Research article

Role of CTLA-4, PD-1 and PD-L1 immune biomarkers among HCV patients undergoing hemodialysis

Ryiam S. Jouda[1], Basim M. Ibrahim[2], Ahmed F. Al-Khafagi[3]

[1,2]Department of Microbiology, College of Medicine, University of Baghdad, Baghdad, Iraq
[3]Iraqi Center of Haemodialysis, Baghdad Teaching Hospital, Baghdad, Iraq

(Received: September 2022 Revised: January 2023 Accepted: February 2023)

Corresponding author: **Ryiam S. Jouda.** Email: Rayam.sadek1210a@comed.uobaghdad.edu.iq

ABSTRACT

Introduction and Aim: One of the most prevalent diseases seen in Iraqi patients undergoing hemodialysis treatment for chronic renal disease is hepatitis C virus, also known as HCV infection (CKD). In this study, we intended to determine whether immune checkpoint biomarkers such as CTLA-4, PD-1, and PD-L1 might be utilized to predict the outcome for hemodialysis patients who had HCV infection and CKD.

Materials and Methods: The study comprised 90 Iraqi patients. The participants were separated into three groups: Group I (control group) consisted of those without viral infection. Group II consisted of 30 HCV-infected patients who did not receive antiviral medication, whereas Group III consisted of 30 HCV-infected patients who had had recent/previous antiviral drug treatment. ELISA was used to evaluate the serum predictive values of immunological biomarkers in all three groups of people. The collected results were statistically analyzed.

Results: Serum levels of the measured biomarkers were seen elevated among all individuals in Group I and II showing a high statistically significant difference between patients' group and healthy controls. The area under the curve (AUC) for CTLA-4, PD-1, and PD-L1 was 93%, 99%, and 96%, respectively.

Conclusion: The prognostic factor-value of immune biomarkers investigated in this study could be used as predictors in patients with CKD and infected with HCV.

Keywords: HCV; CTLA-4; PD-1/PD-L1; immune biomarkers; CKD; hemodialysis.

INTRODUCTION

The illness caused by the hepatitis C virus is widely regarded as the most frequent bloodborne infection (1). There is both an acute and a chronic stage of the infection (2). The most common and widespread method by which viruses are spread and transmitted is through contact with blood and blood components (3). In developing nations, infection with HCV is a major problem for hemodialysis (HD) patients, with rates ranging from 6 to 60 percent and being associated with high mortality rates (4). Chronic hemodialysis patients are at a significant risk for infection, primarily because of a lack of standard infection-precautions, insufficient disinfection of HD machines, tools, and equipment, and prolonged duration of hemodialysis. Because the hemodialysis procedure requires vascular access for extended periods of time, chronic hemodialysis patients are at a significant risk for infection (5).

Immune checkpoints operate as immunological regulators. They play a significant part in the process of self-tolerance, which stops the immune system from arbitrarily attacking cells and stops autoimmune reactions to proteins found in the body's own tissues (6) .The most important and widely studied immune biomarkers are Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), Programmed death-1 (PD-1) and Programmed death-ligand-1 (PD-L1), also known as CD152, CD279 and CD274 protein receptors respectively (7). The expression of these immune checkpoints helps in limiting T-cell proliferation, restoring immunological function in the microenvironment of tumors, and regulating responses to self-proteins. Moreover, the expression of these immune checkpoints helps regulate responses to self-proteins (8). Recent clinical studies have demonstrated that these indicators are associated with several other medical diseases, such as melanoma (9), sepsis (10), and viral infections (11). In Iraq, on the other hand, there are no clinical data available to demonstrate the function that these biomarkers play as predictors for HCV infection. So, the purpose of this study was to assess the prognostic factor value of CTLA-4, PD-1, and PD-L1 immune checkpoint biomarkers among Iraqi patients who were undergoing hemodialysis and who suffered from CKD and HCV infection.

MATERIALS AND METHODS

Study design and population

Ninety people participated in this case-control study; they were split into three groups of thirty each. Those who were virus-free made Group 1 (the "control group"). Patients with HCV infection who did not receive any antiviral treatment were placed in Group II, whereas those with HCV infection who had had antiviral drug therapies in the recent past or at some point in the past were placed in Group III. From

October 3rd, 2021, to the end of December of the same year, researchers from the Iraqi Center of Hemodialysis at the Baghdad Teaching Hospital and the University of Baghdad's Department of Microbiology conducted the study. Individuals were interviewed directly to collect data on age, gender, HD duration, vascular access technique, and the presence or absence of chronic illnesses. Ethical clearance for the study was obtained from the Deanery of the College of Medicine/University of Baghdad and the Head of the Iraqi center of HD at Baghdad Teaching Hospital (Letter dated 2021/9/28).

Inclusion and exclusion criteria

Patients with chronic kidney disease (CKD) who were taking HD, with or without HCV infection, and who did not have any ongoing microbial infections or other serious medical diseases or ailments were considered eligible to participate in this study. Prior to the beginning of the experiment, the patients' permission to participate was sought.

Laboratory analysis

Blood (5 ml) was drawn from all participants included in Group I, II, and III. After serial addition and washing, the optical density (O.D.) was measured using an ELISA reader (MyBioSource, USA) at a wavelength of 450 nm, and the results were recorded. For quantitative sandwich in EIA, biomarkers were captured by an immobilized antibody by first coating a microtiter plate with antibody and then introducing standards and samples via a micropipette into the wells. The wells were rinsed and then refilled with biotinylated monoclonal antibodies to remove any unbound molecules. After the third washing, a substrate solution was added to the wells to stop the reaction. The streptavidin enzyme was linked. The color variations of the bound biomarkers represent their concentrations.

Statistical analysis

The results were analyzed with the SPSS program (version 27; IBM Corp., USA) to determine the rate of immune checkpoint biomarkers in patients with Hepatitis C infection and on hemodialysis (with antiviral drugs and without antiviral drugs) as well as patients who were not infected with the hepatitis C virus but on hemodialysis. Methods of descriptive and inferential analysis were employed to study and make predictions regarding the associations between the variables, and a *P* value <0.005 was regarded as being statistically significant.

RESULTS

Participants totaling ninety were included in the investigation. The population consisted of 48 males (53%), while the population consisted of 42 females (47%). The participants' ages ranged anywhere from 19 to 69 years old, with a mean age of 51.1±14.7 years and a standard deviation of 14.7 years. The number of men to women was approximately 1:1.35. As a means of vascular access, the A.V. fistula was utilized by a total of 79 (88%) of the patients, whereas the dual lumen catheter was utilized by a total of 11 (12%) of the patients. The association with the presence of other chronic diseases was also explored; the results revealed that 78 (87%) of patients were hypertensive, with differences that did not reach statistical significance. The clinical studies that were conducted across all of the groups are outlined in Table 1.

Table 1: Quantitative parameters and clinical profile of the studied groups

Parameters		Groups			*P* value
		Group III Control No. (%)	Group II HCV infected and Without treatment No. (%)	Group III HCV infected and with treatment No. (%)	
Gender	Male	15 (50)	16 (53)	17 (57)	0.964
	Female	15 (50)	14 (47)	13 (43)	
Route of HD	A.V fistula	29 (97)	27 (90)	23 (77)	0.203
	Dual lumen	1 (3)	3 (10)	7 (23)	
Hypertension	Yes	26 (87)	26 (87)	26 (87)	1
	No	4 (13)	4 (13)	4 (13)	

Table 2: Post-hoc analysis of difference in HD duration for the studied groups

Clinical Group	Group I	Group II	Group III
	Hemodialysis duration		
Group I- Control	-	0.005*	0.002*
Group II -HCV infected and without treatment	0.005*	-	1.0
Group III- HCV infected and with treatment	0.002*	1.0	-

***** Significant difference between clinical groups using Bonferroni post-hoc test at 0.05 level

Data analysis shows that the mean duration of HD of the participants was (4.31±2.45) years, which was significantly different between the patients and the control groups, (5.0±2.3) and (3.0±2.1) years, respectively, (controls have longer duration of HD). The results are shown in Table 2.

Three immune checkpoint biomarkers were measured for all the participants in this study. Data revealed the mean level of PD-1, PD-L1 and CTLA-4 to be (41.42±12.61, 134.48±42.6 and 647.5±212.9 respectively, with a high statistically significant difference observed for their concentration among the studied groups. The results are shown in Table 3.

Table 3: Post-hoc analysis of the differences for the studied immune checkpoint biomarkers

Clinical Group	Group I	Group II	Group III
	PD-1		
Group I- Control	-	**< 0.001***	< 0.001*
Group II- HCV infected and without treatment	< 0.001*	-	< 0.001*
Group III- HCV infected and with treatment	< 0.001*	< 0.001*	-
	PD-L1		
Group I- Control	-	< 0.001*	< 0.001*
Group II- HCV infected and without treatment	< 0.001*	-	< 0.001*
Group III- HCV infected and with treatment	< 0.001*	< 0.001*	-
	CTLA4		
Group I- Control	-	< 0.001*	< 0.001*
Group II- HCV infected and without treatment	< 0.001*	-	
Group III- HCV infected and with treatment	< 0.001*	< 0.001*	-

* Significant difference between clinical groups using Bonferroni post-hoc test at 0.05 level

Table 4: Results of ROC analysis for the studied immune checkpoint biomarkers

Biomarker	Cut-off value	Sensitivity	Specificity	AUC
PD-1	≥ 20.0	100%	0%	99%
	≥ 32.5	100%	56.7%	
	≥ 34.5	95.0%	96.7%	
	≥ 37.5	76.7%	100%	
PD-L1	≥ 63.0	100%	0%	96%
	≥ 64.5	100%	3.3%	
	≥ 90.5	96.7%	50.0%	
	≥ 109.0	93.3%	93.3%	
	≥ 133.5	78.3%	100%	
CTLA-4	≥ 295	100%	0%	93%
	≥ 343.5	98.3%	3.3%	
	≥ 415.0	96.7%	50.0%	
	≥ 541.5	91.7%	80.0%	
	≥ 596.5	73.3%	100%	

The receiver operating characteristic (ROC) analysis was used to assess the prognostic factor value of the studied biomarkers among patients with HCV infection and on HD. The values of AUC for PD-1, PD-L1 and CTLA-4 biomarkers were 99%, 96% and 93%, respectively (Table 4). The sensitivity and specificity for each biomarker studied using ROC analysis is also presented in Table 4.

DISCUSSION

The current study is the first in Iraq to detect serum levels of three immune checkpoint biomarkers in HCV-infected patients with CKD and on hemodialysis. The main goal was to demonstrate the prognostic factor value of these biomarkers among these patients, in addition to identify any possible association between the demographic data and the measured markers. In this study, men had a higher infection rate than women, with a peak of infection occurring between the ages of 50 and 59. These findings were consistent with the findings of other studies indicating that infection appears to be more prevalent in men than in women (3) especially among the aged due to decreasing physiological and mental abilities making them susceptible to age-related diseases (12). We observed women to have lower HCV infection which is in line with a previous study (13), which found that women had spontaneously

better HCV clearance rates and slower rates of disease progression if they develop chronic HCV infection.

According to the National Kidney Foundation of the United States, the A.V. fistula is the ideal treatment and gold standard for HD since it lasts longer and has fewer complications like infections and thrombotic complications (14). Regarding the vascular access for HD, we observed that 88% of the patients were using arteriovenous (A.V.) fistulas, which was consistent with prior studies that determined this type of access to be the optimal vascular access for HD patients' longevity (15).

Increased arterial stiffness due to volume overload/sodium retention, sleep apnea and use of recombinant human erythropoietin have been implicated as risk factors predisposing these patients to the risk of hypertension (16). Regarding the duration of HD, we found that the mean duration of HD of the studied groups to be 4.31±2.45 years, with a statistically significant difference existing between patients and control groups. A study done in Italy in 2012 and later supported by a CDC observation in 2018 exhibited that >50% of HCV outbreaks from 2008-2015 appeared in HD settings (17). This observation, however, confirms the fact that the risk of HCV infection is elevated as patients stay longer in HD units, which support our findings concerning the mean duration of HD. HCV diagnosis in patients with CKD is often delayed due to many reasons, including presence of nonspecific signs and symptoms, fluctuating levels of liver enzymes, lower sensitivity of detection tests and lower viremia seen among those patients (18). In this study, a highly statistical significance difference was observed between the serum levels of the studied biomarkers among participants in the three groups, with a *P* value <0.001 for all the markers. The significant use of these biomarkers was proved to be efficient not only among ESRD patients (19) and HCV infected patients (7), but also to other diseases/medical conditions, including melanoma (9), sepsis (10) and viral infections including COVID-19 (11). Immune checkpoints have been demonstrated to be utilized in the immune escape of HCV, causing T-cell dysfunction, and the expression of these molecules on suppressor cells, thereby causing difficulties in excluding such infections (20).

A comparison of infection outcomes with PD-1 levels in this study revealed that during the acute phase of infection PD-1 expression in HCV-specific T-cells differs and varies greatly during the acute stage of infection, implying that it is one of the independent-determinants of outcomes. As a result, we conclude that upregulation of PD-1 in the acute stage of infection is associated with infection fighting, whereas in the chronic stage, it is associated with infection impairing (21). However, the findings of this study contradict previous research that found PD-1 levels to be lowered despite persistently high HCV-RNA levels (22). We recommend the diagnosis of HCV by molecular techniques that give more accurate results, because it was applied in different areas of medicine to diagnose diseases (23-25). However, given that the participants in this study were CKD patients taking regular HD, such discrepancies in outcomes could be caused by variations in study design, regional differences, patient populations, assays used, and conditions linked to such diseases. Nonetheless, the study provides evidence that immune evasion mechanisms allowing HCV to survive include either epitope escape or signals keeping the expression of checkpoint receptors on virus-specific T cells higher (26).

CONCLUSION

From the above-mentioned outcomes, we could conclude that CTLA-4, PD-1 and PD-L1 immune checkpoint biomarkers have an excellent prognostic factor-value as predictors for CKD patients on HD with HCV infection. Men were more predisposed than women to kidney diseases and their complications. The A.V. fistula was the most predominant vascular access method. Moreover, hypertension appears as a common chronic disease among the CKD patients on HD, and the risk of HCV infection is elevated as patients stay more time in HD units.

ACKNOWLEDGMENT

The authors are grateful to the Iraqi Hemodialysis Centre, Baghdad and Teaching Hospital, Baghdad, Iraq, for their assistance and help.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Jalota, A., Lindner, B.K., Thomas, B., Lerma, E.V. Hepatitis C and treatment in patients with chronic kidney disease. Disease-A-Month. 2021; 7: 101017.
2. Chan, S.T., Ou, J.H.J. HCV-induced autophagy and host innate immune response. Viruses. 2017; 67(9): 224; https://doi.org/10.3390/v9080224.
3. Salman, H.S., Ibrahim, B.M., Al-Khafagi, A.F. Serum levels of ICAM-1, N-terminal pro-BNP and TnI among HD patients with HCV. J. Cardio. Dis. Res. 2021; 12 (5): 696-700.
4. Shehadeh, F., Kalligeros, M., Byrd, K., Shemin, D., Mylonakis, E., Martin, P., *et al.*, Efficacy and safety of sofosbuvir in the treatment of hep C among patients on HD: A systematic review and meta- analysis. Sci. Rep. 2020;10: https://doi.org/10.1038/s41598-020-71205-5.
5. Ledezma-Morales, M., Amariles, P. Treatment of CHC in elderly patients with CKD: A structured review. Revista Colombiana de Gastroenterologia. 2018; 33: 269-84.
6. Malnick, S.D.H.,Abdullah, A., Neuman, M.G. Checkpoint Inhibitors and Hepatotoxicity. Biomedicines. 2021; 9:101. https://doi.org/10.3390/biomedicines9020101
7. Alkrekshi, A., Tamaskar, I. Safety of immune checkpoint inhibitors in patients with cancer and HCV infection. Oncologist. 2021;26: e827- e830.
8. Gu, D., Xiang, A., Yu, Y., Zhuo, C., Xiang, X. Soluble immune checkpoints in cancer: Production, function, and biological significance. J. Immuno. Cancer. 2018; 6:1-14.

9. Carlino, M.S., Larkin, J., Long, G.V. Immune checkpoint inhibitors in melanoma. Lancet. 2021; 398:1002-1014.
10. McBride, M.A., Patil, T.K., Bohannon, J.K., Hernandez, A., Sherwood, E.R., Patil, N.K. Immune checkpoints: Novel therapeutic targets to attenuate sepsis-induced immunosuppression. Front. Immunol. 2021; 11:1-14.
11. Cai, H., Liu, G., Zhong, J., Zheng, K., Xiao, H., Li, C., *et al.*, Immune checkpoints in viral infections. Viruses. 2020; 12:1051.
12. Franceschi, C., Garagnani, P., Morsiani, C., Conte, M., Santoro, A., Grignolio, A., *et al.*, The continuum of aging and age-related diseases: Common mechanisms but different rates. Frontiers in Medicine. 2018; 5 (61):1-23.
13. Bakr, I., Rekacewicz, C., El Hosseiny, M., Ismail, S., El Daly, M., El-Kafrawy, S., *et al*., Higher clearance of HCV infection in females compared with males. Gut. 2006; 55:1183-1187.
14. National Kidney Foundation (NKF). 2015. HD access. National Kidney Foundation Inc., NY, USA. Available from: https://www.kidney.org/atoz/content/hemoaccess. Last accessed: 01.07.22
15. Santoro, D., Benedetto, F., Mondello, P., Pipitò, N., Barillà, D., Spinelli, F., *et al.*, Vascular access for HD: Current perspectives. Int. J. Nephrol. Renovascul. Dis. 2014; 7:281-294.
16. Terada, K., Sumi, Y., Aratani, S., Hirama, A., Kashiwagi, T., Sakai, Y. Plasma erythropoietin level and heart failure in patients undergoing peritoneal dialysis: A cross-sectional study. Renal Replacement Therapy. 2021; 7(3):1-8.
17. Fabrizi, F., Martin, P., Dixit, V., Messa, P. HCV infection and kidney disease: A meta-analysis. Clin. J. Am. Soc. Nephrol. 2012; 7: 549-557.
18. Bernieh, B. Viral hepatitis in HD: An update. J. Trans. Int. Med. 2015; 3: 93-105.
19. Cheun, H., Kim, M., Lee, H., Oh, K.H., Keam, B. Safety and efficacy of immune checkpoint inhibitors for ESRD patients undergoing dialysis: A retrospective case series and literature review. Investigational New Drugs. 2019;37: 579-583.
20. Hashimoto, M., Kamphorst, A.O., Im, S.J., Kissick, H.T., Pillai, R.N., Ramalingam, S.S., *et al.*, CD8 T-cell exhaustion in chronic infection and cancer: Opportunities for interventions. Ann. Rev. Med. 2018; 69:301-318.
21. Andrews, L.P., Yano, H., Vignali, D.A.A. Inhibitory receptors, and ligands beyond PD-1, PD-L1 and CTLA-4: Breakthroughs or backups. Nat. Immunol. 2019; 20: 1425-1434.
22. Barrett, M.T., Anderson, K.S., Lenkiewicz, E., Andreozzi, M., Cunliffe, H.E., Klassen, C.L., *et al.*, Genomic amplification of 9p24.1 targeting JAK2, PD-L1, and PD-L2 is enriched in high-risk triple negative breast cancer. Oncotarget. 2015; 6 (28): 26483-26493.
23. Abbas, M.S., Ahmed, A.G., Ali, S.Q., AL-Rubaii, B.A.L. Immunological inflammatory factors in patients diagnosed with COVID-19. Biomedicine, 2023; 43(1):230-235.
24. Ahmed, A.A., Khaleel, K.J., Fadhel, A.A. and Al-Rubaii, B.A.L. Chronic Myeloid Leukemia: A retrospective study of clinical and pathological features. Bionatura, 2022; 7(3):1-3.
25. Abdulrazaq, R.A., Mahmood, W.S., Alwan, B., Saleh, T.H., Hashim, S.T., Al-Rubaii, B.A.L. Biological study of protease produced by clinical isolates of Staphylococcus aureus. Res J Pharm Technol., 2022; 15(12):5415-5420.
26. Zong, Z., Zou, J., Mao, R., Ma, C., Li, N., Wang, J., *et al.*, M1 macrophages induce PD-L1 expression in HCC cells through IL-1β signaling. Front. Immunol. 2019; 10: Article 1643.