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ORIGINAL RESEARCH

PD-L1 is Fascinating but IDO Needs Attention in Non-HCV and Non-HBV-Associated Hepatocellular Carcinoma Patients

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Background/Aim: Hepatocellular carcinoma (HCC) is one of the most common forms of liver cancer that is modulated by the immune system. Programmed cell death ligand-1 (PD-L1) has emerged as a novel therapeutic target in various cancers. Indoleamine 2,3-dioxygenase (IDO) is an immunosuppressive enzyme that is associated with poor prognoses in various cancer types. The aim of this study was to investigate the PD-L1 expression, and clinicopathological features of non-HCV and non-HBV-associated HCC patients, including IDO expression.

Patients and Methods: In this study, immunohistochemical analysis was performed to analyze the expression of PD-L1 and IDO. Formalin-fixed paraffin-embedded HCC tumor tissues (n=50) were obtained from the pathology department, at Shaukat Khanum Memorial Cancer Hospital and Research Centre (SKMCH&RC) in Lahore, Pakistan between 2005 and 2022. All the patients were HBV and HCV negative. Furthermore, it was a rare group of patients with no previous history of any viral hepatitis. In addition, for categorical and continuous variables chi-square or Fisher exact test and Mann–Whitney *U*-test was performed.

Results: Of 50 tissue specimens, PD-L1+ was observed in 21 [high: 12 (24%), low: 9 (18%)] and PD-L1- was observed in 29 HCC patients. IDO+ was observed in all 50 specimens [high: 42 (84%), low: 8 (16%)]. Additionally, both PD-L1 and IDO had high expression in 11 (22%) patients. While both PD-L1 and IDO had low expression in 2 (4%) patients. Furthermore, in IDO+/PD-L1- group, 20 (69%) out of 29 patients died while in the IDO+/PD-L1+ group, 9 (43%) out of 21 patients died.

Conclusion: Evaluation of IDO and PD-L1 expression may add therapeutic advantage in non-HCV and non-HBV-associated HCC patients that overexpress IDO. Further validation in a larger cohort is warranted.

Keywords: programmed cell death ligand-1, indoleamine 2, 3-dioxygenase, non-HCV HCC, non-HBV HCC, immune checkpoint molecules

Introduction

Hepatocellular carcinoma (HCC) is one of the most aggressive malignancies of the liver and is becoming the leading cause of cancer-related mortality and morbidity worldwide.[1] It continues to be a global public health and economic burden, with a projected peak incidence of more than 1 million deaths by 2030.[2] The incidence rate of HCC is growing worldwide, including in Pakistan.[3] Hepatitis C virus (HCV) and Hepatitis B virus (HBV) infections primarily contribute to HCC development and its progression.[4] HCV and HBV account for the majority of HCC incidence worldwide.[4]

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However, some studies have also reported non-HCV and non-HBV HCC cases that confirm the non-viral etiological factors responsible for HCC development.[5–8] HCC may evade the anti-cancer immune response due to the presence of a diverse range of immunity and immune tolerance in the liver.[9] However, the actual underlying immunosuppressive mechanisms are still unknown.

Checkpoint blockades have emerged as a paradigm-shifting therapeutic modality in immunotherapy for HCC. In various malignancies, programmed death ligand-1 (PD-L1) checkpoint blockades have shown considerable clinical efficacy.[10–12] PD-L1 protein expression on the surface of tumor cells is crucial for these cells to evade immunosuppression.[13] The overexpression of PD-L1 on tumor cells can avoid T-cell cytolysis and facilitate cancer formation.[14–16] Several studies have revealed a significant association of PD-L1 overexpression with antitumor immunity, tumor aggressiveness, and poor prognosis in HCC patients.[17,18] However, a lot of cancer patients failed to respond to the PD-L1 checkpoint blockades.[10] Immune checkpoint inhibitors (ICIs) such as pembrolizumab, nivolumab, durvalumab, atezolizumab, and others have been evaluated in HCC patients, but single-agent ICI trials have not yielded promising results.[19] On the other hand, combination therapies involving ICIs have shown more favorable outcomes.[20] The Phase III IMbrave150 trial has established a new standard of care for advanced HCC patients with a combination of bevacizumab and atezolizumab.[19] This combination has resulted in significant benefits in clinical outcomes such as objective response rate (ORR), progression-free survival (PFS), and overall survival (OS).[19] Despite the success of combination therapies, the lack of validated biomarkers of response in HCC immunotherapy remains a significant issue. PD-L1 expression, tumor mutational burden (TMB), microsatellite instability (MSI) status, gut microbiota, and other potential biomarkers need further exploration to identify patients who are most likely to benefit from immunotherapy.[21] The heterogeneous tumor immune microenvironment in HCC may also account for the inconsistent outcomes observed with ICIs. Hence, the need for reliable markers of response is crucial.[22]

PD-L1 expression in predicting the response to immunotherapy in HCC remains controversial.[23] Shrestha et al reported that only 65 out of 751 HCC patients expressed PD-L1, indicating the need for further research to determine whether PD-L1 expression can be used as a predictor of ICI efficacy in HCC patients.[24]

Thus, targeting immune checkpoints is an emerging field of research for novel cancer therapies.[25–27] One such potential target is indoleamine 2, 3-dioxygenase (IDO), a checkpoint protein that contributes to an immunosuppressive tumor microenvironment.[28] IDO is a heme-containing enzyme that degrades L-tryptophan into kynurenine.[29] Local deprivation of tryptophan impedes the cytotoxicity of T-cells, resulting in the inhibition of T-cell immune responses by inducing regulatory T-cell differentiation.[30,31] High IDO expression has been reported in many cancers, including breast, colorectal, ovarian, and gastric cancer.[32–35] It is also associated with cancer metastasis and poor prognosis in HCC patients.[36–40]

PD-L1 expression is crucial in predicting which tumors are responsive to anti-PD-L1 immunotherapy. IDO overexpression is associated with poor prognosis in several tumors. Therefore, determining the expressions of PD-L1 and IDO might help to predict which individual patients may benefit from anti-IDO/anti-PD-L1 therapy or combinational therapies. In the current study, we investigated the expression of PD-L1 and IDO in HCC patients by immunohistochemical analysis.

Materials and Methods

Study Material

Formalin-fixed paraffin-embedded tissue samples of non-HCV and non-HBV HCC patients (n=50) were obtained from the archives of the Department of Pathology, Shaukat Khanum Memorial Cancer Hospital and Research Centre (SKMCH&RC) in Lahore, Pakistan, between 2005 and 2022. The patients chosen for the study were treatment-naive. The tumor tissue block for each sample was reviewed and confirmed by the histopathologist. The most appropriate tissue block was selected if the sample contained multiple tumors. The histologic cell types were allocated according to the criteria given in the WHO classification. All the clinicopathological and radiological parameters of non-HCV and non-HBV HCC patients were collected from the medical records of the hospital information system (HIS). The current retrospective study was approved by the institutional review board (IRB) of SKMCH & RC. IRB granted the waiver for

informed consent for this study, which is in accordance with the Declaration of Helsinki. The patient data accessed complied with relevant data protection and privacy regulations.

Immunohistochemistry

Two sections of formalin-fixed paraffin-embedded (FFPE) tumor specimens of the same patients were cut at a thickness of 4 µm. IDO staining was performed as described in[41] using an anti-Indoleamine 2, 3-dioxygenase antibody (Abcam, # ab55305); heat-mediated epitope retrieval with a Tris-EDTA buffer was performed. The immunoreactivity was detected by using the Dako EnVision kit (K5007). Normal human tonsils served as a positive control. PD-L1 immunoreactivity was assessed by an immunohistochemical assay for formalin-fixed, paraffin-embedded tumor specimens.[42,43] Slides were stained using an autostainer Link 48 (Dako Denmark) as per the manufacturer's protocol. Slides were deparaffinized and antigen was retrieved simultaneously with the target retrieval solution (#GV805 Dako). PD-L1 antibody (clone 22C3; Cat# M3653) and an automated staining procedure developed by DAKO. PD-L1 labeling was visualized using Envision Flex detection kit DAKO (K8000). Normal human tonsils served as a positive control. Slides were visualized by an optical microscope (Provis AX-70, Olympus, Melville, NY).

Scoring

Pathologists assessed all the results. They performed a blind histopathologic evaluation. The discrepancies between the pathologists were examined mutually to reach a consensus and the mean score of both of them was considered a decisive score. The total IDO immunostaining scores were calculated as described earlier.[38] The intensity was scored for IDO as negative (0), weak (1), moderate (2), or strong (3). The percentage of tumor cells with positive staining (range, 0–9) were classified as diffuse (3+, 50–75%), focal (2+, 25–50%), sporadic (1+, 5–25%), and negative (0, 0%). The immunohistochemical expression of PD-L1 was calculated as described earlier.[44] PD-L1 staining intensity was assessed as strong (3), moderate (2), weak (1), or negative (0). The percentage of tumor cells with positive staining was categorized according to the following formula: PD-L1 expression score (H score) (range, 0–9)=0×% of non-stained tumor cells +1×% of weakly stained tumor cells +2×% of moderately stained tumor cells +3×% of strongly stained tumor cells.

Statistical Analysis

Statistical analysis was performed by using SPSS software (version 20.0; SPSS, Chicago, IL, USA). Frequency and percentage were used for categorical variables while the median and range (min-max) were used for continuous variables. Bivariate analysis was done using chi-square or Fisher exact test (where necessary). For continuous explanatory variables such as age, the Mann–Whitney *U*-test was performed. Statistical significance was defined as a two-tailed P-value of 0.05.

Results

Clinicopathological Profiles of Non-HCV and Non-HBV-Associated HCC Patients

Over the last 17 years (2005 to 2022), we identified 50 patients of non-HCV and non-HBV-associated HCC at SKMCH&RC. Among these, 39 were male and 11 were female. Of the 50 patients, the majority 42 (84%), belonged to the Punjab province. The mean age of patients was 65 years. As per the medical history of the patients, 16 (32%) were smokers and 4 (8%) were consuming alcohol. Diabetes was observed as the major comorbidity (54%) in the current data set. Out of 50 patients, 29 (58%) had died as of 2022. In addition, further clinicopathological characteristics of patients are given in Table 1 and Table 2.

Immunohistochemical Staining of PD-L1 and IDO in Tissue Samples

To evaluate the expression of PD-L1 and IDO, formalin-fixed paraffin-embedded (FFPE) non-HCV and non-HBV-associated HCC tissues (n=50) were used for immunohistochemical analysis. Patients were categorized into PD-L1+ (42%) and PD-L1- (58%) groups as shown in Figure 1A and B. In addition, the PD-L1+ group was further classified into high (24%) and low (18%). IDO+ was observed in all 50 tissue specimens. High IDO expression was observed in 42

Table 1 Demographics and Baseline Characteristics of Patients with PD-L1

Demographics	Total (n=50)	Negative 29 (58.0)	PD-L1 Low 9 (18.0%)	PD-L1 High 12 (24.0%)	*P*-value
Age (years)					0.44
Median (min-max)	65 (19–88)	65 (19–88)	60 (41–74)	64 (51–78)	
Gender					0.65
Male	39 (78.0)	21 (72.4)	8 (88.9)	10 (83.3)	
Female	11 (22.0)	8 (27.6)	1 (11.1)	2 (16.7)	
Ethnicity					0.28
Afghanistan	2 (4.0)	–	1 (11.1)	1 (8.3)	
Balochistan	2 (4.0)	1 (3.4)	1 (11.1)	–	
KPK	4 (8.0)	2 (6.9)	1 (11.1)	1 (8.3)	
Punjab	42 (84.0)	26 (89.7)	6 (66.7)	10 (83.3)	
Histological grade					0.13
Poorly differentiated	4 (8.0)	2 (6.9)	–	2 (16.7)	
Moderately differentiated	14 (28.0)	6 (20.7)	2 (22.2)	6 (50.0)	
Well differentiated	32 (64.0)	21 (72.4)	7 (77.8)	4 (33.3)	
BCLC staging					0.19
0	2 (4.0)	1 (8.3)	–	1 (3.4)	
A	6 (12.0)	2 (6.9)	1 (11.1)	3 (25.0)	
B	18 (36.0)	8 (27.6)	6 (66.7)	4 (33.3)	
C	18 (36.0)	14 (48.3)	2 (22.2)	2 (16.7)	
Unknown	6 (12.0)	4 (13.8)	–	2 (16.7)	
Child-Pugh score					0.75
A	44 (88.0)	25 (86.2)	9 (100.0)	10 (83.3)	
B	5 (10.0)	3 (10.3)	–	2 (16.7)	
Unknown	1 (2.0)	1 (3.4)	–	–	
ECOG					0.03
0	13 (26.0)	7 (24.1)	1 (11.1)	5 (41.7)	
1	13 (26.0)	4 (13.8)	4 (44.4)	5 (41.7)	
Unknown	24 (48.0)	18 (62.1)	4 (44.4)	2 (16.7)	
Alcoholic status					0.36
No	46 (92.0)	25 (86.20)	9 (100.0)	12 (100.0)	
Yes	4 (8.0)	4 (13.80)	–	–	

(*Continued*)

Table 1 (Continued).

Demographics	Total (n=50)	Negative 29 (58.0)	PD-L1 Low 9 (18.0%)	PD-L1 High 12 (24.0%)	*P*-value
Status					0.30
Alive	12 (24.0)	5 (17.2)	2 (22.2)	5 (41.7)	
Death	29 (58.0)	20 (69.0)	5 (55.6)	4 (33.3)	
Unknown	9 (18.0)	4 (13.8)	2 (22.2)	3 (25.0)	
Diabetes					0.79
No	23 (46.0)	12 (41.4)	5 (55.6)	6 (50.0)	
Yes	27 (54.0)	17 (58.6)	4 (44.4)	6 (50.0)	
Smoking status					0.91
No	34 (68.0)	19 (65.5)	6 (66.7)	9 (75.0)	
Yes	16 (32.0)	10 (34.5)	3 (33.3)	3 (25.0)	
Metastasis status					1.00
No	41 (82.0)	24 (82.8)	7 (77.8)	10 (83.3)	
Yes	9 (18.0)	5 (17.2)	2 (22.2)	2 (16.7)	
Recurrence					0.21
No	8 (16.0)	3 (10.3)	3 (33.3)	2 (16.7)	
Yes	10 (20.0)	4 (13.8)	2 (22.2)	4 (33.3)	
Unknown	32 (64.0)	22 (75.9)	4 (44.4)	6 (50.0)	
Tumor size (cm)					0.41
Median (min-max)	7.70 (1–20)	9.20 (1–20)	7.90 (2–15)	4.90 (3–16)	
AFP					0.88
Median (min-max)	25.70 (1–30,000)	24.60 (2–30,000)	186.35 (1–5582)	42.0 (3–1646)	
ALT					0.98
Median (min-max)	48.0 (10–759)	48.0 (10–759)	48.0 (25–185)	47.50 (23–119)	
AST					0.99
Median (min-max)	47.0 (18–649)	47.0 (18–649)	45 (26–262)	54.50 (19–139)	
Albumin					0.76
Median (min-max)	3.63 (2.05–4.85)	3.55 (2.05–4.49)	3.67 (2.82–4.56)	3.79 (2.26–4.85)	
Bilirubin					0.87
Median (min-max)	0.80 (0.22–6.83)	0.80 (0.25–6.23)	0.58 (0.35–1.71)	0.77 (0.22–0.83)	
INR					0.55
Median (min-max)	1.15 (1–4)	1.17 (1–2)	1.15 (1–4)	1.04 (1–2)	

Abbreviations: AFP, Alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate transaminase; ECOG, Eastern Cooperative Oncology Group; INR, international normalized ratio.

Table 2 Demographics and Baseline Characteristics of Patients with IDO

Demographics	Total (n=50)	IDO Low 8 (16.0%)	IDO High 42 (84.0%)	*P*-value
Age (years)				0.85
Median (min-max)	65 (19–88)	65 (51–80)	65 (19–88)	
Gender				1.00
Male	39 (78.0)	6 (75.0)	33 (78.6)	
Female	11 (22.0)	2 (25.0)	9 (21.4)	
Ethnicity				0.58
Afghanistan	2 (4.0)	–	2 (4.8)	
Balochistan	2 (4.0)	1 (12.5)	1 (2.4)	
KPK	4 (8.0)	–	4 (9.5)	
Punjab	42 (84.0)	7 (87.5)	35 (83.3)	
Histological grade				0.85
Poorly differentiated	4 (8.0)	1 (12.5)	3 (7.1)	
Moderately differentiated	14 (28.0)	2 (25.0)	12 (28.6)	
Well differentiated	32 (64.0)	5 (62.5)	27 (64.3)	
BCLC staging				0.69
0	2 (4.0)	–	2 (4.8)	
A	6 (12.0)	2 (25.0)	4 (9.5)	
B	18 (36.0)	3 (37.5)	15 (35.7)	
C	18 (36.0)	3 (37.5)	15 (35.7)	
Unknown	6 (12.0)	–	6 (14.3)	
Child-Pugh score				0.64
A	44 (88.0)	8 (100.0)	36 (85.7)	
B	5 (10.0)	–	5 (11.9)	
Unknown	1 (2.0)	–	1 (2.4)	
ECOG				0.06
0	13 (26.0)	5 (62.5)	8 (19.0)	
1	13 (26.0)	1 (12.5)	12 (28.6)	
Unknown	24 (48.0)	2 (25.0)	22 (52.4)	
Alcoholic status				0.51
No	46 (92.0)	7 (87.5)	39 (92.9)	
Yes	4 (8.0)	1 (12.5)	3 (7.1)	

(*Continued*)

Table 2 (Continued).

Demographics	Total (n=50)	IDO Low 8 (16.0%)	IDO High 42 (84.0%)	*P*-value
Status				1.00
Alive	12 (24.0)	2 (25.0)	10 (23.8)	
Death	29 (58.0)	5 (62.5)	24 (57.1)	
Unknown	9 (18.0)	1 (12.5)	8 (19.0)	
Diabetes				0.71
No	23 (46.0)	3 (37.5)	20 (47.6)	
Yes	27 (54.0)	5 (62.5)	22 (52.4)	
Smoking status				0.25
No	34 (68.0)	4 (50.0)	30 (71.4)	
Yes	16 (32.0)	4 (50.0)	12 (28.6)	
Metastasis status				1.00
No	41 (82.0)	7 (87.5)	34 (81.0)	
Yes	9 (18.0)	1 (12.5)	8 (19.0)	
Recurrence				0.73
No	8 (16.0)	2 (25.0)	6 (14.3)	
Yes	10 (20.0)	1 (12.5)	9 (21.4)	
Unknown	32 (64.0)	5 (62.5)	27 (64.3)	
Tumor size (cm)				
Median (min-max)	7.70 (1–20)	7.05 (3–14)	8.80 (1–20)	0.49
AFP				
Median (min-max)	25.70 (1–30,000)	25.85 (2–543)	25.70 (1–30,000)	1.00
ALT				
Median (min-max)	48.0 (10–759)	45 (10–86)	48.50 (12–759)	0.65
AST				
Median (min-max)	47.0 (18–649)	52 (18–64)	47 (19–649)	0.35
Albumin				
Median (min-max)	3.63 (2.05–4.85)	3.62 (2.33–4.49)	3.63 (2.05–4.85)	0.78
Bilirubin				
Median (min-max)	0.80 (0.22–6.83)	0.89 (0.46–6.83)	0.58 (0.22–6.23)	0.23
INR				
Median (min-max)	1.15 (1–4)	1.29 (1–2)	1.08 (1–4)	0.22

Abbreviations: AFP, Alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate transaminase; ECOG, Eastern Cooperative Oncology Group; INR, international normalized ratio.

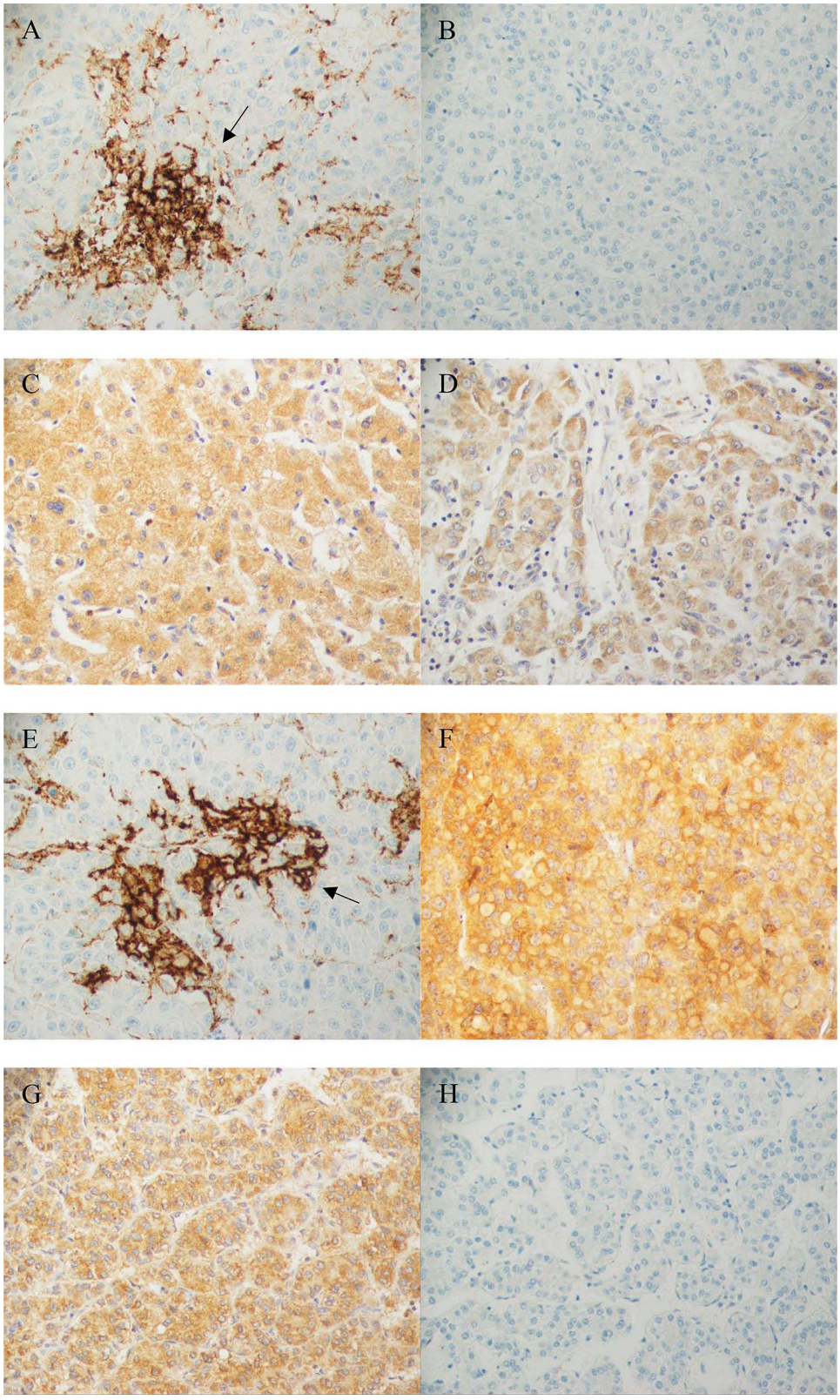

Figure 1 The expression of PD-L1 and IDO detected by immunohistochemical staining. Representative images of immunohistochemical staining of PD-L1 and IDO in HCC cases: (**A**) Positive strong membranous PD-L1 staining in tumor cells with high PD-L1 expression indicated by black arrow. (**B**) Negative PD-L1 expression. (**C**) Strong diffuse high cytoplasmic IDO expression. (**D**) Weak patchy low cytoplasmic IDO expression. (**E** and **F**) High PD-L1 and IDO expression in the same tissue specimen respectively. (**G** and **H**) IDO+ and negative PD-L1- in the same tissue specimen respectively. All images were captured at 40X magnification.

(84%) and low IDO expression was observed in 8 (16%) tissue specimens as shown in Figure 1C and D. Additionally, both PD-L1 and IDO had high expression in 11 (22%) patients as shown in Figure 1E and F. While IDO+ and PD-L1- expressions are shown in Figure 1G and H. There was no statistically significant association between PD-L1 and IDO expression with age, sex, histology, or smoking and alcoholic status in these patients (Table 1 and Table 2).

Discussion

In the present study, we examined the PD-L1 expression, and clinicopathological and radiological features of non-HCV and non-HBV-associated HCC patients, including IDO expression. To evaluate the expression of PD-L1 and IDO, immunohistochemistry was performed on the HCC tissues. In recent years, it has been observed that the number of patients with non-HCV and non-HBV-associated HCC has increased, however, the incidence and the number of patients with viral-associated HCC tended to decrease.[8] Furthermore, Toyoda et al revealed that the patient's survival improved significantly in the viral HCC group as compared to the non-viral HCC group.[45] One of the reasons could be less efficacy of surveillance in non-viral HCC patients. In addition, there is a lack of measures to preserve liver function in non-viral HCC.[45]

The immune system plays a pivotal role in regulating cancer progression.[46] Over the past few years, immuno-oncology has been a paradigm shift in cancer therapy, including HCC.[47] Although there are several treatment options for HCC, such as surgery, trans-arterial chemoembolization, radioembolization, radiofrequency ablation, and chemotherapy, however, they will only assist a limited percentage of patients.[48] The capability of immunotherapy to elicit efficient anti-tumor responses makes it remarkably well-suited for the treatment of HCC.[48] Immune checkpoint molecules have considerably transformed the clinical management of HCC.[49] Several studies have demonstrated the role of PD-L1 in HCC.[14–18] Moreover, the role of IDO has also been identified in HCC.[36–40] Keeping the previously reported data in the view, we investigated the expression of PD-L1 and IDO in non-HCV and non-HBV-associated HCC patients. This rare group of patients was identified from the hospital record for the last seventeen years.

Jung et al observed overexpression of PD-L1 in 27% of HCC specimens.[17] In our data set, PD-L1 was overexpressed in 24% of HCC specimens. These percentages were lower as compared to the other malignancies, including cancers of the lung (50%), esophagus (44%), and stomach (42%).[17,50] Furthermore, Jung et al reported PD-L1+ expression in 11 [high: 2 (18.2%), low: 9 (81.8%)] non-HCV and non-HBV-associated HCC patients.[17] We identified PD-L1+ in 21 [high: 12 (24%), low: 9 (18%)] and PD-L1- in 29 (58%) non-HCV and non-HBV-associated HCC patients. However, no significant association was found between PD-L1 expression and clinicopathological characteristics: age, gender, histological grading, and liver function tests. Our results were consistent with a previous study conducted by Jung et al.[17]

Pan et al observed overexpression of IDO in 35.5% of HCC specimens.[38] In our data set, IDO was overexpressed in 84% of HCC specimens. Moreover, Pan et al reported IDO expression in 22 [high: 5 (22.7%), low: 17 (77.2%)] in only non-HBV-associated HCC patients.[38] We identified IDO expression in all 50 [high: 42 (84%), low: 8 (16%)] non-HCV and non-HBV-associated HCC patients. Nevertheless, no significant association was found between IDO expression and clinicopathological features: age, gender, histological grading, and liver function tests. Our results were in compliance with Pan et al except for the gender.[38]

We further analyzed the clinicopathological and radiological characteristics of non-survivor HCC patients along with PD-L1/IDO expression as shown in Table 3. We evaluated that in the IDO+/PD-L1- group, 20 (69%) out of 29 patients died. All the non-survivor HCC patients were IDO+ [high: 24 (82.7%), low: 5 (17.3%)]. IDO was overexpressed in HCC specimens with histological grading of moderate to poor differentiation. Low IDO expression was only observed in the well-differentiated HCC specimens. Diabetes mellitus is a global endemic and one of the major risk factors for HCC.[51,52] In the current study, out of 29 non-survivor HCC patients, 14 (48.3%) had diabetes. 13 (92.8%) out of 14 diabetic non-survivor HCC patients had high IDO expression. Smoking and alcohol consumption are also considered risk factors for HCC.[53,54] In our data set, 10 non-survivor HCC patients were smokers, while only one patient had a history of alcohol consumption. Out of 10 smokers, 8 (80%) had high IDO expression. Liang et al reported that tobacco smoke induces IDO expression, which leads to immunosuppression and cancer progression.[55] Early HCC can be managed by surgical resection.[56] Nevertheless, multifocal HCC is challenging to treat with frequent recurrences that influence its outcome.[56] We identified 9 multifocal HCCs among the non-survivors (Figure 2). 7 (77.7%) out of 9 had high IDO expression. IDO overexpression is associated with poor prognosis and metastasis in HCC patients.[38,40] We observed metastasis in 8 out of

Table 3 Clinicopathological and Radiological Characteristic of Non-Survivor HCC Patients Along with PD-L1/IDO Expression

Case	Histological Grade	Diabetes	Smoking Status	Alcoholic Status	Multifocal	Metastasis	PD–L1	IDO
01	Poorly differentiated	+	–	–	–	–	High	High
02	Moderately differentiated	–	–	–	+	+	High	High
03	Well differentiated	+	–	–	+	–	High	High
04	Moderately differentiated	–	+	–	+	–	High	High
05	Well differentiated	–	+	–	–	–	Low	High
06	Well differentiated	+	+	–	–	+	Low	High
07	Well differentiated	+	–	–	+	–	Low	High
08	Well differentiated	+	+	–	+	+	Low	Low
09	Well differentiated	–	–	–	+	–	Low	Low
10	Well differentiated	–	+	–	–	–	–	Low
11	Well differentiated	–	–	–	–	–	–	Low
12	Well differentiated	–	–	–	–	–	–	Low
13	Well differentiated	–	+	–	–	–	–	High
14	Well differentiated	+	–	–	+	+	–	High
15	Well differentiated	–	–	–	UNK	–	–	High
16	Well differentiated	+	+	–	–	–	–	High
17	Well differentiated	+	–	–	–	–	–	High
18	Well differentiated	+	+	–	–	+	–	High
19	Well differentiated	–	–	–	–	–	–	High
20	Well differentiated	+	+	+	–	–	–	High
21	Moderately differentiated	+	–	–	–	–	–	High
22	Well differentiated	+	–	–	–	–	–	High
23	Moderately differentiated	+	–	–	+	+	–	High
24	Moderately differentiated	–	+	–	–	+	–	High
25	Well differentiated	–	–	–	+	+	–	High
26	Well differentiated	+	–	–	–	–	–	High
27	Moderately differentiated	–	–	–	–	–	–	High
28	Poorly differentiated	–	–	–	–	–	–	High
29	Well differentiated	–	–	–	–	–	–	High

Abbreviations: +, Positive; –, Negative; UNK, Unknown.

29 non-survivors among which 7 (87.5%) had high IDO expression. Our results are in compliance with those previously published by Pan et al.[38]

There were some limitations in the current study. The sample size was small because all the patients were HBV and HCV-negative. Furthermore, it was a rare group of patients with no previous history of any viral hepatitis. The data of

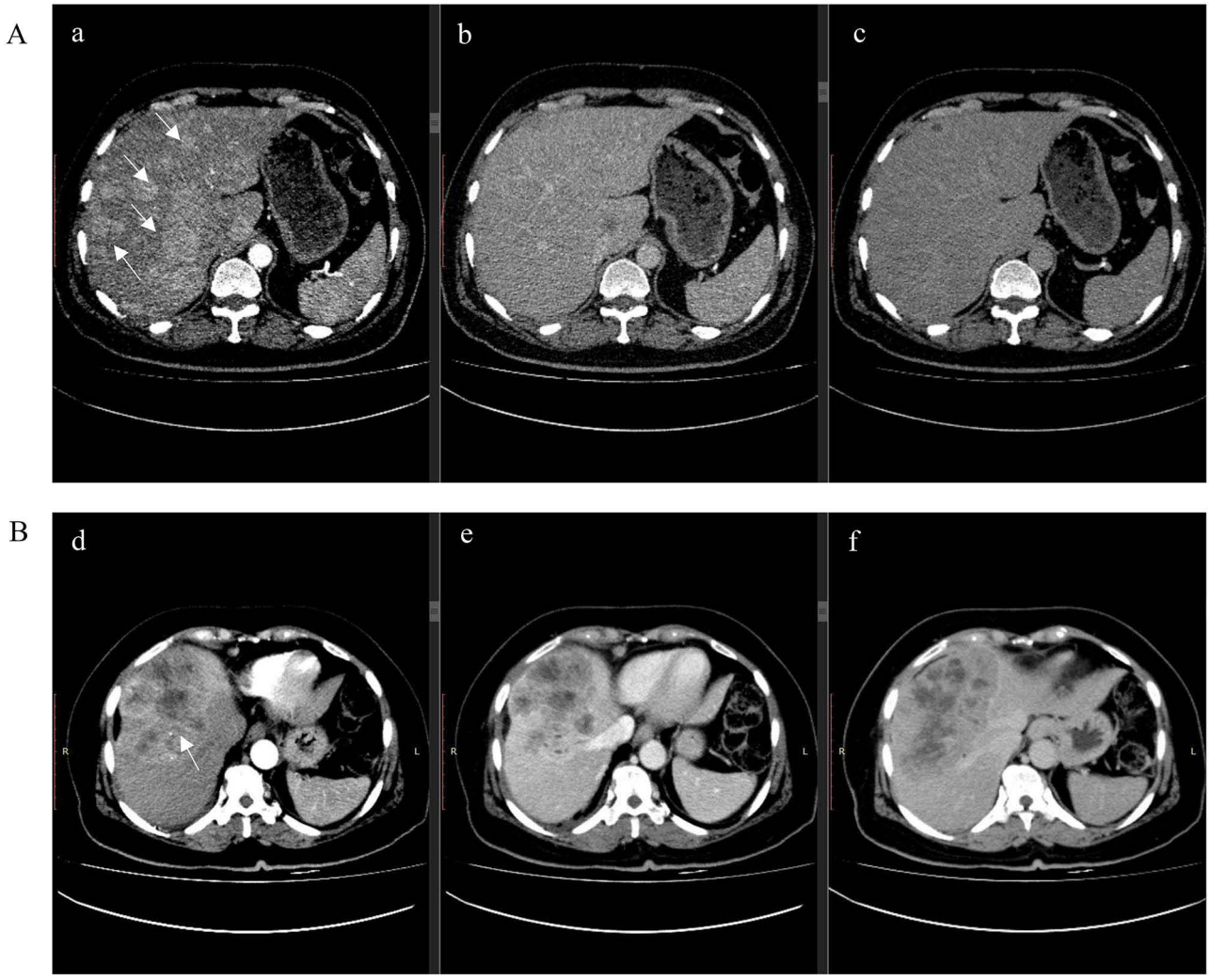

Figure 2 Computed tomography (CT) of Multifocal HCC vs Solitary HCC. (**A**) CT post-contrast axial a: arterial-phase b: portal venous phase and c: delayed phase images. Innumerable arterially enhancing hepatic lesions of variable sizes are seen demonstrating washout on the portal venous phase, consistent with multifocal HCC. (**B**); CT post-contrast d: arterial-phase e: portal venous phase and f: delayed phase images, showing relatively circumscribed centrally necrotic mass measuring 8.4×13.9 cm demonstrating peripheral arterial enhancement with venous washout and an enhancing pseudo capsule; this is centered in hepatic segments 4, 5 and 8, and approaches the capsule. Imaging features are compatible with solitary HCC.

this retrospective study was collected over the last 17 years (2005 and 2022) from a specialized cancer care hospital in Pakistan. Another limitation was the retrospective nature of our data and lack of awareness to seek medical care and early diagnosis among the patient population. Gao et al examined PD-L1 expression in 240 HCC patients. Among them, only 16 were both HBV and HCV negative.[18] Pan et al observed IDO expression in 138 HCC patients out of which only 22 patients were negative for HBV.[38] We have analyzed the expression of IDO and PD-L1 in 50 non-HCV and non-HBV-associated HCC patients. To the best of our knowledge, although there have been studies on IDO expression status and its immunosuppressing effect in HCC,[36,38,57] this is the first study to examine its expression along with PD-L1.

Conclusion

In conclusion, we report that among non-survivors, non-HCV, and non-HBV-associated HCC patients, PD-L1 was negative while IDO was overexpressed in the majority of patients. PD-L1 expression is controversial in predicting which tumor subtypes might be responsive to anti-PD-L1 immunotherapy. Hence, it is suggested to investigate PD-L1 expression prior to treatment to determine which individual patients may benefit from therapy. Recently, IDO has gained attention as a novel immunotherapeutic and prognostic marker in cancer. Combinational therapy is a cornerstone of

cancer therapy as it enhances therapeutic efficacy more than the mono-therapeutic approach. IDO and PD-L1 inhibitors might be a promising strategy for HCC management. Nevertheless, further multicenter studies on larger cohorts are warranted to fortify these findings.

Data Sharing Statement

The data generated in the present study may be requested from the corresponding author.

Ethics Approval and Informed Consent

The institutional review board (IRB) of SKMCH&RC approved the current retrospective study (#EXMPT-09-03-18-01). IRB granted the waiver for informed consent for this study, which is in accordance with the Declaration of Helsinki. The patient data accessed complied with relevant data protection and privacy regulations.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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