Role of Programmed Cell Death-1 and Programmed Cell Death Ligand-1 immune checkpoint biomarkers among chronic Hepatitis C virus patients under Hemodialysis

Background: Hepatitis C virus (HCV) infection is one of the most common infections associated with chronic kidney disease (CKD) patients undergoing hemodialysis (HD) in Iraq.

Aim of the study: To determine the prognostic factor value of Programmed Cell Death-1 (PD-1) and Programmed Cell Death Ligand-1 (PD-L1) immune checkpoint biomarkers among CKD patients with HCV infection under HD.

Methodology: ELISA technique was used for the measurement of the above-mentioned biomarkers in the serum of 90 Iraqi patients. The participants were divided into three groups; Group I included 30 patients infected with HCV without antiviral treatment, group II included 30 patients infected with HCV with recent/previous antiviral treatment, and Group III included 30 patients without viral infection (control group).

Results: Serum levels of the measured biomarkers were elevated among all the participants, and highly statistically significant differences were found between patients with no treatment. The area under the curve (AUC) of PD-1 was 99% and for PD-L1 was 96%.

Conclusions: The PD-1 and PD-L1 immune checkpoint biomarkers have excellent prognostic factor value as predictors for patients with CKD on HD infected with HCV.

Keywords: HCV in HD; PD-1/PD-L1 and HD; Immune checkpoint biomarkers; Immune biomarkers in CKD with/out HCV.

Introduction:

Hepatitis C virus infection is considered the most common bloodborne infection [1]. The infection has two phases; acute and chronic [2]. The most common method of virus spread is exposure to blood and blood products [3]. Infection with HCV is a major problem among HD patients in developing countries, ranging from 6-60%, and is related to high mortality rates [4]. The main reasons behind this prevalence are the duration of HD, the need for transfusions, lack of standard infection precautions, insufficient disinfection of HD machines, tools, and equipment, and the need for vascular access to perform the HD [5, 6]. Immune checkpoints are immunity regulators. They play a crucial role in self-tolerance, preventing the immune system from attacking cells randomly, in addition to blocking autoimmunity reactions to self-proteins [7]. The most common important and studied biomarkers, Programmed death-1 (PD-1) and Programmed death-ligand-1 (PD-L1), which are termed clusters of differentiation (CD279 and CD274), respectively [8]. Their expression aid in controlling T-cell proliferation, restoring immune function in tumor micro-environment as well as regulating responses to self-proteins [9]. Recent clinical data demonstrate that these biomarkers have been implicated in many medical conditions, including melanoma [10], sepsis [11], and viral infections [12, 13]. In Iraq, however, there were no clinical data to demonstrate the role of these biomarkers as predictors for HCV infection. Thus, this study aimed to determine the prognostic factor value of PD-1 and PD-L1 immune checkpoint biomarkers for CKD patients with HCV infection under HD.

Patients and Methods

Study Design and Population: A case-control study was carried out at the Department of Microbiology, College of Medicine, University of Baghdad and the Iraqi Center of Hemodialysis at Baghdad Teaching Hospital from the 3rd of October to the end of December 2021, and included 90 patients, group
one(30) patients infected with HCV not take antiviral treatment, group two (30) patients take antiviral treatment, and group three non-infected with HCV. Data (age, sex, HD duration, route of vascular access and existence of chronic diseases) were collected by direct interview with all the participants.

Inclusion and Exclusion Criteria: Patients with CKD on HD with/without HCV infection only clear from any other microbial infection and/or other medical diseases/conditions were included in this study.

Laboratory Analysis: Blood specimens were collected from 90 patients with CKD undergoing HD, and were divided into three groups group one(30) patients infected with HCV not take antiviral treatment, group two (30) patients take antiviral treatment, and group three non-infected with HCV. The serum was transferred to the wells of the microtitration plate and after serial adding and washing the concentration was read by the ELISA reader at a specific wavelength.

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control No. (%)</th>
<th>Without treatment No. (%)</th>
<th>With treatment No. (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>15 (50)</td>
<td>16 (53)</td>
<td>17 (57)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>15 (50)</td>
<td>14 (47)</td>
<td>13 (43)</td>
</tr>
<tr>
<td>Route of HD</td>
<td>A.V fistula</td>
<td>29 (97)</td>
<td>27 (90)</td>
<td>28 (77)</td>
</tr>
<tr>
<td></td>
<td>Dual lumen</td>
<td>1 (3)</td>
<td>3 (10)</td>
<td>7 (23)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Yes</td>
<td>26 (87)</td>
<td>26 (87)</td>
<td>26 (87)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>4 (13)</td>
<td>4 (13)</td>
<td>4 (13)</td>
</tr>
</tbody>
</table>

Analysis using Pearson’s chi-square test with application of Fisher’s correction whenever applicable HD: Hemodialysis; A.V fistula: Arteriovenous fistula

Data analysis shows that the mean duration of HD of the participants was (4.3±2.4) years, which was significantly different between the control and the patients’ groups, (5.0±2.3) and (3.0±2.1) years, respectively, (control havea longer duration of HD). The results are shown in Table 2.

Table (2) Characteristics of study groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Study Groups</th>
<th>HCV - Ve</th>
<th>HCV + Ve Without treatment</th>
<th>HCV + Ve With treatment</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53.80 ± 13.18 (16-74)</td>
<td>49.00 ± 15.63(19-80)</td>
<td>50.53 ± 15.45(20-75)</td>
<td>0.442</td>
<td></td>
</tr>
<tr>
<td>HCV duration (years)</td>
<td>-</td>
<td>3.37 ± 2.34 (0.67-11)</td>
<td>3.38 ± 2.10 (0.67-9)</td>
<td>0.768</td>
<td></td>
</tr>
<tr>
<td>HD duration (years)</td>
<td>2.99 ± 2.18 (0.42-12)</td>
<td>4.90 ± 2.53 (1-12)</td>
<td>5.06 ± 2.13 (2-10)</td>
<td>0.001*</td>
<td></td>
</tr>
</tbody>
</table>

* Significant difference in parameters between study groups using one-way ANOVA test at 0.05 level

Three immune checkpoint biomarkers were measured for all the participants in this study. Data analysis revealed that the mean level of PD-1, PD-L1 and were (41.4±12.61), (134.5±42.6), respectively, with highly significant differences in their concentration between the controls and the study groups. The results are shown in Table 3.

Table (3): Serum levels of the studied immune checkpoint biomarkers

<table>
<thead>
<tr>
<th>Immune checkpoint biomarkers</th>
<th>Study groups</th>
<th>HCV - Ve</th>
<th>HCV + Ve Without treatment</th>
<th>HCV + Ve With treatment</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-1</td>
<td>Mean (Range) ± SD</td>
<td>30.73 ± 3.68 (21-37)</td>
<td>39.10 ± 4.99 (33-52)</td>
<td>54.43 ± 12.27 (40-79)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>PD-L1</td>
<td>92.03 ± 15.43 (64-133)</td>
<td>132.83 ± 23.80 (65-170)</td>
<td>178.57 ± 29.81 (111-299)</td>
<td>&lt; 0.001*</td>
<td></td>
</tr>
<tr>
<td>CTLA4</td>
<td>444.13 ± 80.12 (325-590)</td>
<td>604.53 ± 116.39 (296-910)</td>
<td>893.83 ± 108.123 (749-1275)</td>
<td>&lt; 0.001*</td>
<td></td>
</tr>
</tbody>
</table>

* Significant difference between HCV +ve and -ve patients using one-way ANOVA test at 0.05 level

The receiver operating characteristic (ROC) analysis was used to assess the prognostic factor value of the studied biomarkers among patients with HCV infection on HD. The values of the area under the curve (AUC) for PD-1, PD-L1, and biomarkers were (99%), (96%), respectively. These findings, however, indicate the excellent predictive power of these biomarkers. The results are shown in Table 4.
The current study found a significant difference between patients and controls regarding the duration of HD. A study done in Italy in 2012 and later supported by a CDC observation in 2018 reported that > 50% of HCV outbreaks from 2008-2015 appeared in HD settings [27, 28]. This observation, however, confirms the fact that the risk of HCV infection increased as patients stayed longer in HD units, which supports our findings concerning the mean duration of HD. The diagnosis of HCV infection in patients with CKD seems not to be made on time due to many reasons, including the presence of nonspecific signs and symptoms, fluctuating levels of liver enzymes, the lower sensitivity of detection tests, and lower viremia seen among those patients [29, 30]. Immune checkpoint molecules are regulators of the immune system. Via self-tolerance, they prohibit autoimmune reactions and the immune system from randomly attacking cells [31]. The current study demonstrated that there were highly statistically significant differences between the serum levels of the studied biomarkers and the three groups of participants. The significant use of these biomarkers was proved to be efficient not only among End Stage Renal Disease patients [32] and HCV-infected patients [8, 33], but also to other diseases/ medical conditions, including melanoma [10], sepsis [11] and viral infections including COVID-19 [12, 13].

It has been revealed that immune checkpoints were utilized in the immune escape of HCV by causing dysfunction of T-cells, and the expression of these molecules on suppressor cells will influence its secretion, and that was the reason beyond difficulties in excluding such infections [34]. A comparative analysis of infection outcomes with PD-1 levels during the acute phase of infection exhibited that PD-1 expression in HCV-specific T-cells differs and varies highly through the acute stage of infection, suggesting that it is one of the independent determinants of outcomes, hence, we could conclude that upregulating PD-1 in the acute stage of infection was associated with fighting the infection [35], whereas, in the chronic stage, it was associated with an impaired T-cells function, resulting in viral infection of a persistent type, a conclusion that shows relevance to intervention with blocking-antibodies [36]. The findings of the current study were not compatible with those of a molecular study done in the USA in 2015 which showed that PD-1 levels were lowered regardless of continual high HCV-RNA levels [37]. Inconsistencies of results might be due to differences in study design, geographic differences, patient populations, the assay used, and certain conditions related to such diseases since the participants enrolled in this study were CKD patients on regular HD. These results, however, provide evidence that immune evasion mechanisms permitting HCV to persist either include epitope escape or signals maintaining higher expression of checkpoint receptors on virus-specific T-cells [38].
Conclusion
Based on these results we can conclude that PD-L1 and PD-L1 immune checkpoint biomarkers have an excellent prognostic factor value as predictors for CKD patients on HD with HCV infection.

Authors’ declaration:-
Conflicts of Interest: None
We hereby confirm that all the Figures and Tables in the manuscript are ours. Besides, the Figures and images, which are not ours, have been given permission for re-publication attached with the manuscript.-Authors sign on ethical consideration’s approval-Ethical Clearance: The project was approved by the local ethical committee in the College of Medicine/ University of Baghdad according to the code number 1439.6.11.2021).

Authors’ contributions:
Ryiam S. Jouda: MSc students
Basim M. Ibrahim: first supervisor
Ahmed F. Al-Khafagi: second supervisor

References:

Last accessed: 01.07.22

Isolation and Identification of \textit{H. pylori} among Iraq patients with chronic gastric inflammation

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The objectives: To determine the value of the prediction of the immunological markers Po1 and Po2a, related to patients infected with viral hepatitis under dialysis treatment.

The approach: Using ELISA technique to measure the mentioned above markers in 90 Iraqi patients. The participants were divided into three groups; the first group of 30 patients infected with viral hepatitis without any antiviral treatment, the second group of 30 patients infected with viral hepatitis under antiviral treatments, and the third group of 30 patients without any viral infection (control group).

Results: The levels of the measured biological markers were raised between all participants, and there were statistical significant differences in the group of patients without treatment.

Conclusions: The biological markers for the immunological examination that were examined are a good predictor for patients suffering from chronic kidney disease on HD and infected with viral hepatitis.

Keywords: Viral hepatitis Type B; Po1 in hemodialysis for kidney; Po2a; Biological markers of immunological examination; biological markers of immunological examination in chronic kidney disease.