levels, which were connected to worse clinical outcomes, based on the comparability of data between investigations. The severe group had considerably higher blood levels of IL-6 than the non-severe group, in studies describing the immunological features of severe cases of COVID-19, significant levels of circulating inflammatory cytokines, especially interleukin-6 (IL-6), as well as high concentrations of various inflammatory indicators as complete blood count values have been documented (20).
One of the many function performed by IL6 is to encourage liver cells to produce acute phase proteins, which exacerbates the systemic effects of inflammation. (21). Interleukin-6 (IL-6) is involved in both innate and adaptive immune responses. Immune and non-immune cells that generate and secrete IL-6 include Mast cells, keratinocytes, mesangial cells, macrophages, DCs, monocytes, macrophages, and fibroblasts. Uncontrolled inflammatory reactions can result in a cytokine storm, ARDS, multi-organ failure, and even death. (22). Growing evidence points to a dysregulated host immune response to invasive pathogenic microorganisms as a critical element in the emergence of target organ dysfunction and as a significant contributor to morbidity and death.
T-cell activation is necessary for both inflammatory reactions and autoimmune diseases. An inhibitory mechanism called PD-1-PDL-1, which belongs to the CD28-B7 family, speed up T-cell death during an immunological response. According to the study's findings, patients with sPDL-1 levels were greater in COVID-19 than in controls. Even though this growth is significant, P. value (0.001), this could be explained by the possibility that inflammatory cells might up-regulate PDL-1 expression in order to stop the progression of the inflammation caused by plaque development and inflammatory cell buildup (23). The findings of this investigation were consistent with those of Mimura et al., who demonstrated that TNF-alpha can upregulate both PDL-1 and HLA class I. They found a substantial positive association between sPDL-1 and the inflammatory cytokine TNF-alpha (24). This association could be explained by the fact that PDL-1 is expressed and produced on the surface of activated T-cells, B-cells, and other cells when TNF-alpha from inflammatory immune cells in the plaque area interacts with the PDL-1 production promoter, leading to an increase in PDL-1 levels (24). Increased PDL-1 expression maintains vital balance during an inflammatory response by playing a crucial role in encapsulating activated T and B cells in lymph nodes, which in turn prevents the clonal expansion of antigen-specific T and B cells through apoptosis and the activation of regulatory T cells that produce inhibitory cytokines like IL-17 and other cytokines (Dong et al., 2021 (25). The interaction between inflammatory cells and pro-inflammatory cytokines TNF-alpha, IL-2, and others that are able to maintain the cells in an activated state may be the reason for the down regulation of PD-1 in activated T and B cells. In this study, an increased level of sPD-1 was found in case of COVID-19 and this may represent the main cause behind the continuous of the inflammation and plaque formation (26). A controlled balance between the expression of PDL-1 and PD-1, which they correlate positively in the control group, is evidenced by the strong positive correlation between sPDL-1 and sPD-1. This correlation refers to the positive increased and decreased to gather information regulating the immune response (25). TNF-alpha shows a positive link between both PD-1 and PDL-1, which are the most important inflammatory markers in this condition. (27). Our findings, which showed that elevated TNF-alpha levels boosted the production of PD-1 and PDL-1, together with an increase in damage and inflammation in COVID-19 patients who were already afflicted with a chronic illness, made it clear that TNF-alpha can stimulate the expression of PD-1 (28).

4. Conclusion

The increased serum levels of IL6 and increased level of PD-1,PDL-1compared to the control group, indicate the host's immune responses against the coronavirus infection and the "cytokine storm "that caused by a sudden acute rise in circulating levels of different pro-inflammatory cytokines like IL-6 and may lead to acute lung injury and ARDS with death As a result, the serum concentration of these biomarkers could be considered a reflection of COVID-19 severity, and these findings point to different immuno-regulatory events during SARS-CoV-2 infection, which could be useful in early detection of severe COVID-19 infected patients, as well as early recognition of Cytokine Strom, which could lead to a better outcome.

References

1-Umakanthan, S., Sahu, P., Ranade, A.V., Bukelo, M.M., Rao, J.S., Abrahao- Machado, L.F., Dahal, S., Kumar, H. and Dhananjaya, K.V., 2020. Origin, transmission, diagnosis and management of coronavirus disease 2019 (COVID- 19). Postgraduate medical journal, 96(1142), pp.753-758.
2-Chan, J. F. et al. Is the discovery of the novel human betacoronavirus 2cEMC/2012 (HCoV-EMC) the beginning of another SARS-like pandemic? J.Infect. 65, 477–489 (2012).
3-Birhanu Ayelign.Meaza Genetu. Tadelo Wondmagegn. Gashaw Adane. Markos Negash. Nega Berhane. TNF-α (−308) Gene Polymorphism and Type 2 Diabetes Mellitus in Ethiopian Diabetes

Patients. dovepress. 2019;12: 2453–2459.
4-Cheng, M. P., Papenburg, J., Desjardins, M., Kanjilal, S., Quach, C., Libman, M., Dittrich, S., & Yansouni, C. P. (2020). Diagnostic Testing for Severe Acute Respiratory Syndrome-Related Coronavirus 2: A Narrative Review. Annals of Internal Medicine, 172(11), 726–734.
5-WHO 2022
6-Prompetchara, E., Ketloy, C., & Palaga, T. (2020). Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic. Asian Pacific Journal of Allergy and Immunology, 38(1), 1–9.
7-Nicholas L, Syn; Teng, Michele W L; Mok, Tony S K; Soo, Ross A (2017). "De-novo and acquired resistance to immune checkpoint targeting". The Lancet Oncology. 18 (12): e731–e741
8-Chae Y. K., A. Arya, W. Iams et al., (2018): Current landscape and future of dual anti-CTLA4 and PD-1/PD-L1 blockade immunotherapy in cancer; lessons learned from clinical trials with melanoma and non-small cell lung cancer (NSCLC), J Immunother Cancer,6(1):39. 6- 1: 39.
9-Tseng S. Y., M. Otsuji, K. Gorski et al., (2001): "B7-DC, a new dendritic cell molecule with potent costimulatory properties for T cells193, Exp Med,193(7):839-46
10-Caio Marcio Barros de Oliveira, Rioko Kimiko Sakata, TSA, Adriana Machado Issy, Luis Roberto Gerola, Reynaldo Salomão. Cytokines and Pain. Rev Bras Anestesiol. 2011; 61(2): 255-265
11-Tadamitsu Kishimoto. IL-6: from its discovery to clinical applications. International Immunology. 2010; 22(5) :347–352
12-Birhanu Ayelign.Meaza Genetu. Tadelo Wondmagegn. Gashaw Adane. Markos Negash. Nega Berhane. TNF-α (−308) Gene Polymorphism and Type 2 Diabetes Mellitus in Ethiopian Diabetes Patients. dovepress. 2019;12: 2453–2459.
13-Yang, X., et al. (2020). "Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study." The Lancet. Respiratory medicine 475-481.
14- Kong, J., et al. (2020). "Analysis of hematological indexes of COVID-19patients from fever clinics in Suzhou, China." International journal of laboratory hematology 42(5): e204-e206.
15-Sayah, W., et al. (2021). "Interleukin-6, procalcitonin and neutrophil-tolymphocyte ratio: Potential immune-inflammatory parameters to identify severe and fatal forms of COVID-19." Cytokine 141: 155428-155428.
16-Zhang, J.-j., et al. (2020). "Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China." Allergy 75(7): 1730-1741.
17- WHO (2020)
18-Vargas-Vargas M and Cortés-Rojo C. Ferritin levels and COVID-Rev Panam Salud Publica. 2020;44: e72.
19-Liu, D. X., Liang, J. Q., & Fung, T. S. (2021). Human Coronavirus-229E, -OC43, -NL63, and -HKU1 (Coronaviridae). Encyclopedia of Virology, January, 428–440.
20-Soraya GV, Ulhaq ZS, Bintang AK. Akbar M, et al. Among authors: ulhaq zs. Front Aging Neurosci. 2022 May 17; 14:896371. doi: 10.3389/fnagi.2022.896371. eCollection 2022. Front Aging Neurosci. 2022. PMID: 35656536 Free PMC article. No abstract available
21-M Shohrati, The Role of Serum Level of Interleukin-6 in Severity of Pulmonary Complications of Sulfur Mustard Injuries July 2014.Iranian Journal of Medical Sciences
22-Abbasifard M, Khorramdelazad H. The bio-mission of interleukin-6 in the pathogenesis of COVID-19: A brief look at potential therapeutic tactics. Life sciences. 2020 Sep 15; 257:118097.
23-Canavan, M., Floudas, A., Veale, D.J. et al. (2021): The PD-1: PD-L1 axis in Inflammatory Arthritis. BMC Rheumatol, 11;5(1):1.
24-Mimura, K., Kua, L. F., Shiraishi, K., Kee Siang, L., Shabbir, A., Komachi, M., Suzuki, Y., Nakano, T., Yong, W. P., So, J., & Kono, K. (2014). Inhibition of mitogen-activated protein kinase pathway can induce upregulation of human leukocyte antigen class I without PD-L1-upregulation in contrast to interferon-γ treatment. Cancer science, 105(10), 1236–1244.
25-Dinesh, R. K., Hahn, B. H., & Singh, R. P. (2010). PD-1, gender, and autoimmunity. Autoimmunity reviews, 9(8), 583–587.
26-Sun Y, Li L, Wu Y, Yang K. (2020): PD-1/PD-L1 in cardiovascular disease. Clin Chim Acta.;505:26-30.
27-Pascal J H Kusters, Esther Lutgens, Tom T P Seijkens. (2018): Exploring immune checkpoints as potential therapeutic targets in atherosclerosis.,Cardiovasc Res. 114, 3, 01 368–377.
28-Gotsman I, Grabie N, Dacosta R, Sukhova G, Sharpe A, Lichtman AH. (2007): Proatherogenic immune responses are regulated by the PD-1/PD-L pathway in mice. J Clin Invest,117(10):2974-82.