RESEARCH ARTICLE

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# High Indoleamine 2,3-Dioxygenase Expression along with Low Bridging Integrator-1 Expression in Hepatocellular Carcinoma Patients

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## Abstract

**Background:** Hepatocellular carcinoma (HCC) adopts several tumor immune escape mechanisms; therefore, it has the potential to be targeted by immunotherapy. Indoleamine 2, 3-dioxygenase (IDO) is an immunosuppressive enzyme that has been observed to be overexpressed in HCC patients with poor prognoses. Bridging integrator 1 (*Bin1*) loss promotes immune escape in cancer by deregulating IDO. Our aim is to investigate IDO expression along with *Bin1* expression to find evidence of immunosuppression in HCC patients. **Materials and Methods:** In this study, we investigated IDO and *Bin1* expression in HCC tissue specimens and the correlation of IDO and *Bin1* expression with clinicopathological characteristics and prognosis of HCC patients (n=45). Immunohistochemical analysis was performed to analyze the expression of IDO and *Bin1*. **Results:** IDO was overexpressed in 38 (84.4%) out of 45 HCC tissue specimens. In addition, tumor size was significantly increased with an increase in the IDO expression (P=0.03). Low *Bin1* expression was observed in 27(60%) HCC tissue specimens, whereas the remaining 18(40%) showed high *Bin1* expression. **Conclusion:** Our data showed that expression of IDO along with *Bin1* expression could be investigated for clinical evaluation in HCC. IDO might be used as an immunotherapeutic target for HCC. Therefore, further studies in larger patient cohorts are warranted.

**Keywords:** Indoleamine 2- 3-dioxygenase- bridging integrator-1- hepatocellular carcinoma- immunosuppression

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## Introduction

HCC is a leading cause of cancer-associated morbidity and mortality (Sim et al., 2018). HCC is one of the most common cancers and it accounts for the second most cancer-associated deaths worldwide (Park et al., 2015). The incidence of HCC is increasing in Pakistan (Hafeez et al., 2016). The prognosis of HCC is poor even after surgical resection because of a high recurrence rate (Pan et al., 2008). Several studies have revealed that the tumor immunosuppressive microenvironment mediates the tumor immune escape (Mapara et al., 2004; Zou et al., 2005). However, the exact underlying immunosuppressive mechanisms are not completely understood. Cancer cells escape from normal immune destruction. One mechanism by which this is achieved is through the immunosuppression that is created by the up-regulation of IDO (Nelp et al., 2018). IDO is an immunosuppressive enzyme that degrades tryptophan into kynurenine (Takikawa et al., 1986; El-Fattah et al., 2022) and it is generally identified in several types of tumors (Uyttenhove et al., 2003; Théate et al., 2015). Overexpression of IDO was observed in the tissue samples of HCC patients (Pan et al., 2008). Furthermore, high-IDO tumors were linked with poor overall survival (Korangy et al., 2010). Recently, published data revealed that IDO expression in HCC is associated with the host antitumor immune responses (Li et al., 2018).

*Bin1* is located on human chromosome 2 and encodes numerous tissue-specific isoforms of the Myc-interacting

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adaptor protein (Sakamuro et al., 1999; Elliott et al., 1999; Sakamuro et al., 1999; Prendergast., 1999; Prokic et al., 2014). Several studies demonstrated that *Bin1* expression is abrogated in various primary tumors, such as prostate and breast cancers, however, its ectopic expression could promote apoptosis and inhibit cell proliferation (Sakamuro et al., 1996; Ge et al., 1999; Ge et al., 2000; Ghaneie et al., 2007; Galderisi et al., 1999; Hogarty et al., 2000; Lundgaard et al., 2011). Previously, a lack of *Bin1* expression was observed in the HCC cell line, and overexpression of *Bin1* was shown to suppress tumor cell growth (Sakamuro et al., 1996). These results suggested that *Bin1* might play a crucial role as a tumor suppressor in HCC. Recent data has shown that abnormal gene expression, including overexpression of oncogene and down-expression of tumor suppressor, is responsible for the development of HCC (Pan et al., 2012). Muller et al. reported that *Bin1* is involved in regulating the expression of the Indo gene, which encodes the IDO enzyme (Muller et al., 2005). IDO-dependent tryptophan depletion inhibits T-cells proliferation. Tryptophan shortage leads to T-cells' arrest in the G1 phase of their cell cycle. Therefore, IDO expression can inhibit T-lymphocytes and can promote immune escape in the tumor microenvironment (Uyttenhove et al., 2003). However, the immunosuppressive mechanisms involved in the development of HCC remain elusive. Our aim was to investigate IDO expression along with *Bin1* expression to find evidence of immunosuppression in HCC patients. Therefore, we investigated the IDO and *Bin1*, expression in the tissue samples of HCC by performing immunohistochemical analysis. Furthermore, we evaluated the IDO, *Bin1* expression, and clinicopathological parameters of HCC.

## Materials and Methods

*Tissue samples and data collection*

Formalin-fixed paraffin-embedded (FFPE) HCC tumor tissues (n=45) were obtained from the pathology department, at Shaukat Khanum Memorial Cancer Hospital and Research Centre (SKMCH&RC) Lahore, Pakistan between 2005 and 2020. All the patients selected for the current study were HBV and HCV negative and registered at SKMCH&RC. Furthermore, they had no previous history of any viral hepatitis. These patients did not receive any preoperative treatment (chemotherapy and radiotherapy). The histologic cell types were allocated according to the criteria given in the WHO classification (Bosman et al., 2010). The information about the clinical characteristics of the HCC patients was retrieved from pathology and radiology reports of the hospital medical record system. The radiological images are shown in Figure-1. To the best of our knowledge, this is the first study that investigates the association between IDO expression and *Bin1* in HCC patients. 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.

*Immunohistochemistry and scoring of IDO and Bin1*

Two sections of FFPE tumor specimens of the same patients were cut at a thickness of 4 µm. Sections were deparaffinized in xylene and rehydrated. Further, the sections were treated with hydrogen peroxide (3%) at RT for 10 min. For heat-induced epitope retrieval, in a Tris-EDTA buffer pH 9.0 for 20 min by using a microwave oven. Then treated with phosphate-buffered saline (PBS) two times for 5 min. Then sections were incubated for 40 min at 37°C with the primary antibodies IDO1 (Abcam, # ab55305, anti-Indoleamine 2, 3-dioxygenase antibody, @1:200) and *Bin1* (Abcam, # ab137459, anti-*Bin1* antibody, @1:1000) independently. The immunoreactivity was detected by using the Dako EnVision kit (K5007). Peroxidase activity was detected by incubation with a DAB kit (K5007). Finally, the sections were counterstained with hematoxylin, dehydrated, cleared in xylene, and mounted with Vitro-Clud. Slides were visualized by an optical microscope (Provis AX-70, Olympus, Melville, NY).

The total IDO and *Bin1* immunostaining scores were calculated as described earlier (Pan et al., 2008; Pan et al., 2012). The intensity was scored for IDO as strong (3), moderate (2), weak (1), or negative (0). The percentage of tumor cells with positive staining was classified as diffuse (3+, 50–75%), focal (2+, 25–50%), sporadic (1+, 5–25%), and negative (0, <5%). The total score of staining intensity and percentage ranged from 0 to 9, (>4: high expression and ≤4: low expression). *Bin1* staining score was also based on two factors i) the staining intensity and ii) positively stained tumor cells. Briefly, the intensity was scored as 0 (no staining), 1 (weakly stained), 2 (moderately stained), or 3 (strongly stained). The percentage of positively stained tumor cells was scored as 3 (>50%, diffuse), 2 (25–50%, focal), 1 (5–25%, sporadic), or 0 (<5%, negative), The total immunostaining score was calculated with the value of staining intensity score × percent positivity score (range: 0 to 9). We classified the *Bin1* expression levels as follows: +++ (score >6) ++ (score 4–6), + (score 2–3), and – (score 0–1). The patients were divided into two groups according to their expression levels of *Bin1*: the high (++ and +++) and low (– and +). Two 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.

*Statistical analysis*

Statistical analysis was performed by using SPSS software (version 20.0; SPSS, Chicago, IL, USA). Percentages (proportions) were used for categorical variables while the mean and standard deviation was used for continuous variable. Bivariate analysis was done using chi-square or Fisher exact test (when necessary). For continuous explanatory variables such as age, Mann Whitney U test was performed. The Kaplan-Meier method was used to observe the survival difference between the groups (IDO and *Bin1*). Statistical significance was defined as a two-tailed P-value of 0.05.

## Results

*Immunohistochemical staining of IDO in HCC patients' samples*

To investigate the relationship between the IDO expression and clinicopathological features in HCC, paraffin-embedded HCC tissues (n=45) were used for immunohistochemical analysis. IDO expression was cytoplasmic. High IDO expression was observed in 38(84.4%) HCC tissue specimens, whereas the remaining 7(15.6%) showed low IDO expression (Table 1). The distant normal liver tissues did not display IDO-positive staining (Figure 2A). Additionally, a stepwise increase in the expression of IDO was observed in well-differentiated to poorly differentiated HCC tumor tissue (Figure 2 B-D). However, IDO expression was identified in the tumor cells and neighboring non-tumorous tissue (Figure 2E). The relationship between IDO expression and clinicopathological characteristics was listed in Table-1. We observed that tumor size was significantly increased with an increase in the IDO expression (P=0.03). Tumor size in low IDO expression was 6 (3-8) median and high IDO expression was 9 (1-20) median respectively as shown in Table 1.

*Immunohistochemical staining of Bin1 in HCC patients' samples*

To further examine the relationship between the *Bin1* expression and clinicopathological characteristics in HCC, paraffin-embedded HCC tissues (n=45) of same patients were used for immunohistochemical analysis. *Bin1* expression was cytoplasmic. Low *Bin1* expression was observed in 27(60.0%) HCC tissue specimens, whereas the remaining 18 (40.0%) showed high *Bin1* expression (Table

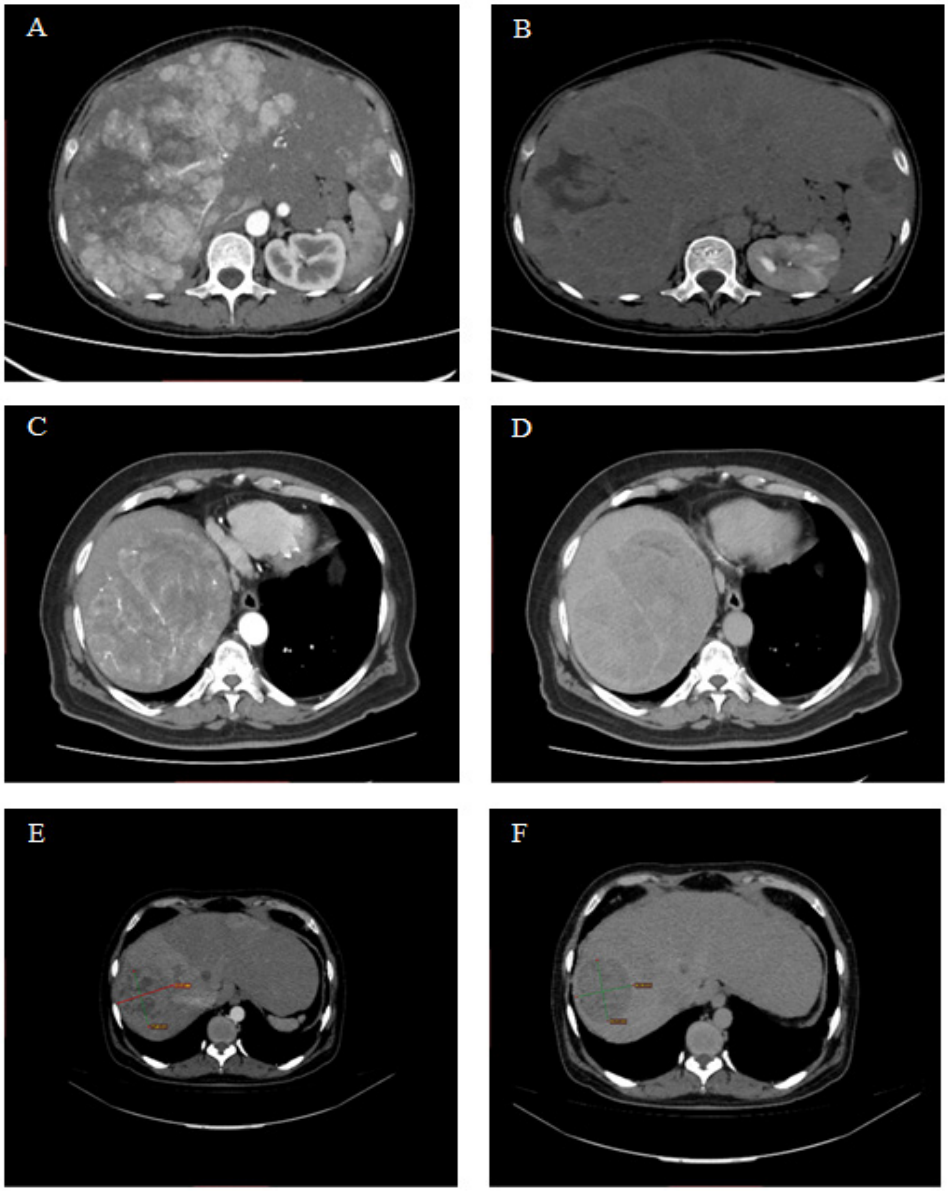


Figure 1. CT Images of HCC Patients. (A-B) Innumerable round circumscribed nodular enhancing lesions throughout the hepatic parenchyma, particularly in the right hepatic lobe, which are demonstrating arterial enhancement and delayed washout, worrisome for HCC. (C-D) Huge 14 x 11.5 x 8.7 cm size hypervascular avidly enhancing lobulated mass in segment 8 and 7 of the liver. This shows washout on the venous and delayed phase images and has a capsule around it suggestive of HCC. (E-F) Large angio invasive HCC in segment 8/7 of the liver with infiltrates disease in segment 5 and 8 of the liver.

Table 1. Demographics and Baseline Characteristics of Patients with IDO

| | Total (n=45) | IDO Low 7 (15.6%) | IDO High 38 (84.4%) | P-value |
|---|---|---|---|---|
| Demographics | | | | |
| Age (years) | | | | 0.26 |
| Median (min-max) | 65 (19-88) | 67 (63-80) | 65 (19-88) | |
| Gender | | | | 0.78 |
| Male | 34 (75.6%) | 5 (71.4) | 29 (76.3) | |
| Female | 11 (24.4%) | 2 (28.6) | 9 (23.7) | |
| Ethnicity | | | | 0.66 |
| Afghanistan | 2 (4.4) | 0 (0.0) | 2 (5.3) | |
| Balochistan | 2 (4.4) | 1 (14.3) | 1 (2.6) | |
| Khyber Pakhtunkhwa | 2 (4.4) | 0 (0.0) | 2 (5.3) | |
| Punjab | 39 (86.7) | 6 (85.7) | 33 (86.6) | |
| Histological grade | | | | 0.62 |
| Poorly differentiated | 3 (6.7) | 1 (14.3) | 2 (5.3) | |
| Moderately differentiated | 11 (24.4) | 1 (14.3) | 10 (26.3) | |
| Well differentiated | 31 (68.9) | 5 (71.4) | 26 (68.4) | |
| ECOG | | | | 0.07 |
| 0 | 10 (22.2) | 4 (57.1) | 6 (15.8) | |
| 1 | 12 (26.7) | 1 (14.3) | 11 (28.9) | |
| Unknown | 23 (51.1) | 2 (28.6) | 21 (55.3) | |
| Alcoholic status | | | | 0.51 |
| No | 41 (91.1) | 6 (85.7) | 35 (92.1) | |
| Yes | 4 (8.9 | 1 (14.3) | 3 (7.9) | |
| Status | | | | 0.8 |
| Alive | 8 (17.8) | 1 (14.3) | 7 (18.4) | |
| Death | 29 (64.4) | 5 (71.4) | 24 (63.2) | |
| Unknown | 8 (17.8) | 1 (14.3) | 7 (18.4) | |
| Diabetes | | | | 1 |
| No | 19 (42.2) | 3 (42.3) | 16 (42.1) | |
| Yes | 26 (57.8) | 4 (57.1) | 22 (57.9) | |
| Smoking status | | | | 0.22 |
| No | 29 (64.4) | 3 (42.9) | 26 (68.4) | |
| Yes | 16 (35.6) | 4 (57.1) | 12 (31.6) | |
| Metastasis status | | | | 1 |
| No | 36 (80.0) | 6 (85.7) | 30 (78.9) | |
| Yes | 9 (20.0) | 1 (14.3) | 8 (21.1) | |
| Angio-invasion | | | | 1 |
| No | 36 (80.0) | 6 (85.7) | 30 (78.9) | |
| Yes | 9 (20.0) | 1 (14.3) | 8 (21.1) | |
| Tumor size (cm) | | | | 0.03* |
| Median (min-max) | 8 (1-20) | 6 (3-8) | 9 (1-20) | |
| AFP | | | | 0.81 |
| Median (min-max) | 25 (1-30,000) | 26 (2-543) | 24 (1-30,000) | |
| ALT | | | | 0.54 |
| Median (min-max) | 46 (10-759) | 43 (10-59) | 47 (12-759) | |
| AST | | | | 0.31 |
| Median (min-max) | 46 (18-649) | 43 (18-58) | 46 (19-649) | |

ECOG, Eastern Cooperative Oncology Group; AFP, alpha-fetoprotein; ALT, alanine transaminase; AST, aspartate transaminase

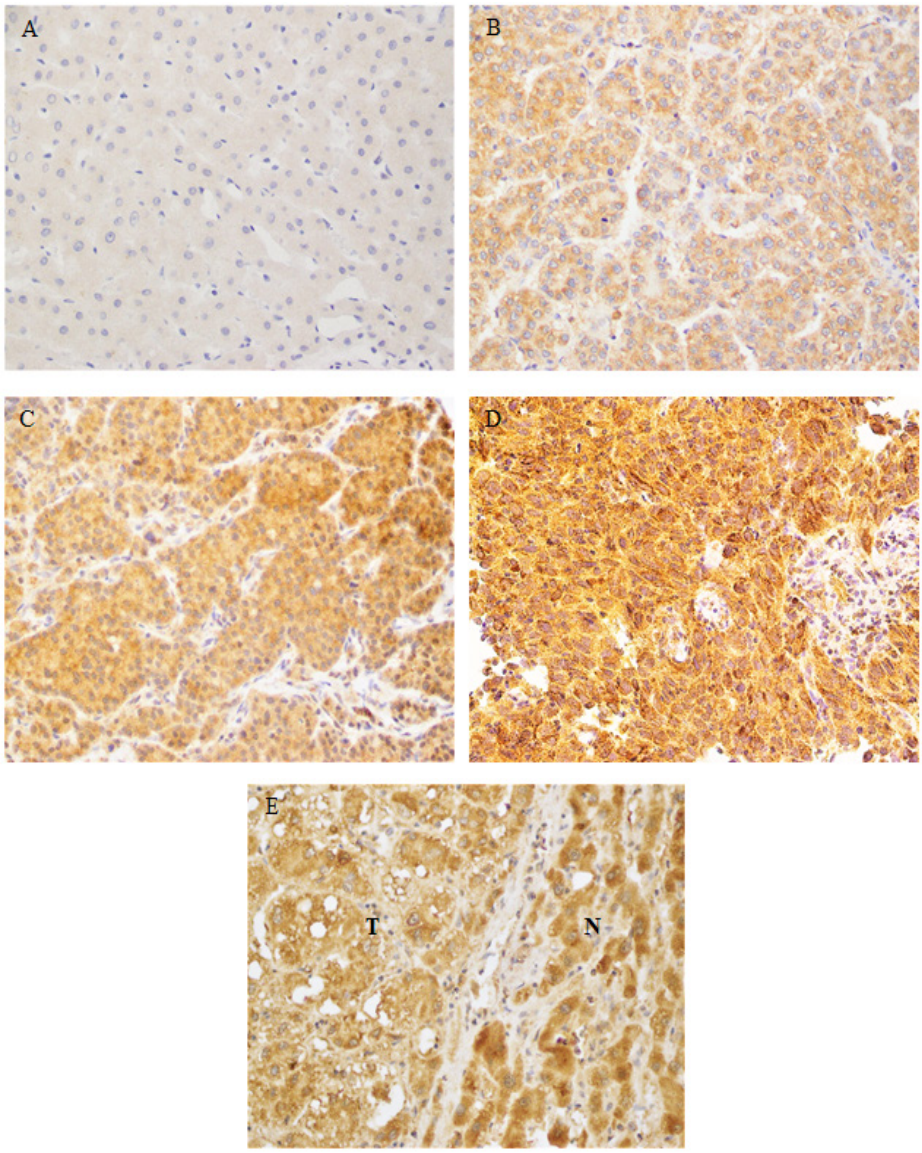


Figure 2. The Expression of IDO Detected by Immunohistochemical Staining. (A) Distant normal liver tissue. (B) Well differentiated HCC. (C) Moderately differentiated HCC. (D) Poorly differentiated HCC. (E) Surrounding noncancerous tissue; N, tumor tissue; T. Images were captured at 40X magnification.

2). We found *Bin1* expression in the distant normal liver tissues and adjacent non-tumorous tissue (Figure 3 A, B) however, *Bin1* expression was decreased in tumor tissue as compared to the non-tumorous tissue (Figure 3B). Additionally, a stepwise decrease in the expression of *Bin1* was observed in well-differentiated to poorly differentiated HCC tumor tissue (Figure 3 C-E). Out of 45, 3 (6.7%) patients were having poorly differentiated grades, 11 (24.4%) patients had moderately differentiated grades and well-differentiated was reported in 31 (68.9%) patients. Additionally, moderately differentiated grades showed a significant difference with respect to *Bin1* low and high status. Furthermore, out of 11 (24.4%) moderately differentiated grades, the majority of the patients, 10 (91%), showed low *Bin1* expression as shown in Table 2.

*Survival Analysis*

To investigate the survival difference of IDO and *Bin1* in HCC, overall survival rates were analyzed by Kaplan–Meier survival curves (Figure 4). The two-year overall survival was 35%. Furthermore, overall survival was bifurcated between IDO and *Bin1* low and high respectively. Two-year overall survival between IDO low and high was 33% and 35% and *Bin1* low and high was 31% and 40% respectively. In addition, clinically a survival difference was observed in IDO (low vs high) and *Bin1* (low vs high) expression but statistically the difference was not significant between IDO expression (P=0.63) and *Bin1* (P=0.38) as shown in Figure 4.

## Discussion

HCC is one of the common malignancies with a disappointing prognosis (Sim et al., 2018; Rastogi et al., 2018). HCC is generally developing on a background of chronic liver disease (Sim et al., 2018). Unfortunately, patients with advanced HCC have few therapeutic options (Sim et al., 2018). Despite enormous advances in the treatment and early diagnosis of HCC, the overall

Table 2. Demographics and Baseline Characteristics of Patients with *Bin1*

| | Total (n=45) | Bin1 Low 27 (60.0%) | Bin1 High 18 (40.0%) | P-value |
|---|---|---|---|---|
| Demographics | | | | |
| Age (years) | | | | 0.62 |
| Median (min-max) | 65 (19-88) | 67 (19-88) | 64 (30-78) | |
| Gender | | | | 0.3 |
| Male | 34 (75.6%) | 22 (81.5) | 12 (66.7) | |
| Female | 11 (24.4%) | 5 (18.5) | 6 (33.3) | |
| Ethnicity | | | | 0.88 |
| Afghanistan | 2 (4.4) | 2 (7.4) | 0 (0.0) | |
| Balochistan | 2 (4.4) | 1 (3.7) | 1 (5.6) | |
| Khyber Pakhtunkhwa | 2 (4.4) | 1 (3.7) | 1 (5.6) | |
| Punjab | 39 (86.7) | 23 (85.2) | 16 (88.9) | |
| Histological grade | | | | 0.03* |
| Poorly differentiated | 3 (6.7) | 1 (3.7) | 2 (11.1) | |
| Moderately differentiated | 11 (24.4) | 10 (37.0) | 1 (5.6) | |
| Well differentiated | 31 (68.9) | 16 (59.3) | 15 (83.3) | |
| ECOG | | | | 0.41 |
| 0 | 10 (22.2) | 4 (14.8) | 6 (33.3) | |
| 1 | 12 (26.7) | 8 (29.6) | 4 (22.2) | |
| Unknown | 23 (51.1) | 15 (55.6) | 8 (44.4) | |
| Alcoholic status | | | | 0.64 |
| No | 41 (91.1) | 24 (88.9) | 17 (94.4) | |
| Yes | 4 (8.9 | 3 (11.1) | 1 (5.6) | |
| Status | | | | 0.9 |
| Alive | 8 (17.8) | 5 (18.5) | 3 (16.7) | |
| Death | 29 (64.4) | 18 (66.7) | 11 (61.1) | |
| Unknown | 8 (17.8) | 4 (14.8) | 4 (22.2) | |
| Diabetes | | | | 0.11 |
| No | 19 (42.2) | 14 (51.9) | 5 (27.8) | |
| Yes | 26 (57.8) | 13 (48.1) | 13 (72.2) | |
| Smoking status | | | | 0.76 |
| No | 29 (64.4) | 18 (66.7) | 11 (61.1) | |
| Yes | 16 (35.6) | 9 (33.3) | 7 (38.9) | |
| Metastasis status | | | | 0.72 |
| No | 36 (80.0) | 21 (77.8) | 15 (83.3) | |
| Yes | 9 (20.0) | 6 (22.2) | 3 (16.7) | |
| Angio-invasion | | | | 0.72 |
| No | 36 (80.0) | 21 (77.8) | 15 (83.3) | |
| Yes | 9 (20.0) | 6 (22.2) | 3 (16.7) | |
| Tumor size (cm) | | | | 0.61 |
| Median (min-max) | 8 (1-20) | 8.50 (1-20) | 7.50 (2-16) | |
| AFP | | | | 0.61 |
| Median (min-max) | 25 (1-30,000) | 22 (1-30,000) | 26 (3-5582) | |
| ALT | | | | 0.85 |
| Median (min-max) | 46 (10-759) | 46 (10-188) | 46 (14-759) | |
| AST | | | | 0.41 |
| Median (min-max) | 46 (18-649) | 54 (18-262) | 41 (21-649) | |

ECOG, Eastern Cooperative Oncology Group; AFP, alpha-fetoprotein; ALT, alanine transaminase; AST, aspartate transaminase

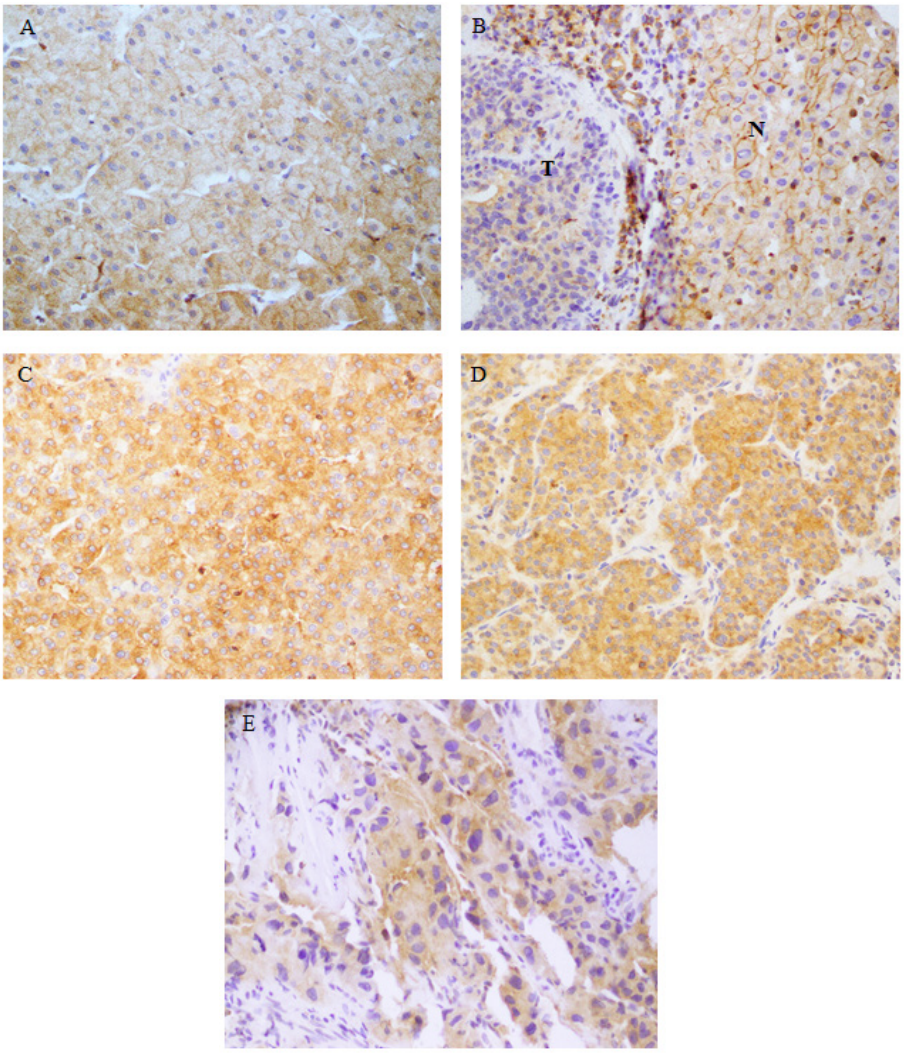


Figure 3. The Expression of *Bin1* Detected by Immunohistochemical Staining. (A) Distant normal liver tissue. (B) Surrounding noncancerous tissue; N, tumor tissue; T. (C) Well differentiated HCC. (D) Moderately differentiated HCC. (E) Poorly differentiated HCC. Images were captured at 40X magnification.

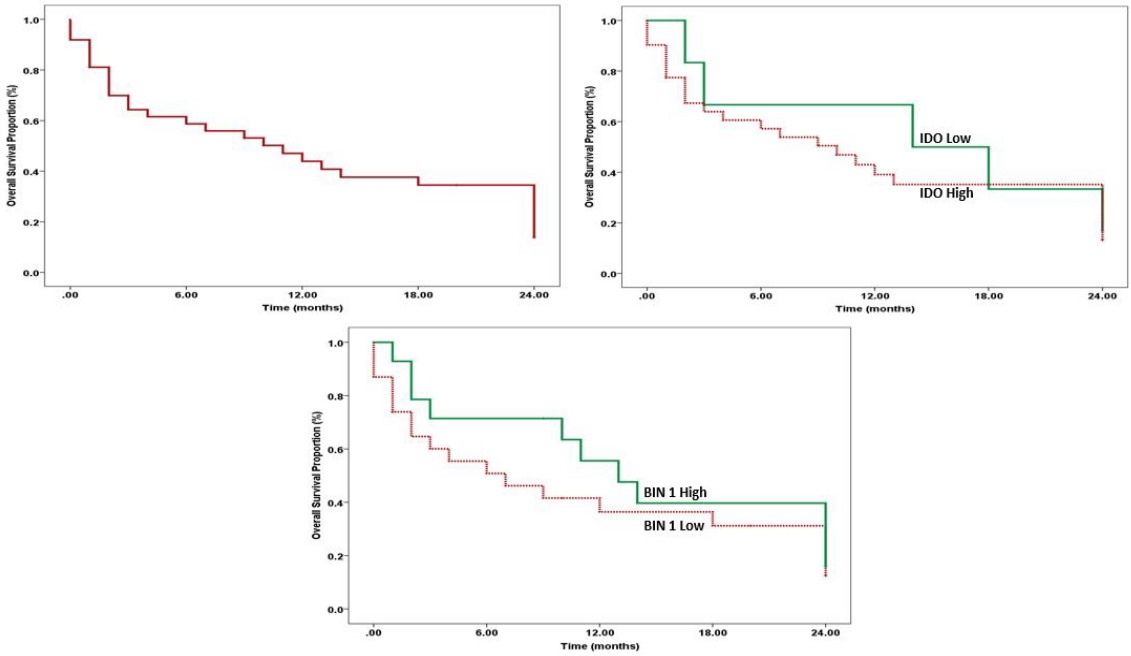


Figure 4. Kaplan-Meier Analysis of Overall Survival of HCC Patients (A) Kaplan–Meier survival curves of IDO-high versus IDO-low expression in the patients with HCC (B). Kaplan–Meier survival curves of Bin1-high versus Bin1-low expression in the HCC patients (C).

survival of patients remains unsatisfactory. Therefore, it is emergent to identify the new immunotherapeutic targets.

Immune escape is a hallmark of cancer (Muller et al., 2005). The debilitated immune system is a characteristic feature of solid tumors (Mortezaee et al., 2020). There is a complex interplay between cancer and the host immune system (Muenst et al., 2016). Amongst the several mediators responsible for the escape of tumors from the host's immune responses, the immunoregulatory enzyme IDO has attracted distinctive consideration (Zamanakou et al., 2007). Elevation of IDO in human cancer can assist immune escape (Muller et al., 2005). IDO is under the genetic control of *Bin1*, which is broadly attenuated in several malignancies (Sakamuro et al., 1996; Ge et al., 1999; Tajiri et al., 2003; Muller et al., 2004; DuHadaway et al., 2003). Furthermore, mouse knockout studies point out that *Bin1* loss promotes the STAT1- and NF-κB-dependent expression of IDO (Muller et al., 2005). Muller et al. revealed that *Bin1* loss promotes immune escape in cancer by deregulating IDO (Muller et al., 2005). Moreover, *Bin1* restricts IDO at the level of IFN-γ regulated transcription by restraining the induction of IDO (Muller et al., 2005). In the tumor microenvironment (TME), the presence of IFN-γ may elucidate the upregulation of IDO and the attenuation of *Bin1* during cancer progression (Muller et al., 2005).

In the current study, we evaluated the IDO and *Bin1* expressions and clinical outcomes of HCC patients by tissue samples (n=45) for the first time and observed that the high IDO expression was associated with low *Bin1* expression. Tissue immunohistochemical analysis showed a high IDO expression of 84.4% in HCC samples. High IDO expression was associated with the larger tumor size 9 (1-20) as compared to the low IDO expression 6 (3-8), Table 1. Furthermore, we found the elevated expression of IDO in 67% of poorly differentiated, 91% of moderately differentiated and 84% of well-differentiated HCC. In addition, high IDO expression was clinically but not statistically associated with metastasis, angio-invasion and patient status (alive/dead). Overexpression of IDO was associated with a high rate of metastasis (89%) and angio-invasion (89%) as compared to the low IDO expression. Importantly, the high IDO expression was associated with mortality (83%) as compared to the low IDO expression (17%). We observed the IDO expression in the tumor cells and neighboring non-tumorous tissue and this may create an immunosuppressive environment, helping tumor cells to evade the immune system. Our results are consistent with a previous study conducted by Pan et al (Pan et al., 2008).

Low *Bin1* expression was observed in 60% of primary HCC samples. Low *Bin1* expression was clinically but not statistically associated with a larger tumor size, metastasis, angio-invasion and patient status (alive/dead). Additionally, Low *Bin1* expression showed larger tumor size of 8.50 (1-20) as compared to the high *Bin1* expression of 7.50(2-16), (Table-2). Moreover, we found low *Bin1* expression in poorly differentiated (33%), moderately differentiated (91%), and well-differentiated (52%) HCC. Furthermore, low *Bin1* expression was associated with a high rate of metastasis (67%) and angio-invasion (67%) as compared to the high *Bin1* expression. Low *Bin1* expression was associated with mortality (62%) as compared to the high *Bin1* expression (38%). Our findings are consistent with the previous study conducted by Pan et al (Pan et al., 2012).

All the patients (n=45) selected for the current study were HBV and HCV negative. However, in the previously performed independent studies to identify the IDO and *Bin1* expression status in HCC patients, the sample size was 22 and 17 respectively. In addition, these patients were only HBV negative (Pan et al., 2008; Pan et al., 2012). The sample size of previously published data was far smaller than ours; furthermore, all the patients were treatment-naive and had no history of viral hepatitis. To the best of our knowledge, although there have been studies on IDO expression status and its immunosuppressing effect in HCC (Pan et al., 2008; Li et al., 2018; Brown et al., 2018; Zhao et al., 2016; Lin et al., 2013; Han et al., 2014) , this is the first study to examine its expression in association with *Bin1*. In our data, we found that 27 (60%) patients showed low *Bin1* expression and 18 (40%) patients showed high *Bin1* expression (Table 2). In addition, out of 27 (60%) with low *Bin1* expression majority of patients, 23 (85.2%), showed high IDO expression. We observed that high IDO and low *Bin1* expression was associated with mortality. Furthermore, there was a clinical survival difference observed in low vs high IDO and *Bin1* expression as shown in Figure-4. Our results are similar with the study conducted by Jia et al. in which they observed that low *Bin1* expression along with high expression of IDO was associated with poor prognosis in esophageal squamous cell cancer (Jia et al., 2015).

There were some limitations in our study. A major limitation was the retrospective nature of our data. The sample size was limited because the HCC patients were HBV and HCV negative and had no previous history of viral hepatitis. Additionally, the clinical and pathological data were available for only those patients who were admitted to the hospital. Future studies are warranted on a larger cohort.

Our results demonstrated that both HCC cancer cells and adjacent non-cancer cells exhibited high IDO expression (Figure 2E), and this may create an immunosuppressive tumor microenvironment and tumor immune escape. Our results are consistent with a previous study conducted by Pan et al. (Pan et al., 2008). In conclusion, we suggest that IDO and *Bin1* should be investigated for clinical evaluation in HCC. IDO inhibition may add a therapeutic advantage to HCC. Therefore, further studies in larger patients' cohorts are warranted.

## Author Contribution Statement

KA, IAR, MAB, KH, MT, SB, SM, AF, KS, AL, are authors responsible for the conceptualization and execution of the study, as well as the evaluation and interpretation of patient data. They also participated in the development of project plans and revision of the manuscript, and the analysis of data. The authors reviewed and ratified the final version of the manuscript.

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*Ethics approval and consent to participate*

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.

*Data Availability*

The datasets used during the current study are available from the corresponding author upon request.

*Conflict-of-interest statement*

The authors declare that they have no conflict of interest.

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