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ORIGINAL ARTICLE

Evaluation of Cytotoxic T-Lymphocyte Antigen 4 Polymorphism and Soluble Immune Checkpoint Level Among A Sample of Sars-Cov-2 Iraqi Patients

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ABSTRACT

Objective: To Evaluate the Roley of Cytotoxic T-Lymphocytek antigen 4 Polymorphism and soluble immune checkpoint level (PD-1,PDL-1 and CTLA-4) in SARS-Cov-2 patients.

Methods: Fromt October 2020 to April 2021, the currentk study was conducted in Baghdad-Iraq. Ninety patients with Confirmatory SARS-Cov-2 by PCR were inclusion in the 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 Sever SARS-Cov-2 symptom and those with mild - moderate SARS-Cov-2 symptoms (cross sectional study. Patients with another form of autoimmune illness, malignant, diabetes, under the age of 18 and pregnant women were excluded.

Results: Data regarding serum level of CTLA-4f, PD-1j and PD-L1 in mild-moderate and severe covid19 patients were found to be non-normally distributed. The median serum level of CTLA-4, PD-1 and PD-L1 mild-moderate groups were much lower than that of severe cases with highly significant differences. 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). Allele specific of CTLA-4(+49G/A) PCR was used for gene amplification and genotyping under Gel electrophoresis of PCR products revealed that this SNP had three genotypes in mild/moderate and severe cases of COVID-19. These were GG, GA and AA. The wild homozygous genotype (AA) was more frequent among severe group than mild-moderate group with a significant difference.

Conclusion: Soluble Immune checkpoint markers are significantly increased in patients with covoid-19 in severe cases and soluble immune checkpoint markers are positively significant correlation with age and The genotyping CTLA-4(+49G/A) gene SNP (AG and GG by allele specific PCR) was significantly higher in mild-moderate covid-19 cases in which may indicate that this SNP mostly protective with good prognosis.

Keywords: Immune checkpoint, CTLA-4, PD1, PDL-1 and COVID-19.

INTRODUCTION

A novel coronavirus (2019-nCoV) at the end of 2019, an epidemic was discovered in Wuhan, Hubei Province, China (1). The same tropism receptor, angiotensin-converting enzyme 2, was used by both SARS-CoV2 and SARS-CoV 1 to penetrate the host human cell (ACE2)(2). Most COVID-19 patients were relatively mild and moderate symptoms, but nearly exactly 715% progress to severe pneumonial and around 5% develop acute respiratory distress syndrome (ARDS), both of which require immediate treatment (3). Immune checkpoint blockade (ICB), which targets the (CTLA-4) and (PD-1)/PD-L1) axis, has been a recognized 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-11 , PDL-12 and CTLA-44 were shown, and checkpoint signaling modulation has shown early promise to enhance responses to infections that last a long time (5). Infection with Covid-19 causes the inducement of an many inhibitory immunological checkpoint proteins such as CTLA-4 and PD-1, which is preserved in place by the progressive loss of T cell effector activity during the SARS-Cov-2 human infection(6).

MATERIAL AND METHODS

Ninety patients with Confirmatory SARS-Cov-2 by PCR were inclusion 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 Sever SARS-Cov-2 symptoms (lowest oxygen saturated) and those with mild - moderate SARS-Cov-2 symptoms. Patients with another form of autoimmune illness, malignant, diabetes, under the age of 18 and pregnant women were excluded. The (3 ml) were obtained from each patients to evaluate and monitor the levels of soluble PDL-1, PD-1, and CTLA-4 since they had previously been placed in a gel tubes, The samples were separated by centrifugation at 3000 rpm for five minutes and kept at -20 C after being utilized for just an enzyme linked immunological sorbent test (ELISA).

After DNA extraction for molecular testing, the entire 2 ml (whole blood) was maintained in an ethylene diamine tetra acetic acid (EDTA) tube (90) sample for CTLA-4 genotyping and (77) sample for CTLA-4 sequencing with specific primers (+49 G/A rs231775 and -1722 T/ C rs733618).

Statistical Analysis: SPSSD software version 25.01 was used for statistical analysis (SPSS1, Chicago2). The normality testt (Shapiro Wilk test) was performed on continuous data. Information with normally distributions were provided as mean2 and standard2 deviation1 and evaluated using the Student t-test. Non-normally distributed data were presented as median and range and analyzed using the Mann3 Whitney U2 test (for two-group 2comparison) or1 the Kruskal Wallis test (for three groups comparison). Categorical variables had been recorded as numbers and percentages were examined using the Chi-square test. The Spearman's correlation test was employed to investigate the probable relationship between check point markers and age. A statistically significant difference was defined as a p-value less than 0.05.

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).

Spearman's1 correlation was2 used to explore the possible correlation between age and checkpoint markers and between checkpoint markers themselves. Age demonstrated a positive significant correlation with each ofCTLA-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).

Table 1: Demographic characteristics of the study population

Check point markers	Mild-moderate (n=45)	Severe (n=45)	P-value1
Age, years Mean ±SDD Range	 44.58±15.96 19.0-87	 56.73±10.85 24-85	 <0.001
Gender Male Female	 22(48.89%) 23(51.11%)	 30(66.67%) 15(33.33%)	 0.088

Table 2: Spearman's correlation between age and check point markers

Variable	CTLA-44		PD-11		PD-L1	
	r	p-value1	r	p-value3	r	p-value4
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

Data regarding serum level of CTLA-4, PD-1 and PD-L1 in mild-moderate and severe patients were found to be non-normally distributed**.**

The median serum level of CTLA-4, PD-1 and PD-L1 mild-moderate groups were 0.57ng/ml, 0.51 ng/ml amd 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 (figure 1).

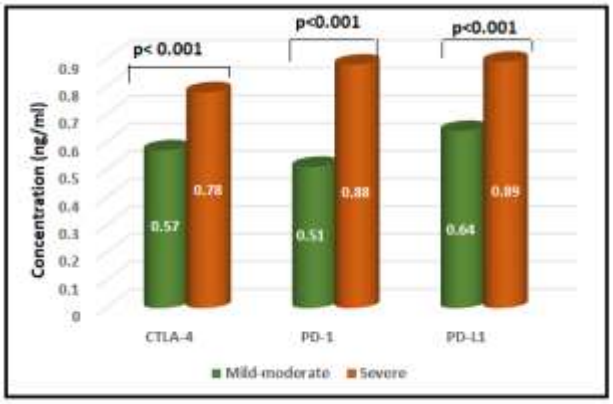

Figure 1: Median concentration of CTLA-4, PD-1 and PD-L1 in mild-moderate and severe cases of COVID-19.

The wild homozygous genotype (AA) was more frequent among severe group than mild-moderate group (73.33% vs. 49.89%) with a significant difference. In contrast, the heterozygous genotype (AG) and mutant homozygous genotype (GG) were more common among mild-severe group (33.33% and 17.78%, respectively) than severe group (24.44% and 2.22%, respectively) with significant differences (OR= 0.06, 95%CI= 0.01-5.0, p= 0.009 and OR= 0.1, 95%CI=0.01-0.9, p= 0.040, respectively).

Analysis of allele distribution revealed a higher frequency of G allele among mild-moderate group than severe group (34.44% versus 14.44%) with a highly significant difference (OR= 0.32, 95%CI= 0.15-0.67, p= 0.002) shown in (Table 2).

Direct sequencing was used for genotyping of the PCR products,

The polymorphism of (rs733618) appeared in two genotypes only: TT and TC that shown in (Figure 2).

Table 3: The frequency1 of2 different genotypes3 and allele of CTLA-4(+49G/A) polymorphism in mild-moderate0 and severe1 cases of COVID-19

CTLA-4(+49G/A)	Mild-moderate (n=45)	Severe (n=45)	P-value	OR(95%CI)
Genotypes3 AA AG GG HWE	 22(48.89%) 15(33.33%) 8(17.78%) 0.079	 33(73.33%) 11(24.44%) 1(2.22%) 0.816	 0.028 0.009 0.040	 1.0 0.06(0.01-5.0) 0.1(0.01-0.9)
Dominant model AA+GA GG	 37(82.22%) 8(17.78%)	 44(97.78%) 1(2.22%)	 0.038	 1.0 0.11(0.01-0.88)
Recessive model AA GG+GA	 22(48.89%) 23(51.11%)	 33(73.33%) 12(26.67%)	 0.019	 1.0 0.35(0.14-0.84)
Alleles A G	 59(65.56%) 31(34.44%)	 77(85.56%) 13(14.44%)	 0.002	 1.0 0.32(0.15-0.67)

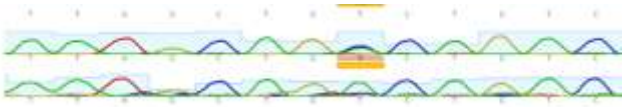

Figure 2: Sequence analysis of the CTLA-4 gene polymorphism (rs733618), the forward strand. The T in the higher frame represents heterozygous genotype (TC), the T1 in the lower frame2 represents homozygous genotype (TT2).

The frequency 2of different genotypes and alleles of this SNP was very close between patients with mild/moderate and severe COVID-19 with no significant differences.

DISCUSSION

In this study, the characteristics of age groups Covid-19 Patients 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 was3 not significant. The current study's1 findings was agree with another study like the previously research was already observed to be rather significant in adults ages 655 and over, in (China4 and Italy4) show a case mortality rate of 2.30 % in COVID-19 individuals, with more than half of the deaths occurring in patients 50 years of age or older (7). Also agree with another previous studies from Northern Italy, that shows the total case-fatality rate in individuals 644 years or older were 36.0%, compared to 155% in younger patients (8).

In the current study, the data regarding soluble level of CTLA-4, PD-1 and PD-L1 in mild-moderate and severe patients were found to be non-normally (abnormal increase) distributed. 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 agreed with another study in estimation of soluble immune checkpoint in SARS-Cov-2 patients (9, 10). The study appeared that gene amplification and genotyping of (CTLA-4(+49G/A) by allele specific (ARMS) PCR was performed. Gel electrophoresis of PCR results demonstrated that this SNP had three genotypes in mild/moderate and severe COVID-19 patients. These were GG, GA, and AA. The wild homozygous genotype (AA) was more frequent among severe group than mild-moderate group (73.33% vs. 49.89%) with a significant difference. while the heterozygous genotype (AG) and mutant homozygous genotype (GG) were more common among mild-severe group (33.33% and 17.78%, respectively) than severe group (24.44% and 2.22%, respectively) with significant differences (p= 0.009 and p= 0.040, respectively). the SNP (rs733618) homozygote genotype (TT) by direct sequencing

(Forward) analysis was higher in mild-moderate Covid-19 than the sever groups while the heterozygote genotyping (CT) was higher in the sever groups and this suggest the SNP is associated with the severity.

CONCLUSIONS

1- Soluble Immune checkpoint markers are significantly increased in patients with covoid-19 in severe cases.
2- Soluble Immune checkpoint markers are positively significant correlation with age.
3- The genotyping CTLA-4(+49G/A) gene SNP (AG and GG by allele specific PCR) was significantly higher in mild-moderate covid-19 cases in which may indicate that this SNP mostly protective with good prognosis.
4- The genotyping CTLA-4 (rs733618) (CT by direct sequencing analysis) was higher in sever covid-19 cases.

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REFERENCES

1 Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395:497–506. doi:10.1016/S0140-6736-30183-5.

2 Zhou P, Yang X, Wang X, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020; 579:270–3. doi:10.1038/s41586-020-2012-7.

3 Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF and Tan W. A Novel Coronavirus from Patients with Pneumonia in China, 2019. The New England journal of medicine. 2020; 382, 727-733, doi: 10.1056/NEJMoa 2001017.

4 Kruger S, Legenstein ML, Rosgen V, Haas M, Modest DP, Westphalen CB, 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, M. N. & Lewin, S. R. Immune checkpoint blockade in infectious diseases. National library of Immunology.2018;18,91–104. doi: 10.1038/nri.2017.112.

6 Aghbash PS, Eslami N , Shamekh A, Entezari-Maleki T , Baghi HB. SARS-CoV-2 infection: The role of PD-1/PD-L1 and CTLA-4 axis. 2021; 270: 119124. doi: 10.1016/j.lfs.119124.

7 Porcheddu R, Serra C, Kelvin D, Kelvin N and Rubino S. 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.

8 Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, et al. COVID-19 Lombardy ICU Network. 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.

9 Westmeier J , Paniskaki K, Karaköse Z, Werner T, Sutter K, Dolff S,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.

10 Diao B, Wang C, Tan Y, Chen X,Liu Y, Ning L, et al. Reduction and Functional Exhaustion of T Cells in Patients With Coronavirus Disease 2019(COVID-19).FrontImmunology.2020;11:827. doi/10.3389/fimmu.00827.