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Role of Diagnostic and Prognostic Immunohistochemical Markers in Hepatocellular Carcinoma

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ABSTRACT

As the primary cause of cancer-related death globally, Hepatocellular Carcinoma requires accurate diagnostic and prognostic markers.Immunohistochemical indicators have been identified as promising instruments to improve the precision of hepatocellular Carcinoma diagnosis and forecast patient outcomes. **Objectives:** To evaluate the relationships between clinicopathological characteristics associated with hepatocellular carcinoma, such as tumor grade, vascular invasion, and patient characteristics, and the expression of immunohistochemical markers. **Methods:** A cross-sectional study was conducted for six months from Feb 2024 to Jul 2024 in the Department of Pathology at a tertiary care hospital. There were 323 patients with Hepatocellular Carcinoma diagnoses in all. Immunohistochemical was used to examine specimens of tissue for the markers Ki-67, CK19, Glypican-3, alpha-fetoprotein (AFP), HepPar-1, and CD34. Kaplan-Meier survival analysis, t-tests, and chi-square tests were used to evaluate correlation with clinicopathological characteristics and survival results. **Results:** High percentages of positive expression were seen for CD34 (88.2%), Glypican-3 (75.9%), and HepPar-1 (82.7%). There were noteworthy associations discovered between tumor size, vascular invasion, and serum AFP levels and IHC markers. Notably, HepPar-1 positive predicted a better prognosis (HR 0.72, p=0.032), but Glypican-3 (HR 1.58, p=0.001) and Ki-67 (HR 2.10, p=0.002) were linked to poor overall survival. **Conclusions:** It was concluded that the significant associations between specific immunohistochemical markers (e.g., HepPar-1, Glypican-3, and Ki-67) and clinicopathological characteristics, as well as their impact on prognosis in Hepatocellular Carcinoma patients.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related deaths worldwide and one of the most common types of liver cancer. Alcohol misuse, metabolic problems, and chronic liver diseases, in particular, cirrhosis carried on by viral hepatitis (HCV and HBV), are commonly associated with it. Clinical management and treatment of HCC are significantly challenged because of the disease's sneaky development and sometimes delayed diagnosis. To improve patient outcomes and customize treatment plans, early identification and precise prognostication are essential [1, 2]. In countries like China and some regions of Southeast Asia where the hepatitis B virus (HBV) is endemic, HCC is particularly widespread, making up a substantial percentage of morbidity and death due to cancer. Nevertheless, in Western nations, where hepatitis C, NAFLD, and virus (HCV) infection pose serious risk variables, the prevalence of HCC has been increasing steadily within the past few decades [3, 4]. Since HCC frequently manifests as asymptomatic or with nebulous symptoms that are easily confused with other illnesses, many patients receive their diagnosis of the disease at an advanced stage. The disease may have advanced to the point that curative measures, including surgical excision or

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transplantation, are no longer a possibility by the time a diagnosis is made. Because HCC develops slowly, routine screening is crucial but often neglected, particularly in high-risk populations [5, 6]. Treatment outcomes could be greatly improved by early detection achieved by routine imaging and biomarker investigations. Treatment choices become considerably limited for people with advanced HCC. Options for treatment could also be made more complex by parameters like the functioning of the liver, treatment obstructions, and complexity of the tumor [7, 8]. A useful method for evaluating the histopathology of HCC is immunohistochemical (IHC) staining. HepPar-1, Glypican-3, alpha-fetoprotein (AFP), CK19, CD34, and Ki-67 are examples of IHC markers that can be used to determine the biological behavior, differentiation state, and possible metastasis of a tumor. The diagnostic and treatment choices can be guided by these markers, which can help differentiate HCC from other liver diseases [9]. The primary risk factors for HCC include chronic viral infections, particularly hepatitis B virus (HBV) and hepatitis C virus (HCV), along with alcohol abuse, metabolic disorders, and non-alcoholic fatty liver disease (NAFLD). These risk factors often lead to liver cirrhosis, which is a major predisposing condition for the development of HCC.

This study aims to evaluate the relationships between clinicopathological characteristics associated with hepatocellular carcinoma, such as tumor grade, vascular invasion, and patient characteristics, and the expression of immunohistochemical markers.

METHODS

A cross-sectional study was conducted for six months from Feb 2024 to Jul 2024 in the Department of Pathology at a tertiary care hospital. The study was approved by the Institutional Review Board (KMC/RERC78) of the hospital, and informed consent was obtained from all participants before their inclusion in the study. Inclusion criteria were participants diagnosed with HCC were confirmed by histological findings, demographic information, laboratory results, imaging findings, and treatment history, and follow-up data were carefully extracted. Exclusion criteria were patients with metastatic liver cancer, incomplete clinical data, inadequate biopsy samples. The sample size for a cross-sectional study, such as evaluating immunohistochemical markers in hepatocellular carcinoma, was determined by the frequency of an outcome in particular (e.g., the expression of certain markers, such as HepPar-1, Glypican-3, etc.). The following formula was used to determine the sample size in cross-sectional studies: $n = (Z^2 * p * (1-p)) / d^2$, where Z was the 95% confidence level, p=estimated the prevalence (0.07), and d was the margin of error (5%). The sample size of 323 participants was calculated using the G*Power tool for correlation analysis. The calculation was based on prior studies assessing GP73 levels to fibrosis staging. The gold standard study/reference was used for justification [10]. Liver tissue specimens (FFPE) from 323 participants with histopathologically confirmed hepatocellular carcinoma (HCC) were used. Following tissue collection by biopsy or surgical resection, the tissues were dehydrated, cleaned in xylene, and embedded in paraffin before being fixed in 10% neutral buffered formalin for 24 to 48 hours. Glass slides were prepared with sections that were 4-5 microns thick. Using citrate or EDTA buffer, antigen retrieval was carried out following deparaffinization and rehydration. Using 3% hydrogen peroxide, endogenous peroxidase activity was inhibited. A panel of commercially available antibodies targeting specific markers associated with HCC, including alpha-fetoprotein (AFP), glypican-3 (GPC3), heat shock protein 70 (HSP70), and cytokeratin 19 (CK19), was selected based on their relevance and established utility in HCC diagnosis and characterization. Before staining, optimization of IHC protocols was conducted to ensure optimal antigen retrieval, antibody specificity, and signal detection. For the HCC IHC panel, the primary antibodies include HepPar-1 (OCH1E5, mouse, Dako, M7158), Glypican-3 (GPC3) (1G12, mouse, Abcam, ab66596), AFP (Merc Millipore Cat. No. MABX5512-10KC), CK19 (Zeta corporation-Catalogue Number Z2134ML.), CD34 (QBEnd/10, mouse, Dako, M7165), and Ki-67 (MIB-1, mouse, Dako, M7240) and goat anti-mouse IgGfor secondary antibodies. For DAB staining, HRP-conjugated anti-mouse IgG (Abcam, ab6789) was used. For immunofluorescence, Alexa Fluor 488-conjugated anti-mouse IgG (Invitrogen, A11001) was used following the manufacturer's instructions. The ABC technique with DAB chromogen was used for visualization. Slides were mounted and counterstained with hematoxylin so that a pathologist could examine them. There were positive and negative controls for every antibody in the quality control system. The clinical information was assessed by the immunohistochemically stained slides [11]. HepPar-1, Glypican-3, AFP, and CK19 were among the markers whose staining intensity and distribution were evaluated. Staining intensity (0: negative, 1+: mild, 2+: moderate, 3+: strong) and the percentage of positively stained samples are included in a semi-quantitative scoring system that was used to standardize the tumour cells' interpretation of the findings of staining. The study utilized electronic medical records and pathology reports to collect and record clinicopathological information, patient demographics, tumor characteristics (size, number, and grade), laboratory parameters (AFP levels), imaging findings, therapeutic approaches, and clinical results. Patient demographics and clinicopathological features were summarized using descriptive statistics.

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The relationships between the expression levels of immunohistochemical markers and several clinicopathological variables were examined statistically. Chi-square and t-tests were used to evaluate the significance of these relationships and find independent predictors of clinical outcomes. The IHC marker's predictive significance in predicting patient survival outcomes was assessed using the Kaplan-Meier technique and log-rank analysis.

RESULTS

The majority of HCC patients (68.4%) were aged 50 or older. Males were predominantly affected (76.1%), aligning with global trends. Tumors larger than 5 cm were found in 58.2% of cases, while 41.8% had smaller tumors. A single tumor was present in 66.6% of patients, suggesting a potentially better prognosis. Vascular invasion was observed in 28.5% of cases. Cirrhosis was present in 60.1% of patients. AFP levels ≥400 ng/mL were seen in 54.2% of cases. HBV was detected in 48.3% of patients, while HCV was found in 37.8% (Table 1).

Table 1: Demographic Characteristics of HCC Patients

Characteristics	(n=323)
Age (Years)	
<50	102 (31.6%)
≥50	221 (68.4%)
Gender	
Male	246 (76.1%)
Female	77 (23.9%)
Tumor Size (cm)	
≤5	135 (41.8%)
>5	188 (58.2%)
Number of Tumors	
Single	215 (66.6%)
Multiple	108 (33.4%)
Vascular Invasion	
Present	92 (28.5%)
Absent	231 (71.5%)
Cirrhosis Status	
Cirrhosis Present	194 (60.1%)
No Cirrhosis	129 (39.9%)
Serum AFP Levels (ng/mL)	
<400	148 (45.8%)
≥400	175 (54.2%)
Virus	
Hepatitis B Virus (HBV) Positive	156 (48.3%)
Hepatitis C Virus (HCV) Positive	122 (37.8%)

For IHC expression, robust positive expression of HepPar-1 in 82.7% (267 patients) and the negative expression of 17.3% (56 patients) was observed in HCC. 75.9% of HCC patients overexpressed Glypican-3 with a negative expression in 78 cases (24.1%). For AFP, 61.3% positive expression of AFP, and (38.7%) of negative expression was observed in patients. Although only 30% of patients have CK19 positivity, those who do have the protein have a more aggressive form of HCC with cholangiocarcinoma-like characteristics (biliary differentiation), and 70.0% of them express CK19 negatively. The abundant vascularity of HCC tumors is reflected by the high expression of CD34, an angiogenesis marker (88.2%) in HCC tissues. The cell proliferation marker Ki-67 was used. In around 35% of patients, the proliferation index was less than 10%. The tumor growth may be comparatively slower in the remaining 65% of cases with a lower proliferation index (<10%)(Table 2).

Table 2: Expression of IHC Markers HepPar-1. Glypican-3, AFP, CK19, CD34 and Ki-67 in HCC Tissues

IHC Markers	Positive Expression n (%)	Negative Expression n (%)
HepPar-1	267 (82.7%)	56 (17.3%)
Glypican-3 (GPC3)	245 (75.9%)	78 (24.1%)
AFP	198 (61.3%)	125 (38.7%)
CK19	97 (30.0%)	226 (70.0%)
CD34 (Angiogenesis)	285 (88.2%)	38 (11.8%)
Ki-67 (Proliferation Index)		
<10%	210 (65.0%)	–
≥10%	113 (35.0%)	-

HCC patients show significant correlations between IHC markers and clinicopathological features (Table 3). AFP (p=0.022*) and Ki-67 ≥10% (p=0.029*) are higher in patients aged ≥50 years. Larger tumors (>5 cm) express AFP, CD34, HepPar-1, Glypican-3, and Ki-67 ≥10% at significantly higher levels (p<0.05). Vascular invasion is linked with increased expression of HepPar-1, Glypican-3, AFP, CK19, CD34, and Ki-67 ≥10% (p<0.05). Cirrhosis correlates with AFP, CK19, and Ki-67 ≥10% (p<0.01). Higher AFP levels (≥400 ng/mL) are associated with elevated expression of HepPar-1, Glypican-3, CK19, CD34, and Ki-67 ≥10%, suggesting more aggressive tumor behavior (Table 3).

Table 3: Correlation between Clinic-Pathological Parameters and IHC Marker Expression in HCC

Parameter	HepPar-1 (p-value)	Glypican-3 (p-value)	AFP (p-value)	Ck19 (p-value)	Cd34 (p-value)	Ki-67 ≥10% (p-value)
Age (<50 vs ≥50)	0.141	0.342	0.022*	0.518	0.113	0.029*
Tumor Size (≤5 vs >5 cm)	0.002*	0.015*	0.001*	0.061	0.039*	0.001*

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Vascular Invasion	0.031*	0.018*	0.003*	0.014*	0.042*	0.002*
Cirrhosis	0.277	0.125	0.009*	0.002*	0.111	0.008*
AFP Levels (<400 vs ≥400 ng/mL)	0.001*	0.002*	-	0.016*	0.033*	0.005*

HepPar-1 was expressed in 82.7% of HCC cases and correlated with better prognosis (HR=0.72, p=0.032*), reducing mortality risk by 28%, with a median survival of 32 months. GPC3 was expressed in 75.9% of cases and linked to worse outcomes (HR=1.58, p=0.001*), increasing mortality risk by 58%, with a median survival of 28 months. AFP was positive in 61.3% of cases, associated with a 42% higher mortality risk (HR=1.42, p=0.005*), and a median survival of 26 months. CK19, expressed in 30% of cases, showed the worst prognosis (HR=1.85, p=0.001*), with an 85% increased mortality risk and a median survival of 22 months (Table 4).

Table 4: IHC Markers in HCC Correlating with Survival in HCC Patients

Marker	No. of Patients (n=323)	Median OS (Months)	1-Year Survival Rate (%)	3-Year Survival Rate (%)	Hazard Ratio (HR) [95% CI]	p-value (Log-Rank)
HepPar-1	Positive: 267 (82.7%)	32	80%	40%	0.72 [0.56-0.90]	0.032*
Glypican-3 (GPC3)	Positive: 245 (75.9%)	28	78%	35%	1.58 [1.25-2.00]	0.001*
AFP	Positive: 198 (61.3%)	26	75%	30%	1.42 [1.12-1.80]	0.005*
CK19	Positive: 97 (30.0%)	22	70%	25%	1.85 [1.40-2.45]	0.001*
CD34 (Angiogenesis)	Positive: 285 (88.2%)	30	77%	38%	0.83 [0.65-1.05]	0.015*
Ki-67 (Proliferation Index)	Positive: 113 (35.0%)	20	65%	20%	2.10 [1.65-2.65]	0.002*

Immunohistochemistry Expression of HepPar-1 is shown (Figure 1).

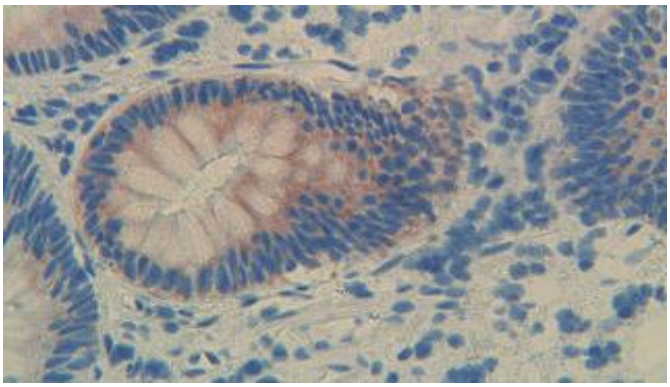

Figure 1: Immunohistochemistry Expression of HepPar-1

Immunohistochemistry expression of Glypican is shown (Figure 2).

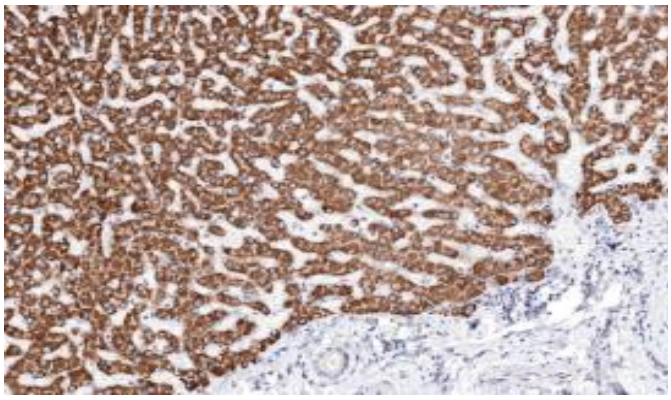

Figure 2: Immunohistochemistry Expression of Glypican

Immunohistochemistry expression of CK19 is shown (Figure 3).

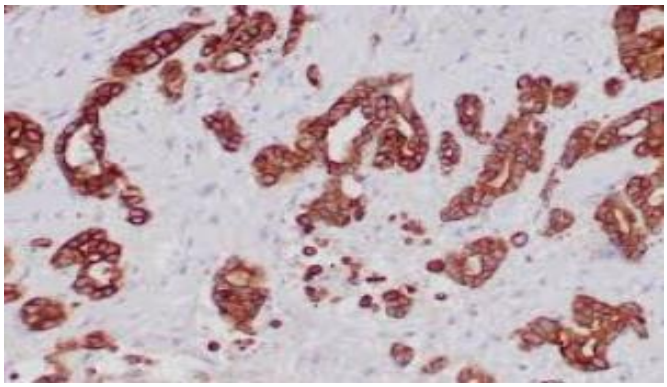

Figure 3: Immunohistochemistry Expression of CK19

Immunohistochemistry expression of Ki-67 is shown (Figure 4).

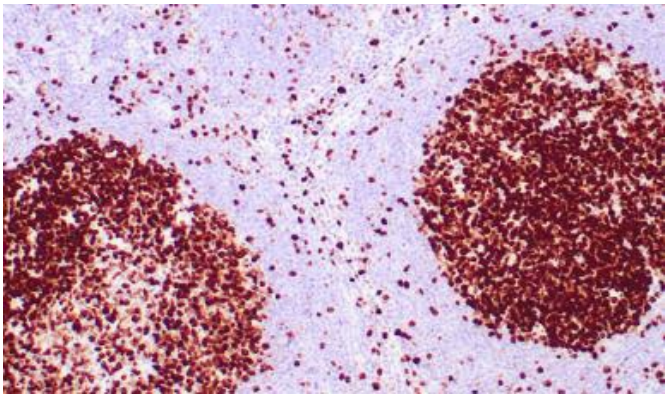

Figure 4: Immunohistochemistry Expression of Ki-67

Immunohistochemistry expression of AFP is shown (Figure 5).

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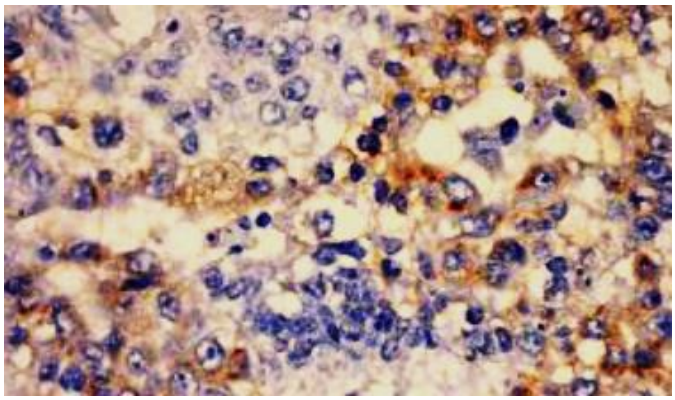

Figure 5: Immunohistochemistry Expression of AFP

Immunohistochemistry expression of CD 34 in HCC is shown, magnification used 40X (Figure 6).

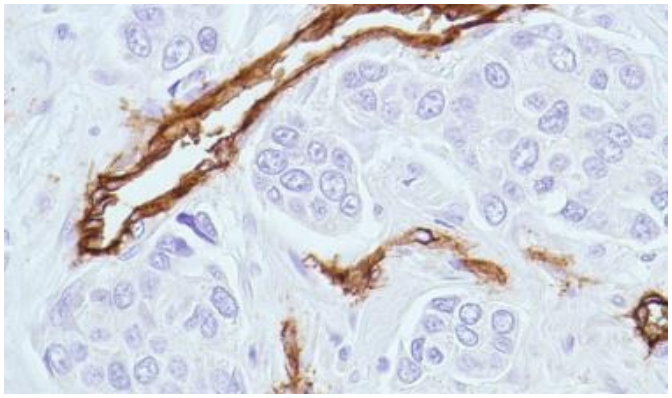

Figure 6: Immunohistochemistry Expression of CD 34 in HCC, Magnification Used 40X

DISCUSSION

Histological and molecular features of hepatocellular carcinoma (HCC) vary, and it poses a substantial worldwide health burden. The present investigation examined the expression of multiple important immunohistochemistry (IHC) markers in hepatocellular carcinoma (HCC) tissues, and assessed the associations between these markers and clinicopathological characteristics as well as overall survival (OS). In order to help with patient care and stratification, the results emphasize the significance of these indicators in predicting the clinical course of HCC [12]. HepPar-1 is a hepatocyte-specific marker that is widely used to confirm hepatocellular differentiation. The high positive expression rate of HepPar-1 (82.7%) observed in this study underscores its role as a reliable diagnostic marker for HCC. HepPar-1 positivity has been linked to well-differentiated HCC tumors, suggesting that its expression reflects a tumor's ability to retain hepatocellular features, which may be associated with less aggressive behavior. Our findings revealed high positive expression rates for several IHC markers, most notably Glypican-3 (75.9%), and CD34 (88.2%). These markers are crucial in the biological characterization and diagnosis of hepatocellular carcinoma (HCC) [13]. We were agreed from the previous research has repeatedly shown, HepPar-1 and Glypican-3 expression is highly expressed in HCC cases making it an essential marker for differentiating HCC from metastatic liver cancers. This highlights the necessity of having a panel of markers in instances when there is no HepPar-1 expression, especially in more aggressive tumor subtypes [14]. As previous study, by interacting with growth factors such as Wnt and Hedgehog, GPC3 has been demonstrated to stimulate cell proliferation and block apoptosis, hence contributing to the malignant transformation of hepatocytes. GPC3 expression was found to be highly expressed in our investigation. GPC3 expression is clinically correlated with worse outcomes; patients who tested positive for GPC3 had a significantly lower median overall survival (28 months). This correlation implies that GPC3 may be a viable target for therapy in addition to acting as a diagnostic marker. GPC3-specific immunotherapies and other GPC3-targeted medicines are the subject of ongoing clinical trials and may offer new therapy options for individuals with high GPC3 expression in their HCC [15]. In 88.2% of HCC cases, neovascularization is crucial in HCC to sustain the growing tumor mass, especially in more advanced tumors. We were observed previous study similar results as our study that, high CD34 positive rate indicates that angiogenesis is a characteristic that is present in most HCC cases, which means that it should be taken into account when making decisions about therapy and prognosis. Patients who are positive for CD34 may be more likely to benefit from anti-angiogenic treatments, such as sorafenib, which are currently being used to treat advanced HCC. Monitoring the response to anti-angiogenic therapy can be facilitated by the function of CD34 in determining the vascularity of tumors [16, 17]. The comparison of correlations between IHC markers and pathological variables such as age, tumor size, vascular invasion, cirrhosis, and AFP levels reveals important relationships that may assist in determining the development, outcomes, and severity of HCC. The strong correlation found between younger age groups and higher AFP levels could suggest that AFP-positive tumors in this age group are more aggressive and physiologically active, which could lead to an earlier beginning and possibly faster development of the disease [18, 19]. This emphasizes the necessity for younger patients with increased AFP to get more careful monitoring and care. Proliferative index is higher in younger patients, as indicated by the association between Ki-67, a well-established marker of proliferation. Consequently, surveillance programs and early detection tests are critical for the timely diagnosis and treatment of HCC. Screening efforts primarily target populations with multiple risk factors, such as known carriers of the hepatitis virus, individuals with cirrhosis, or those with a

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family history of HCC [20]. One important indicator of prognosis in HCC is the size of the tumor, with larger tumors typically denoting more aggressive illness. Since HepPar-1 tends to disappear in later-stage and less distinct cancers, the highly significant correlation between larger tumor size and minimized HepPar-1 expression may be attributed to this occurrence. Alternatively, GPC3 may be linked to more aggressive, quickly growing tumors, consistent with its function in activating oncogenic signaling pathways, as indicated by the positive connection between Glypican-3 and larger tumors [20, 21]. Increased angiogenesis, as shown by CD34 expression (p=0.039), and higher AFP levels (p=0.001) were also associated with stronger tumor sizes. This supports the function of AFP as a gauge of tumor aggressiveness and burden, and the correlation with CD34 emphasizes the role angiogenesis plays in promoting tumor growth in larger tumors. Since larger tumors have higher proliferative activity, which is a sign of more aggressive illness and cause worse outcomes [22]. HepPar-1 expression has decreased in vascular invasion instances (p=0.031), which indicates that an even more invasive tumor has been related to a decline of differentiated as shown by lower HepPar-1. Glypican-3 and AFP have significant associations with vascular invasion, which confirms both of their roles as markers for vigorous tumor dissemination and invasion through their contribution in Wnt/β-catenin signaling. AFP-positive and CK19-positive tumors are more likely to develop in the context of cirrhotic liver tissue, according to the strong association between AFP and CK19 and cirrhosis. The proliferative index (Ki-67 ≥10%) is higher in cirrhotic patients, suggesting a larger potential for tumor growth and aggressive character. This emphasizes the necessity of closely monitoring cirrhotic individuals with elevated Ki-67 expression and maybe more severe treatment [23]. The strong inverse relationship between high levels of AFP and HepPar-1 expression implies that when tumors grow more aggressive and poorly differentiated, they lose HepPar-1 expression and increase their production of AFP. These markers, Glypican-3, CK19, and CD34, are significantly correlated with increased levels of AFP, suggesting that they are frequently expressed in more aggressive tumors that produce AFP. The greater AFP production is associated with more aggressive proliferation rates in tumors, which indicates a worse prognosis and faster disease progression. This is shown in the link between greater AFP levels and increased Ki-67 expression [24]. IHC markers have a significant prognostic impact in HCC; HepPar-1 and CD34 may be predictive of improved outcomes, but GPC3, AFP, CK19, and Ki-67 are associated with a poor prognosis [25, 26]. Comprehending these correlations facilitates better patient matching, customized therapy regimens, and knowledgeable medical judgment, consequently augmenting HCC patients' management approaches. This study's sample size, while adequate, may not fully represent the broader HCC population, and larger multicenter studies are necessary for validation. The retrospective design introduces potential selection bias, and there is a lack of prospective validation. Variability in IHC staining techniques across laboratories could also affect reproducibility. Additionally, without molecular and genetic profiling of the tumors, the study may have missed other contributing factors in HCC progression. Future research should focus on larger, multicenter, prospective studies to validate these IHC markers. Incorporating molecular and genetic profiling of tumors would provide a deeper understanding of their role in HCC. Clinical trials exploring targeted therapies and immunotherapies, particularly focusing on GPC3, are also recommended to improve personalized treatment options for HCC patients.

CONCLUSIONS

It was concluded that significant clinicopathological factors are correlated with the elevated expression rates of immunohistochemistry (IHC) markers in HCC tissues, including HepPar-1 and Glypican-3. The connection between them highlights its potential importance in selecting of diagnosis and duration of treatments.

Authors Contribution

Conceptualization: AQM
Methodology: SA, AA, AHP
Formal analysis: SA, RKR
Writing review and editing: AA, AHP, SAP, RKR

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

All the authors declare no conflict of interest.

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REFERENCES

[1] Konyn P, Ahmed A, Kim D. Current Epidemiology in Hepatocellular Carcinoma.Expert Review of Gastroenterology and Hepatology.2021Nov;15(11): 1295-307. doi: 10.1080/17474124.2021.1991792.

[2] Chidambaranathan-Reghupaty S, Fisher PB, Sarkar D. Hepatocellular Carcinoma (HCC): Epidemiology, Etiology and Molecular Classification.Advances in Cancer Research.2021Jan;149:1-61.doi:10.1016/bs.acr.2020.10.001.

[3] Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S *et al.* Hepatocellular Carcinoma. Nature Reviews Disease Primers.2021Jan;7(1):1-28.doi:10.

DOI: https://doi.org/10.54393/pjhs.v6i3.2428

1038/s41572-020-00240-3.

[4] Mak LY, Liu K, Chirapongsathorn S, Yew KC, Tamaki N, Rajaram RB *et al.* Liver Diseases and Hepatocellular Carcinoma in the Asia-Pacific Region: Burden, Trends, Challenges and Future Directions. Nature Reviews Gastroenterology and Hepatology.2024Aug:1-8.doi: 10.1038/s41575-024-00967-4.

[5] Chakraborty E and Sarkar D. Emerging Therapies for Hepatocellular Carcinoma (HCC). Cancers.2022Jun; 14(11): 2798. doi: 10.3390/cancers14112798.

[6] Brown ZJ, Tsilimigras DI, Ruff SM, Mohseni A, Kamel IR, Cloyd JM *et al.* Management of Hepatocellular Carcinoma: A Review.Journal of American and Medical Association Surgery. 2023 Apr; 158(4): 410-20. doi: 10.1001/jamasurg.2022.7989.

[7] Younossi ZM and Henry L. Epidemiology of Non-Alcoholic Fatty Liver Disease and Hepatocellular Carcinoma. Journal of Hepatology Reports. 2021Aug; 3(4): 100305. doi: 10.1016/j.jhepr.2021.100305.

[8] Doria C, Rogart JN, editors. Hepato-pancreato-biliary Malignancies: Diagnosis and Treatment in the 21st Century. 2022. doi: 10.1007/978-3-030-41683-6.

[9] Sun L, Zhao H, Ding XY, Yang K, Wang GS, Chen JM *et al.* Clinicopathological Features of Hepatocellular Carcinoma with Metabolic Risk Factors.Journal of Hepatocellular Carcinoma.2023Dec:833-46.doi:10.2147/JHC.S412129.

[10] Xu ZJ, Xu T, Ye QX, Li YF, Yang TH, Zhang XM *et al.* Diagnostic and Prognostic Value of Serum Golgi Protein 73 in Patients With Hepatitis B Virus -Associated Acute-on-Chronic Liver Failure. Immunity, Inflammation and Disease.2025 Feb;13(2): e70120. doi: 10.1002/iid3.70120.

[11] Ren QS, Sun Q, Cheng SQ, Du LM, Guo PX. Hepatocellular carcinoma: An Analysis of the Expression Status of Stress Granules and Their Prognostic Value.World Journal of Gastrointestinal Oncology.2024Jun;16(6):2571.doi:10.4251/wjgo.v16.i6.2571.

[12] Kmeid M, Park YN, Chung T, Lukose G, Sullivan L, Brar R *et al.* PSMA Immunohistochemistry in Hepatic Neoplasms: A Promising Diagnostic Marker with Potential Theranostic Applications.The American Journal of Surgical Pathology.2022Dec;46(12):1688-99.doi:10.1097/PAS.0000000000001971.

[13] Guo DZ, Zhang X, Zhang SQ, Zhang SY, Zhang XY, Yan JY, Dong SY, Zhu K, Yang XR, Fan J, Zhou J. Single-cell Tumor Heterogeneity Landscape of Hepatocellular Carcinoma: Unraveling the Pro-Metastatic Subtype and Its Interaction Loop with Fibroblasts.Molecular Cancer.2024Aug;23(1):157.doi:10.1186/s12943-024-02062-3.

[14] Wang C, Shao X, Zhang X, Xie C, Yu J, Xu X *et al.* Diagnostic Value of Glypican-3, Arginase-1 and Hepatocyte Paraffin Antigen-1 in Differentiating Hepatocellular Carcinoma from Intrahepatic Cholangiocarcinoma.Translational Cancer Research. 2020 Jan; 9(1): 128. doi: 10.21037/tcr.2019.11.20.

[15] Wang JY, Wang XK, Zhu GZ, Zhou X, Yao J, Ma XP, Wang B, Peng T. Distinct Diagnostic and Prognostic Values of Glypicans Gene Expression in Patients with Hepatocellular Carcinoma.BioMed Central Cancer. 2021 Apr; 21(1): 462. doi: 10.1186/s12885-021-08104-z.

[16] Chen JL, Wang L, Li R, Jiao YF, Yu WF. High Expression of Endothelial Progenitor Cell-Induced Angiogenic Markers Is Associated with Bile Acid Levels in HCC. Oncology Letters.2020Sep;20(3):2729-38.doi:10.3892/ol.2020.11815.

[17] Jeng KS, Sheen IS, Lin SS, Leu CM, Chang CF. The Role of Endoglin in Hepatocellular Carcinoma.International Journal of Molecular Sciences.2021Mar;22(6):3208. doi: 10.3390/ijms22063208.

[18] Fang YS, Wu Q, Zhao HC, Zhou Y, Ye L, Liu SS *et al.* Do Combined Assays of Serum AFP, AFP-L3, DCP, GP73, and DKK-1 Efficiently Improve the Clinical Values of Biomarkers in Decision-Making for Hepatocellular Carcinoma? A Meta-Analysis.Expert Review of Gastroenterology and Hepatology.2021Sep;15(9): 1065-76. doi: 10.1080/17474124.2021.1900731.

[19] Ahn KS, O'Brien DR, Kim YH, Kim TS, Yamada H, Park JW *et al.* Associations of Serum Tumor Biomarkers with Integrated Genomic and Clinical Characteristics of Hepatocellular Carcinoma. Liver Cancer.2021 Nov; 10(6): 593-605. doi: 10.1159/000516957.

[20] Chan YT, Zhang C, Wu J, Lu P, Xu L, Yuan H *et al.* Biomarkers for Diagnosis and Therapeutic Options in Hepatocellular Carcinoma. Molecular Cancer.2024 Sep; 23(1): 189. doi: 10.1186/s12943-024-02101-z.

[21] Renne SL, Sarcognato S, Sacchi D, Guido M, Roncalli M, Terracciano L *et al.* Hepatocellular Carcinoma: A Clinical and Pathological Overview. Pathologica.2021 Jun; 113(3): 203. doi: 10.32074/1591-951X-295.

[22] Xu W, Huang B, Zhang R, Zhong X, Zhou W, Zhuang S *et al.* Diagnostic And Prognostic Ability of Contrast-Enhanced Unltrasound and Biomarkers in Hepatocellular Carcinoma Subtypes.Ultrasound in Medicine and Biology.2024Apr;50(4):617-26.doi: 10.1016/j.ultrasmedbio.2024.01.007.

[23] Ridder DA, Weinmann A, Schindeldecker M, Urbansky LL, Berndt K, Gerber TS *et al.* Comprehensive Clinicopathologic Study of Alpha Fetoprotein -Expression in A Large Cohort of Patients with Hepatocellular Carcinoma.International Journal of Cancer.2022Mar;150(6):1053-66.doi:10.1002/ijc.33898.

[24] Moudi B, Mahmoudzadeh-Sagheb H, Heidari Z. Hepatocyte Paraffin 1 and Arginase-1 Are Effective Panel of Markers in HBV-Related HCC Diagnosis in

DOI: https://doi.org/10.54393/pjhs.v6i3.2428

Fine-Needle Aspiration Specimens. BioMed Central Research Notes.2020 Dec; 13: 1-6. doi: 10.1186/s13104-020-05230-y.

[25] Sun LY, Cen WJ, Zeng XX, Zhong YY, Deng L, Yang JJ *et al.* The Prognostic Value of Alpha-Fetoprotein Ratio in Patients With Resectable Alpha-Fetoprotein-Negative Hepatocellular Carcinoma.The American Surgeon™.2024Jun;90(6):1240-9.doi:10.1177/00031348241227202.

[26] Kanber Y, Pusztaszeri M, Auger M. Immunocytochemistry for Diagnostic Cytopathology—A Practical Guide.Cytopathology.2021Sep;32(5):562-87. doi: 10.1111/cyt.12993.