Hepatocellular Carcinoma's Characteristics in an Endemic Country: A Closer Examination of Tumor Grade and Microvascular Invasion

Ignasia Andhini Retnowulan, M.D.*, Marini Stephanie, M.D.*, Nur Rahadiani, M.D., Ph.D.*, Ridho Ardhi Syaiful, M.D., Ening Krisnuhoni, M.D., MS***

**Department of Anatomical Pathology, Faculty of Medicine Universitas Indonesia/ Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia, **Department of Surgery, Faculty of Medicine Universitas Indonesia/ Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia.*

ABSTRACT

Objective: Although histological grade and microvascular invasion are known predictors for patient survival and recurrence in hepatocellular carcinoma (HCC), their relationship with various clinical and histomorphological features of HCC remains unclear.

Materials and Methods: Medical records were retrieved from 61 patients who were diagnosed with HCC from 2008-2018. Clinical and histomorphological variables that were hypothesized to be associated with histological grade and microvascular invasion were analyzed statistically using the Chi-square test or the Fisher's exact test as alternatives. Multivariate analysis was performed with logistic regression model.

Results: The majority of the patients had well to moderately-differentiated HCC (67.2%) with some of them presenting microvascular invasion (57.4%). Alpha-fetoprotein level (AFP) ≥100 ng/ml (p=0.036), tumor size >7cm (p=0.031) and mitotic index ≥5 per 10 high power field (p=0.009) were significantly correlated with poorly-differentiated HCC. Mitotic Index ≥5 per 10 high power field was an independent factor for poorly differentiated HCC. Meanwhile BCLC stage B and mitotic index were also an independent factor for the presence of microvascular invasion.

Conclusion: Larger tumor size and higher mitotic index was significantly correlated and independent factors for poorly differentiated HCC and microvascular invasion. In biopsy specimens for which the microvascular invasion is difficult to assess, histological grade, tumor size and mitotic index may be beneficial to depict the prognosis of patients with HCC.

Keywords: Hepatocellular carcinoma; tumor grade; microvascular invasion; Indonesia (Siriraj Med J 2023; 75: 817-826)

INTRODUCTION

Hepatocellular carcinoma (HCC) is the second most frequent cancer in Asia and the second leading cause of cancer-related deaths in East Asia and Sub-Saharan Africa. Moreover, China, Southeast Asia, Japan and Sub-Saharan Africa are areas with a high incidence of HCC with an incidence >20/100,000.[1,2]

The current HCC diagnostic approach relies on radiologic imaging and the use of histopathology biopsy is limited to specific cases.[1,3] Surveillance through ultrasonography (US) examination and alpha-fetoprotein (AFP) measurement should be carried out every 6 months in individuals with a high risk of HCC to achieve early diagnosis.[4] When nodules are found on the liver US with increased AFP level, a computed tomography (CT) scan or three-phase magnetic resonance imaging (MRI) should be performed. Typical features of HCC on CT scan or three-phase MRI are sufficient for diagnosis of

Corresponding author: Marini Stephanie
E-mail: marini2sh@gmail.com
Received 21 August 2023 Revised 8 October 2023 Accepted 9 October 2023
ORCID ID:http://orcid.org/0000-0002-5743-688X
https://doi.org/10.33192/smj.v75i11.264786

HCC, otherwise, tumor biopsy should be carried out for diagnostic purposes.[4]

Although various therapeutic modalities are available, HCC is generally diagnosed at an advanced stage when curative therapy is not possible. Therefore, at present, the prognosis of HCC remains poor.[5] Histopathological examination is involved in determining therapy and prognosis through the assessment of histological parameters which cannot be replaced by radiologic examinations.[3] The present study aimed to provide data on the clinical and histomorphology characteristics of HCC and clarify how these relate to important prognostic factors of HCC, particularly histologic grade and microvascular invasion.

MATERIALS AND METHODS

Medical records of 61 patients were retrieved from 2008-2018 archives for consecutive evaluation. Inclusion criteria were all resection cases with a diagnosis of HCC in the Department of Anatomic Pathology, FKUI/RSCM. Exclusion criteria were biopsy cases, cases with unrepresentative slide and cases with a final diagnosis of non-HCC. The clinical variables assessed were sex, age, hepatitis infection, AFP level, cirrhosis status and Barcelona Clinic Liver Cancer (BCLC) staging. Triphasic CT and additional MRI with contrast for patients with inconclusive CT were performed on all patients and yielded inconclusive radiologic results. Hepatitis infection involves positive serological markers for hepatitis B or C virus (HBV and HCV, respectively) before or during HCC diagnosis. AFP levels were measured at the time of HCC diagnosis. Cirrhosis status was determined radiologically with a US Fibroscan from Echosans and confirmed through histopathological analysis. The BCLC criteria classify clinical stages as 0, A, B, and C.[6]

Two independent pathologists evaluated the pathology reports and histologic specimens of all patients. The histomorphology variables assessed were tumor size, histological pattern, mitotic index, special type carcinomas, histological grade and microvascular invasion. Tumor size was defined as the largest dimension from resection or radiologic findings and was categorized based on the Milan criteria.[7] The histological patterns that were identified included macrotrabecular, microtrabecular, solid and pseudoglandular. Mitotic index was categorized as low or high and defined as <5 per 10 high power field (HPF) and ≥5 per 10 HPF respectively according to a study by Ha *et al.*[8] Special type carcinomas that were identified included fibrolamellar, scirrhous, undifferentiated carcinoma, lymphoepithelial, sarcomatous, steatohepatitic and macrotrabecular massive. HCC was categorized into good-to-moderate or poor differentiation based on histological grade. The cells of HCC with well-to-moderate differentiation display mildly to moderately atypical nuclei, often with nucleoli, and copious eosinophilic to basophilic cytoplasm. Meanwhile, the cells of HCC with poor differentiation were anaplastic with pleomorphic and atypical nuclei, and sparse basophilic cytoplasm.[9] Malignant cells in peritumoral blood vessels indicate microvascular invasion.

The Statistical Package for Social Sciences software (version 25.0; IBM Corp.) was used for analysis. Variables hypothesized to be associated with histological grade or microvascular invasion were analyzed with the Chi-square test or its alternatives Fisher's exact test. Multivariate analysis was performed with logistic regression to draw significant factors associated with features of HCC, the variables were selected from bivariate analysis with p-value ≤0.25.

The Ethics Committee of the Faculty of Medicine Universitas Indonesia and Dr. Cipto Mangunkusumo Hospital have granted ethical approval for this study (protocol number 23-01-0066) under the decision number KET-84/UN2.F1/ETIK/PPM.00.02/2023.

RESULTS

Data from 61 patients with HCC were retrieved. Of these, 80% were male with a mean age of 51.8 years ± 15.643, 20% of them were <40 years old and the youngest patient was 17 years old. Most patients had hepatitis infection. Most patients had stage B HCC according to the BCLC staging system (91.8%). Tumor size ranged from 2.5-25 cm. A similar proportion of cirrhotic and non-cirrhotic patients was found. The median AFP level was 113.5 ng/ml. The mean mitosis count was 6.72 over 10 HPF. The most common histological pattern was the microtrabecular pattern (72.1%). Only 5 patients (8.1%) were found to have steatohepatitic HCC. Most patients had well to moderately-differentiated HCC (67.2%). The microvascular invasion was observed in most cases (57.4%). Clinical and histological characteristics of HCC can be seen in Table 1. Representative microscopic images of the HCC found in the current patients can be seen in Fig 1.

Statistical tests examined the association between sex, age, AFP level, hepatitis infection, BCLC staging, cirrhosis status, tumor size and mitotic index with histological grade, microvascular invasion, and well-differentiated with no microvascular invasion. There was a significant association between AFP level, tumor size and mitotic index with histological grade. AFP level ≥100 ng/ml (p=0.036), tumor size >7cm (p=0.031) and mitotic index ≥5 per 10 HPF (p=0.009) were significantly

TABLE 1. Clinical and histological characteristics of HCC (n=61).

Characteristics	n (%)
Sex	
Male	49 (80.3)
Female	12 (19.7)
Age in years, mean (SD)	51.80 ± 15.643
Hepatitis infection, n (%)	
Non-hepatitis	13 (15.9)
Hepatitis	42 (58.5)
BCLC staging	
Stage 0	0 (0)
Stage A	5 (8.2)
Stage B	56 (91.8)
Stage C	0 (0)
Tumor size in cm, median (minimum-maximum)	10.9 (2.0-25)
Tumor size	
≤7 cm	24 (39.3)
>7 cm	37 (60.7)
Cirrhosis status	
Non-cirrhotic	29 (47.5)
Cirrhotic	32 (52.5)
Median (minimum-maximum) AFP level, ng/ml	113.5 (1.5 – 3898)
Mitosis count in 10 HPF, mean (SD)	6.72 (5.16)
Histological grading	
Well-moderate differentiation	41 (67.2)
Poor differentiation	20 (32.8)
Microvascular invasion	
Positive	35 (57.4)
Negative	26 (42.6)
Histological pattern*	
Macrotrabecular	16 (26.2)
Microtrabecular	44 (72.1)
Solid	31 (50.8)
Pseudoglandular	14 (22.9)
Special type carcinoma	
Fibrolamellar	0 (0)
Scirrhous	0 (0)
Undifferentiated	0 (0)
Lymphoepithelioma-like	0 (0)
Sarcomatoid	0 (0)
Steatohepatitis	5 (8.1%)
Macrotrabecular massive	0(0)

* More than one pattern can be observed in one tumor.
Abbreviations: SD - standard deviation, BCLC - Barcelona Clinic Liver Cancer, AFP - alpha-fetoprotein, HPF - high power field

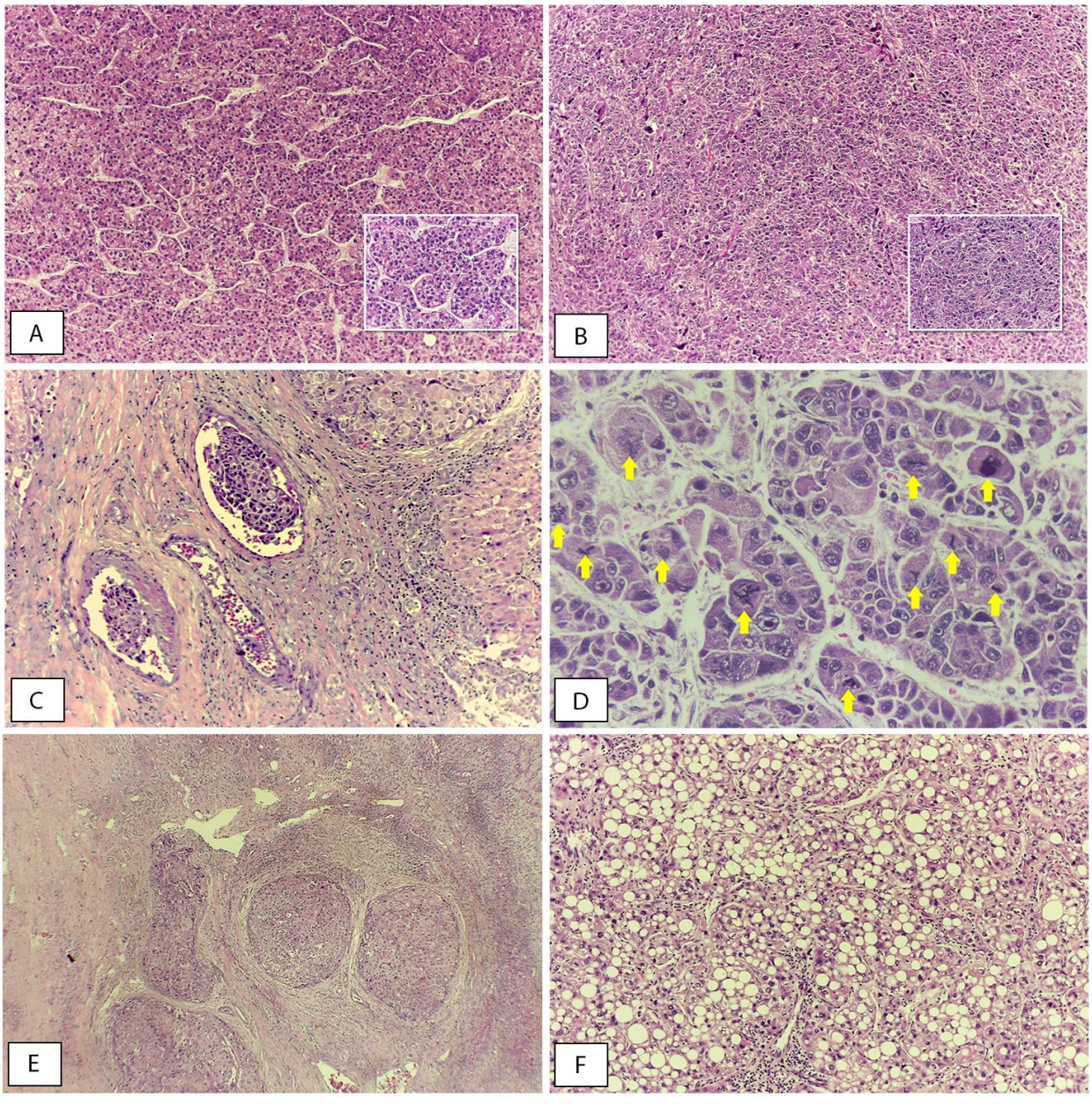

Fig 1. Histomorphology features of HCC. A) Well-moderate differentiation with microtrabecular and pseudoglandular patterns (HE, 100x and 400x). B) Poor differentiation with solid patterns (HE, 100x and 400x). C) Microvascular invasion (HE, 100x). D) Mitosis in poorly-differentiated HCC (HE, 400x). E) Non-tumor liver tissue showing cirrhosis with bridging fibrosis and regenerative nodules (HE, 100x). F) Steatohepatitic HCC with diffuse microvesicular and macrovesicular steatosis (HE, 400x).

correlated with poorly-differentiated HCC. Significant correlations were found between BCLC staging, tumor size and mitotic index with microvascular invasion. BCLC stage B (p=0.011), tumor size >7cm (p=0.046) and mitotic index ≥5 per 10 HPF (p=0.001) were significantly correlated with the presence of microvascular invasion. Significant correlations were also found between age, AFP level, BCLC staging, tumor size, and mitotic index with well-differentiated and no microvascular invasion. Age ≤60 (p=0.022), AFP level < 100 (p=0.000), BCLC stage A (p=0.015), tumor size ≤7 (p=0.001), and mitotic index <5 (p=0.000). Multivariate analysis was conducted from factors with p ≤ 0.25 in bivariate analysis. Mitotic Index ≥ 5 per 10 HPF were independent risk factors for poorly differentiated tumor, with an odds ratio of mitotic index (95% CI: 1.781-64.671; p≤0.005). Mitotic Index ≥ 5 per 10 HPF (95% CI: 1.934-25.853; p≤0.005) was an independent risk for microvascular invasion. On the other hand, AFP level <100 and mitotic index < 5 per 10 HPF were independent risk factors for well-differentiated with no microvascular invasion. Data are presented in Table 2, Table 3 and Table 4.

TABLE 2. Association clinicopathological features with histological grading (n=61).

Factors	Histological Grading			Univariate		Multivariate		
	Poor differentiation, n (%)	Well-moderate differentiation, n (%)	P-value	OR	95% CI	P-value	OR	95% CI
Sex								
Male	16 (26.2)	33 (54.1)						
Female	4 (6.6)	8 (13.1)	1.000[2]	1.031	0.270-3.3941			
Age, years								
≤60	15 (24.6)	29 (47.5)						
>60	5 (8.2)	12 (19.7)	0.727[1]	0.806	0.239-2.716			
AFP level, ng/ml								
≥100	14 (23)	17 (27.9)						
<100	6 (9.8)	24 (39.3)	**0.036**[1]	3.294	1.053-10.305	0.783	1.208	0.315-4.625
Hepatitis infection								
Non-hepatitis	2 (3.3)	11 (18)	Reference					
Hepatitis	18 (29.5)	30 (49.2)	0.189[2]	3.300	0.656-16.608			
BCLC staging								
Stage A	1 (1.6)	4 (6.6)						
Stage B	19 (31.1)	37 (60.7)	1.000[2]	2.054	0.214-19.685			
Tumor size, cm								
>7	16 (26.2)	21 (24.4)						
≤7	4 (6.6)	20 (32.8)	**0.031**[1]	3.810	1.086-13.365	0.460	1.690	0.420-6.804
Cirrhosis status								
Non-cirrhotic	9 (14.8)	20 (32.8)						
Cirrhotic	11 (18)	21 (34.4)	0.781[1]	1.164	0.398-3.403			
Mitotic index, per 10 HPF								
≥ 5	15 (27.8)	19 (35.2)						
< 5	2 (3.7)	18 (33.3)	**0.009**[1]	7.105	1.420-35.550	**0.017**	7.105	1.420-35.550

Abbreviations: OR – odd ratio, CI – Confident Interval, AFP - alpha-fetoprotein, BCLC - Barcelona Clinic Liver Cancer, HPF - high power field. [1]Chi-square test, [2]Fischer's exact test

TABLE 3. Association between clinicopathological features with microvascular invasion (n=61).

Factors	Microvascular Invasion			Multivariate				
	Positive, n (%)	Negative, n (%)	P-value	OR	95% CI	P-value	OR	95% CI
Sex								
Male	30 (49.2)	19 (31.1)						
Female	5 (8.2)	7 (11.5)	0.219[1]	0.452	0.125-1.633	0.234	0.382	0.078-1.864
Age, years								
≤60	27 (44.3)	17 (27.9)						
>60	8 (13.1)	9 (14.8)	0.313[1]	0.560	0.181-1731			
AFP level, ng/ml								
≥100	15 (24.6)	15 (24.6)						
<100	20 (32.8)	11 (18.0)	0.252[1]	1.818	0.651-5.075			
Hepatitis infection								
Non-hepatitis	9 (14.8)	4 (6.6)	Ref					
Hepatitis	26 (42.6)	22 (36.1)	0.330[2]	0.525	0.142-1942			
BCLC staging								
Stage A	0 (0)	5 (8.2)						
Stage B	35 (57.4)	21 (34.4)	**0.011**[2]	n/a[a]	n/a[a]	0.999	2700265285	0.000-.
Tumor size, cm								
>7	25 (41)	12 (19.7)						
≤7	10 (16.4)	14 (23)	**0.046**[1]	2.917	1.006-8.453	0.458	1.673	0.429-6.515
Cirrhosis status								
Non-cirrhotic	18 (29.5)	11 (18)						
Cirrhotic	17 (27.9)	15 (24.6)	0.481[1]	0.693	0.249-1.925			
Mitotic index, per 10 HPF								
≥ 5	27 (50)	7 (13)						
< 5	7 (13)	13 (24.1)	**0.001**[1]	7.163	2.075-24.731	**0.003**	7.071	1.934-25.853

Abbreviations: OR – odd ratio, CI – Confident Interval, AFP - alpha-fetoprotein, Ref - reference value, BCLC - Barcelona Clinic Liver Cancer, HPF - high power field.

[1]Chi-square test, [2]Fisher's exact test, [a]No positive cases of stage A in BCLC Staging, therefore the analysis of OR estimate could not be done.

TABLE 4. Association between clinicopathological features with well-differentiated and no microvascular invasion (n=61).

Factors	Histological Grading		Multivariate					
	Poor differentiation, n (%)	Well-moderate differentiation, n (%)	P-value	OR	95% CI	P-value	OR	95% CI
Sex								
Male	4 (6.6)	37 (60.7)						
Female	12 (4.9)	8 (13.1)	0.715[2]	1.542	0.394-6.040			
Age, years								
≤60	8 (13.1)	36 (59.0)						
>60	8 (13.1)	9 (14.8)	**0.048**[2]	4.000	1.178-13.579	0.787	1.418	0.112-17.925
AFP level, ng/ml								
≥100	15 (24.6)	15 (24.6)						
<100	1 (1.6)	30 (49.2)	**0.000**[1]	0.33	0.004-0.277	**0.014**	0.056	0.006-0.552
Hepatitis infection								
Non-hepatitis	4 (6.6)	9 (14.8)	Ref					
Hepatitis	12 (19.7)	36 (59.0)	0.728[2]	0.750	0.195-2.884			
BCLC staging								
Stage A	4 (6.6)	1 (1.6)						
Stage B	12 (19.7)	44 (72.1)	**0.015**[2]	0.068	0.007-0.668	0.492	0.178	0.001-24.575
Tumor size, cm								
>7	4 (6.6)	33 (54.1)						
≤7	12 (19.7)	12 (19.7)	**0.001**[1]	0.121	0.033-0.449	0.213	0.324	0.55-1.914
Cirrhosis status								
Non-cirrhotic	7 (11.5)	22 (36.1)						
Cirrhotic	9 (14.8)	23 (37.7)	0.727[1]	1.230	0.390-3875			
Mitotic index, per 10 HPF								
≥ 5	2 (3.7)	32 (59.3)						
< 5	11 (20.4)	9 (16.7)	**0.000**[2]	0.051	0.010-0.274	**0.003**	0.065	0.010-0.406

Abbreviations: OR – odd ratio, CI – Confident Interval AFP - alpha-fetoprotein, Ref - reference value, BCLC - Barcelona Clinic Liver Cancer, HPF - high power field.

[1]Chi-square test, [2]Fisher's exact test

DISCUSSION

The average age of patients diagnosed with HCC in areas with high HCC frequency is found to be 10-20 years lower than that in areas with low HCC frequency. This is likely caused by risk factors and age when exposed. HBV is the most common risk factor for HCC and is usually acquired at birth or in childhood.[10] In agreement with the findings of Xiao *et al*, the mean age of the patients in the present study was 51.80 ± 15.643 years and the age was not associated with both histological grade and microvascular invasion.[11] Being an endemic country, the frequency of young patients with HCC (>40 years old) is quite high in the present study (20%).

About 70-90% of patients had a history of chronic liver disease due to HBV and/or HCV infection, alcoholic liver disease or non-alcoholic steatohepatitis (NASH).[12] Atisook *et al* also reported the most common precursor are cirrhosis and chronic hepatitis due to HBV and HCV.[13] The present study showed a similar result in which hepatitis infection was predominantly present in patients with HCC. However, hepatitis infection status was not associated with histological grading, microvascular invasion, and well-differentiated and no microvascular invasion.

Epidemiologically, HCC is more common in males, with male/female ratio ranging from 2:1-4:1.[14] Males are more susceptible to high-risk chronic diseases (HCC) due to exposure to risk factors like alcohol use and Hepatitis infections. In addition, androgens promote DNA damage and oxidative stress, while estrogens suppress tumor by reducing the effect of interleukin-6.[14] Accordingly, the present study showed a higher prevalence of HCC in men. Histological grade and microvascular invasion are associated prognosis and survival in patients with HCC.[9,15] HCC is a heterogeneous tumor due to the gradual dedifferentiation of cells during hepatocarcinogenesis. The histological grade may predict HCC's biological nature.[16] Sasaki *et al*, concluded that any poorly-differentiated components within a tumor increase its degree of malignancy.[9] Tumor recurrence is associated with poor prognosis. According to a study by Lim *et al*, microvascular invasion is a reliable indicator of disease recurrence and poor overall survival.[17] Moreover, it causes frequent recurrence within 30 months of resection because of its role in intrahepatic metastases.[17]

In the present study, tumor size and mitotic index linked to histologic grade and microvascular invasion. Dai *et al* and Nagano *et al* also showed that larger tumor size links to microvascular invasion and higher histological grade.[18,19] In the current study, tumors size >7 cm was associated with poorly-differentiated HCC and positive microvascular invasion. Vascular endothelial growth factor (VEGF) overexpression was found in tumors >5 cm in size. VEGF promotes endothelial cell proliferation, migration and increased vascular permeability.[20] Following this study, Pawlik *et al* found that the larger the size of the tumor, the higher the histological grade.[21] Tumor growth gradually changes its pathobiological nature. In a study about DNA ploidy, the majority of HCC with tumors <3 cm had diploid DNA with a relatively more benign nature and an improved postoperative prognosis. By contrast, HCCs with tumors ≥3 cm were shown to have aneuploid DNA and presented a more aggressive nature and worse survival.[22]

A high proliferation rate is a hallmark of a malignant process; this occurs because of the ability of malignant cells to produce growth factors, insensitivity to anti-growth factors and a high potential for replication.[8] The mitotic index in HCC is associated with early recurrence and a worse prognosis.[23] Concordance with the present study, a higher mitotic rate is more common in tumors with poorly-differentiated components and tumors with microvascular invasion. Meantime, lower mitotic rate is an independent factor for well-differentiated and no microvascular invasion. Ha *et al* and Osorio *et al* concluded that a higher mitotic index was correlated with microvascular invasion and high-grade tumors.[8,23] The mitotic index should be reported in the histopathological report of patients with HCC.

AFP is normally secreted by the yolk sac and fetal hepatocyte cells. The level of AFP decreases from 40 ng/ml in neonates to 20 ng/ml in children of 1 year of age, remaining constant until adulthood. However, in cases of cirrhosis, malignancy and other liver pathologies, the AFP level rises.[24] Increased AFP levels before surgery impact overall and disease-free survival.[25] Tateishi *et al*, found that an AFP level >100 ng/ml significantly predicts tumor progression and recurrence.[26] The present study indicated a significant association between poorly-differentiated HCC and AFP levels ≥100 ng/ml. Meanwhile, survival rate with normal to moderate AFP levels were longer than people with elevated AFP levels>400 ng/ml.[17] This study found that AFP level <100 ng/ml was an independent factor for well-differentiated with no microvascular invasion.

The BCLC staging system is one of the earliest staging systems developed for HCC and has been validated in cohort studies for prognostic stratification.[6] The present study demonstrated a significant difference in the proportion of patients with stage A and B HCC, with and without microvascular invasion, with stage B HCC patients associated with the presence of microvascular

invasion. In contrast to the present study, Huitzil-Malendez *et al* demonstrated that the BCLC staging lacks prognostic significance.[27] The present study had some limitations, including a small sample and lack of patients survival data.

CONCLUSION

Higher mitotic index was significantly associated and independent factor for poorly-differentiated HCC and microvascular invasion suggesting that these factors might influence patients prognosis. In biopsy specimens in which microvascular invasion is difficult to assess, histological grade, tumor size and mitotic index may be beneficial to depict the prognosis of patients with HCC. In approaching patients with HCC, pathologists should carefully assess and report histopathologic features associated with unfavorable prognosis. Physicians should also be aware of these histopathologic features.

ACKNOWLEDGEMENTS

All contributors complied with the authorship criteria.

Conflicts of interest

All authors declare no potential conflicts of interest in this study.

Funding

The present study was funded by the Ministry of Research and Technology/National Agency for Research and Innovation through the SIMLITABMAS and PDUPT grant scheme (grant no. NKB-121, year 2021).

REFERENCES

1. Theise ND, Curado MP, Franceschi S, et al. Hepatocellular carcinoma. In: Bosman FT, Carneiro F, Hruban RH, et al., editors. WHO Classification of Tumours of the Digestive System. 4th ed. Lyon: International Agency for Research on Cancer; 2010.p.205-16.
2. Zhu RX, Seto WK, Lai CL, Yuen MF. Epidemiology of Hepatocellular Carcinoma in the Asia-Pacific Region. Gut Liver. 2016;10(3):332-9.
3. Rastogi A. Changing role of histopathology in the diagnosis and management of hepatocellular carcinoma. World J Gastroenterol. 2018;24(35):4000-13.
4. Perhimpunan Peneliti Hati Indonesia, Perhimpunan Dokter Spesialis Patologi Indonesia, Perhimpunan Dokter Spesialis Onkologi Radiasi Indonesia, et al. Konsensus Nasional Penatalaksanaan Karsinoma Sel Hati. Hasan I, Loho IM, editors. Jakarta: Perhimpunan Peneliti Hati Indonesia; 2017. p.1-77.
5. Loho IM, Hasan I, Rinaldi C, Dewiasty E, Gani RA. Hepatocellular carcinoma in a tertiary referral hospital in Indonesia: Lack of improvement of one-year survival rates between 1998-1999 and 2013-2014. Asian Pac J Cancer Prev. 2016;17(4):2165-70.
6. Pons F, Varela M, Llovet JM. Staging systems in hepatocellular carcinoma. HPB. 2005;7(1):35-41.
7. Mehta N, Yao FY. What Are the Optimal Liver Transplantation Criteria for Hepatocellular Carcinoma? Clin Liver Dis. 2019;13(1):20-5.
8. Ha SY, Choi M, Lee T, Park CK. The Prognostic Role of Mitotic Index in Hepatocellular Carcinoma Patients after Curative Hepatectomy. Cancer Res Treat. 2016;48(1):180-9.
9. Sasaki K, Matsuda M, Ohkura Y, Kawamura Y, Inoue M, Hashimoto M, et al. In Hepatocellular Carcinomas, any Proportion of Poorly Differentiated Components is Associated with Poor Prognosis After Hepatectomy. World J Surg. 2014;38(5):1147-53.
10. El-Serag HB. Hepatocellular Carcinoma. J Clin Gastroenterol. 2002;35(5):S72-8.
11. Xiao J, Li G, Lin S, He K, Lai H, Mo X, et al. Prognostic factors of hepatocellular carcinoma patients treated by transarterial chemoembolization. Int J Clin Exp Pathol. 2014;7(3):1114-23.
12. Wang M, Wang Y, Feng X, Wang R, Wang Y, Zeng H, et al. Contribution of hepatitis B virus and hepatitis C virus to liver cancer in China north areas: Experience of the Chinese National Cancer Center. Int J Infect Dis. 2017;65:15-21.
13. Atisook K, Dechsukum C, Sthapanachai C, Wongwisitsak N. Hepatocellular Carcinoma : Correlation of Histological Graalins with Immunohistochemical Identiffcation of Proliferative Hepatocytes with PC 10. Siriraj Hospital Gaz. 1999;51(2):71-76.
14. Keng VW, Largaespada DA, Villanueva A. Why men are at higher risk for hepatocellular carcinoma? J Hepatol. 2012;57(2):453-4.
15. Qin LX, Tang ZY. The prognostic significance of clinical and pathological features in hepatocellular carcinoma. World J Gastroenterol. 2002;8(2):193-9.
16. Han DH, Choi GH, Kim KS, Choi JS, Park YN, Kim SU, et al. Prognostic significance of the worst grade in hepatocellular carcinoma with heterogeneous histologic grades of differentiation. J Gastroenterol Hepatol. 2013;28(8):1384-90.
17. Lim KC, Chow PKH, Allen JC, Chia GS, Lim M, Cheow PC, et al. Microvascular Invasion Is a Better Predictor of Tumor Recurrence and Overall Survival Following Surgical Resection for Hepatocellular Carcinoma Compared to the Milan Criteria. Ann Surg. 2011;254(1):108-13.
18. Dai CY, Lin CY, Tsai PC, Lin PY, Yeh ML, Huang CF, et al. Impact of tumor size on the prognosis of hepatocellular carcinoma in patients who underwent liver resection. J Chin Med Assoc. 2018;81(2):155-63.
19. Nagano Y, Shimada H, Takeda K, Ueda M, Matsuo K, Tanaka K, et al. Predictive Factors of Microvascular Invasion in Patients with Hepatocellular Carcinoma Larger Than 5 cm. World J Surg. 2008;32(10):2218-22.
20. Wang D, Luo L, Chen W, Chen LZ, Zeng WT, Li W, et al. Significance of the vascular endothelial growth factor and the macrophage migration inhibitory factor in the progression of hepatocellular carcinoma. Oncol Rep. 2014;31(3):1199-204.
21. Pawlik TM, Delman KA, Vauthey JN, Nagorney DM, Lin Ng I, Ikai I, et al. Tumor size predicts vascular invasion and histologic grade: Implications for selection of surgical treatment for hepatocellular carcinoma. Liver Transpl. 2005;11(9):1086-92.
22. Lu XY, Xi T, Lau WY, Dong H, Xian ZH, Yu H, et al. Pathobiological

features of small hepatocellular carcinoma: correlation between tumor size and biological behavior. J Cancer Res Clin Oncol. 2011;137(4):567-75.
23. Osório FMF, Vidigal PVT, Ferrari TCA, Lima AS, Lauar GM, Couto CA. Histologic Grade and Mitotic Index as Predictors of Microvascular Invasion in Hepatocellular Carcinoma. Exp Clin Transplant. 2015;13(5):421-5.
24. Ren YJ, Huang T, Yu HL, Zhang L, He QJ, Xiong ZF, et al. Expression of β-catenin protein in hepatocellular carcinoma and its relationship with alpha-fetoprotein. J Huazhong Univ Sci Technolog Med Sci. 2016;36(6):846-51.
25. Chan MY, She WH, Dai WC, Tsang SHY, Chok KSH, Chan ACY, et al. Prognostic value of preoperative alpha-fetoprotein (AFP) level in patients receiving curative hepatectomy- an analysis of 1,182 patients in Hong Kong. Transl Gastroenterol Hepatol. 2019;4:52-64.
26. Tateishi R, Shiina S, Yoshida H, Teratni T, Obi S, Yamashiki N, et al. Prediction of recurrence of hepatocellular carcinoma after curative ablation using three tumor markers. Hepatology. 2006;44(6):1518-27.
27. Huitzil-Melendez FD, Capanu M, O'Reilly EM, Duffy A, Gansukh B, Saltz LL, et al. Advanced Hepatocellular Carcinoma: Which Staging Systems Best Predict Prognosis? J Clin Oncol. 2010;28(17):2889-95.