Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed.nahrainuniv.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2024; Vol. 22(2)

The Role of Soluble Immune Checkpoint in SARS-CoV-2 Iraqi Patients

Anmar L. Talib[1] *PhD*, **Haider S. Kadhim**[1] *PhD*

[1]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq, [2]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background A novel coronavirus causing corona virus 2019 (COVID-19) disease, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was a serious cause of respiratory failure, cytokine release syndrome or myocarditis. The immune checkpoint blockade (ICB), cytotoxic T-lymphocyte associated proteins 4 (CTLA-4) and programmed cell death protein 1 / programmed death-ligand 1(PD-1)/PD-L1) axis, has been a recognized standard of treatment in various cancers and in the prevention of several viral infections.

Objective To evaluate soluble immune checkpoint levels (PD-1, PDL-1 and CTLA-4) in SARS-Cov-2 patients.

Methods This cross-sectional study included 90 patients with confirmatory SARS-CoV-2 test by polymerase chain reaction (PCR), as they were seeking for treatment at Medical City in Baghdad's Teaching Hospital (BTH). Patients with SARS-Cov-2 were divided into two groups: first group were those with severe SARS-CoV-2 symptoms lowest oxygen saturation (less than 90% O_2 saturation) and the second group are those with mild-moderate SARS-CoV-2 symptoms. The three ml of venous blood were taken from each patient to evaluate the levels of soluble PDL-1, PD-1, and CTLA-4 by using enzyme linked immunological sorbent test (ELISA).

Results Data regarding serum level of CTLA-4, PD-1 and PD-L1 in mild-moderate and severe covid-19 patients were found to be abnormally distributed. The median serum level of CTLA-4, PD-1 and PD-L1 in mild-moderate groups was much lower than that of severe cases with highly significant differences. Age demonstrated a positive significant correlation with each of CTLA-4, PD-1 and PD-L1. Soluble CTLA-4 had a positive significant correlation with each of PD-1 and PD-L1. Finally, PD-1 had a positive significant correlation with PD-L1.

Conclusion Soluble immune checkpoint markers are significantly increased in patients with severe covid-19 cases and soluble immune checkpoint markers are positively significant correlated with age.

Keywords Immune checkpoint, CTLA-4, PD1, PDL-1, SARS-C0v-2, COVID-19

Citation Talib AL, Kadhim HS. The role of soluble immune checkpoint in SARS-CoV-2 Iraqi patients. Iraqi JMS. 2024; 22(2): 268-273. doi: 10.22578/IJMS.22.2.11

List of abbreviations: COVID-19 = Corona virus 2019 disease, CDC = The centers for disease control and prevention, CTLA-4 = Cytotoxic T-lymphocyte associated proteins 4, ELISA = Enzyme linked immune sorbent assay, ICU = Intensive care unit, PCR = Polymerase chain reaction, PD-1 = Programmed cell death protein 1, PD-L1 = Programmed death-ligand 1, SARS-CoV1 = Severe acute respiratory syndrome coronavirus 1, SARS-CoV2 = Severe acute respiratory syndrome coronavirus 2, WHO = world health organization ,

Introduction

A novel coronavirus at the end of 2019, an epidemic was discovered in Wuhan, Hubei Province, China [(1)]. The same tropism receptor, angiotensin-converting enzyme 2 (ACE2), was used by both the severe acute respiratory syndrome coronavirus 2

(SARS-CoV2) and severe acute respiratory syndrome coronavirus 1 (SARS-CoV1) to penetrate the host human cell ACE2 [2]. Most corona virus 2019 disease (COVID-19) patients were relatively mild and moderate symptoms, but nearly exactly 15% progress to severe pneumonia and around 5% develop acute respiratory distress syndrome (ARDS), both of which require immediate treatment [3]. Immune checkpoint blockade (ICB), which targets the cytotoxic T-lymphocyte associated proteins 4 (CTLA-4) and programmed cell death protein 1 / programmed death-ligand 1 (PD-1)/PD-L1) axis, has been a recognized as standard of treatment in various cancers and in the prevention of several viral infections. Additional treatment modalities, such as radiation and chemotherapy, were employed more often in combination regimens that included both PD-1 and CTLA-4 [4]. The immune checkpoints was mainly evolved as a tool to avoid immune attack that was very aggressive injury to healthy tissue, when response to acute and chronic infections, up-regulation of the PD-1, PDL-1 and CTLA-4 were shown, and checkpoint signaling modulation has shown early promise to enhance responses to infections that last a long time [5].

The most common of the SARS-Cov-2 patients were elderly (between 30-79 years) to become mild; however, 14% were severe cases, and about 5% acquired critical illness with a significant death rate [6]. The severe SARS-Cov-2 disease was linked to delayed innate immune responses, early immunosuppression, lymphopenia, typically neutrophilia and cytokine storm [7,8]. The activity of immune cells, and hence the immune response, was closely controlled by a variety of stimulatory and inhibitory receptors that associated with SARS-Cov-2 infection [9].

This study aimed to determine SARS-Cov-2 patients' levels of soluble immunological checkpoints (PD-1, PDL-1, and CTLA-4).

Methods

Ninety patients with confirmed SARS-Cov-2 by Polymerase chain reaction (PCR) were included in this study, and they were seeking treatment at Medical City in Baghdad's Teaching Hospital (BTH). Patients with SARS-Cov-2 were divided into two groups: those with Severe SARS-Cov-2 symptoms (low oxygen saturation) less than 90 % and those with mild-moderate SARS-Cov-2 symptoms. Patients with autoimmune illness, malignant, diabetes, under the age of 18 and pregnant women were excluded.

A (3 ml) were obtained from each patient to evaluate the levels of soluble PDL-1, PD-1, and CTLA-4. The samples were separated by centrifugation at 3000 rpm for five minutes and kept at -20°C to be utilized for the enzyme linked immune sorbent test (ELISA).

Results

In this study, the patients with mild-moderate infection showed lower mean age (44.58±15.96 years) than those with severe infection (56.73±10.85 years) with highly significant differences. Although mild-moderate group had higher frequency of females than severe group (51.11% vs. 33.33%), the difference was not significant (Table 1).

Data regarding serum level of CTLA-4, PD-1 and PD-L1 in mild-moderate and severe COVID-19 cases were found to be non-normally distributed. The median serum level of CTLA-4, PD-1 and PD-L1 mild-moderate group were 0.57 ng/ml, 0.51 ng/ml and 0.64 ng/ml, respectively, which were much lower than that of severe cases (0.78 ng/ml, 0.88 ng/ml and 0.89 ng/ml, respectively) with highly significant differences (Table 2).

Table 1. Demographic characteristics of the study population

Check point markers		Mild-moderate (n=45)	Severe (n=45)	*P* value
Age (years)	Mean±SD	44.58±15.96	56.73±10.85	<0.001
	Range	19.0-87	24-85	
Sex	Male	22 (48.89%)	30 (66.67%)	0.088
	Female	23 (51.11%)	15 (33.33%)	

Table 2. Comparison of CTLA-4, PD-1 and PD-L1 levels between mild-moderate and severe cases of COVID-19

Check point markers		Mild-moderate (n=45)	Severe (n=45)	*P* value
CTLA-4 (ng/ml)	Mean±SD	0.56±0.14	0.86±0.3	<0.001
	Median	0.57	0.78	
	Range	0.22-1.15	0.42-1.73	
PD-1 (ng/ml)	Mean±SD	0.49±0.16	0.89±0.26	<0.001
	Median	0.51	0.88	
	Range	0.19-0.99	0.45-1.65	
PD-L1 (ng/ml)	Mean±SD	0.65±0.26	1.02±0.47	<0.001
	Median	0.64	0.89	
	Range	0.2-1.65	0.64-0.274	

Spearman's correlation was used to explore the possible correlation between age and checkpoint markers and between checkpoint markers themselves. Age demonstrated a positive significant correlation with each of CTLA-4 (r = 0.281, P = 0.007), PD-1 (r = 0.282, P = 0.007) and PD-L1 (r = 0.219, P = 0.039). Soluble CTLA-4 had a positive significant correlation with each of PD-1 (r = 0.714, P <0.001) and PD-L1 (r = 0.602, P <0.001). Finally, PD-1 had a positive significant correlation with PD-L1 (r = 0.626, P <0.001) as shown in table 3.

Table 3. Spearman's correlation between age and check point markers

Variable	CTLA-4		PD-1		PD-L1	
	r	P value	r	P value	r	P value
Age	0.281	0.007	0.282	0.007	0.219	0.039
CTLA-4			0.714	<0.001	0.602	<0.001
PD-1					0.626	<0.001

Discussion

The WHO classified the COVID-19 spread as pandemic on March 11th 2020 [10]. COVID-19 incidence and mortality have been associated with older age and comorbidities, resulting in a worse prognosis for weak patients and more frequently requiring hospitalization, intensive

care unit (ICU) admission, and invasive tracheal intubation [11].

In this study, the characteristics of age groups Covid-19 ڕatients with mild-moderate infection associated with lower mean age than those with severe infection with highly significant differences. Although mild-moderate group had higher frequency of females than severe group but the difference was not significant. In China and Italy showed a case mortality rate of 2.3% in COVID-19 individuals, with more than half of the deaths occurring in patients 50 years of age or older [12]. Another previous study from Northern Italy showed that the total case-fatality rate in individuals 64 years or older were 36%, compared to 15% in younger patients [13].

In addition, the centers for disease control and prevention (CDC) recommended that the older persons should be more concern about COVID-19 and they were at a higher risk of developed sever SARS-Cov-2 disease than younger adults [14]. Also, other study that associated with risk age group includes clinical data on a large sample of COVID-19 patients who died in Italy (2020) that showed less than 9% of COVID-19 patients who died were under the age of 65 years. There were large proportion of patients had relevant chronic disease like cardiovascular disease, diabetes, cancer and respiratory disorders [15]. So, the current study suggest that main age of severe SARS-Cov-2 cases older 50 years of age could due to weaning of the immunity and the presence of associated disease like cardiovascular and other chronic diseases.

The median serum level of CTLA-4, PD-1 and PD-L1 mild-moderate groups, which were much lower than that of severe cases with highly significant differences. This result was in agreement with another study in estimation of soluble immune checkpoint in SARS-Cov-2 patients [16,17]. When SARS-CoV-2 infects target cells, it activates both innate and acquired immune system cells, especially T cells and dendritic cells (DCs) leading to enhance the expression of PD-1 on the surface of monocytes and DCs while suppressing the functions and differentiation of CD4+ T-cells. Furthermore, once DCs deliver antigens to CD8+ T-cells, CTLA-4 attaches to its ligand (CD-86), was located on the surface of DCs and was inhibits CD8+ T-cell activity [18].

Although the immune checkpoint therapies appear to inhibit the immune system and hence may be regarded potentially hazardous in the context of COVID-19, they selectively target individual inflammatory cytokines or mediators rather than a wide panel of immune system components. In fact, cytokine inhibitors have the ability to reduce the hyperinflammatory state associated with COVID-19 and hence have a positive effect. This idea is backed by the fact that the pro-inflammatory cytokines generated in COVID-19 appear to be more important for the host inflammatory response than those engaged primarily in viral clearance [19].

The Spearman's correlation was utilized to investigate the potential relationship between age and checkpoint markers, as well as between checkpoint markers themselves. The age had a strong positive correlation with CTLA-4, PD-1, and PD-L1, and the Soluble CTLA-4 had a positive significant correlation with each of PD-1 and PD-L1. Finally, PD-1 had a positive significant correlation with PD-L1. Aging is also connected with a loss in immune system efficiency and changes to it [20].

The highly level of this checkpoint that agree with a various study recently, established a link between T cell depletion (lymphopenia) and elevated expression of several inhibitory checkpoint molecules on T-cells in severe COVID-19 patients [21]. When SARS-CoV-2 infects target cells, it activates both innate and acquired immune system cells, especially T cells and DCs. Leading to enhance the expression of PD-1 on the surface of monocytes and DCs while suppressing the functions and differentiation of CD4+ T-cells. Furthermore, once DCs deliver antigens to CD8+ T-cells, CTLA-4 attaches to its ligand (CD-86) that located on the surface of DCs and inhibits CD8+ T-cell activity [22].

In conclusions, soluble Immune checkpoint markers are significantly increased in patients with COVID-19 in severe cases, also these markers are positively significant correlated with age.

Acknowledgement

The authors express their gratitude to the participants for their generous contribution to this study.

Author contribution

Dr. Talib: conducted the sampling, work and writing the manuscript. Dr. Kadhim: supervised the work.

Conflict of interest

There are no conflicts of interest.

Funding

Self-funded.

References

1. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395(10223): 497-506. doi: 10.1016/S0140-6736(20)30183-5.
2. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020; 579(7798): 270-3. doi: 10.1038/s41586-020-2012-7.
3. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020; 382(8): 727-33. doi: 10.1056/NEJMoa2001017.
4. Kruger S, Legenstein ML, Rösgen V, et al. Serum levels of soluble programmed death protein 1 (sPD-1) and soluble programmed death ligand 1 (sPD-L1) in advanced pancreatic cancer. Oncoimmunology. 2017; 6(5): e1310358. doi: 10.1080/2162402X.2017.1310358.
5. Wykes MN, Lewin SR. Immune checkpoint blockade in infectious diseases. Nat Rev Immunol. 2018; 18(2): 91-104. doi: 10.1038/nri.2017.112.
6. Wu Z, McGoogan JM. Characteristics of and important lessons from the Coronavirus Disease 2019 (COVID-19) outbreak in China: Summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA. 2020; 323(13): 1239-1242. doi: 10.1001/jama.2020.2648.
7. Vardhana SA, Wolchok JD. The many faces of the anti-COVID immune response. J Exp Med. 2020; 217(6): e20200678. doi: 10.1084/jem.20200678.
8. Blanco-Melo D, Nilsson-Payant BE, Liu WC, et al. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. Cell. 2020; 181(5): 1036-45.e9. doi: 10.1016/j.cell.2020.04.026.
9. Ravetch JV, Lanier LL. Immune inhibitory receptors. Science. 2000; 290(5489): 84-9. doi: 10.1126/science.290.5489.84.
10. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. WHO. URL: https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020.
11. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimmun. 2020; 109: 102433. doi: 10.1016/j.jaut.2020.102433.
12. Porcheddu R, Serra C, Kelvin D, et al. Similarity in Case Fatality Rates (CFR) of COVID-19/SARS-COV-2 in Italy and China. J Infect Dev Ctries. 2020; 14(2): 125-8. doi: 10.3855/jidc.12600.
13. Grasselli G, Zangrillo A, Zanella A, et al. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy Region, Italy. JAMA. 2020; 323(16): 1574-81. doi: 10.1001/jama.2020.5394.
14. Centers for Disease Control and Prevention - CDC (U.S.). Implementation of mitigation strategies for communities with local COVID-19 transmission. 5-8-2020. URL: https://www.cdc.gov/coronavirus/2019-ncov/community/community-mitigation.html
15. Onder G, Rezza G, Brusaferro S. Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. JAMA. 2020; 323(18): 1775-6. doi: 10.1001/jama.2020.4683.
16. Westmeier J, Paniskaki K, Karaköse Z, et al. Impaired Cytotoxic CD8+ T Cell Response in Elderly COVID-19 Patients. mBio. 2020; 11(5): e02243-20. doi: 10.1128/mBio.02243-20.
17. Diao B, Wang C, Tan Y, et al. Reduction and functional exhaustion of T Cells in patients with Coronavirus Disease 2019 (COVID-19). Front Immunol. 2020; 11: 827. doi: 10.3389/fimmu.2020.00827.
18. Aghbash PS, Eslami N, Shamekh A, et al. SARS-CoV-2 infection: The role of PD-1/PD-L1 and CTLA-4 axis. Life Sci. 2021; 270: 119124. doi: 10.1016/j.lfs.2021.119124.
19. Schett G, Sticherling M, Neurath MF. COVID-19: Risk for cytokine targeting in chronic inflammatory diseases? Nat Rev Immunol. 2020; 20(5): 271-2. doi: 10.1038/s41577-020-0312-7.
20. Daste A, Domblides C, Gross-Goupil M, et al. Immune checkpoint inhibitors and elderly people: A review. Eur J Cancer. 2017; 82: 155-66. doi: 10.1016/j.ejca.2017.05.044.
21. Zheng HY, Zhang M, Yang CX, et al. Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe

progression in COVID-19 patients. Cell Mol Immunol. 2020; 17(5): 541-3. doi: 10.1038/s41423-020-0401-3.
22. Aghbash PS, Eslami N, Shamekh A, et al. SARS-CoV-2 infection: The role of PD-1/PD-L1 and CTLA-4 axis. Life Sci. 2021; 270:119124. doi: 10.1016/j.lfs.2021.119124.

Correspondence to Dr. Anmar L. Talib
E-mail: anmarnrl@gmail.com
anmar.layth@nahrainuniv.edu.iq
Received Mar. 23rd 2022
Accepted Jun. 5th 2022