Histopathology and immunohistochemistry in intrahepatic cholangiocarcinoma

Ngo Thi Minh Hanh*, Dong Duc Hoang,**
Nguyen Quang Thi*, Le Thi Thanh Xuan*

**108 Military Central Hospital,*
***Thai Nguyen University of Medicine and Pharmacy*

Summary

Objective: The study aims to describe histopathological and immunohistochemistry characteristics in identifying intrahepatic cholangiocarcinoma. *Subject and method:* A total of 52 patients with intrahepatic cholangiocarcinoma between June 2018 and March 2020 were consecutive in the study. 16G core needle biopsies were implemented for all the patients and ensured the length of the biopsy specimens was at least 1.5cm. All liver specimens were processed according to standard histologic methods with Hematoxylin Eosin (HE) staining, and immunohistochemical staining on an automated Benchmark Ultra machine of Ventana (Roche). Histopathological classification according to The 2019 WHO classification. *Result:* Most of the intrahepatic cholangiocarcinoma was well-differentiated adenocarcinoma accounted for 40.4%, fibrous connective tissue accounted for 61.5%, and tumor necrosis accounted for only 15.4%. CK19 and CK7 were 100% positive and their expression frequently diffuse. CK20 and Hepar-1 were focal positive expressions for 13.5% and 18.2%, respectively. TTF1 only was positive at 12.5%. *Conclusion:* Histopathological features and strongly and diffusely positive expression of CK7, CK19, and positive Hepar-1 are very useful immunohistochemistry makers for diagnosis of ICC. However, TTF1 and CK20 also are positive expression at few rates.

Keywords: Intrahepatic cholangiocarcinoma, histopathology, immunohistochemistry.

1. Background

Cholangiocarcinoma accounts for about 2% of all cancers in humans. Although less common than hepatocellular carcinoma, the rate of intrahepatic cholangiocarcinoma (ICC) is increasing [6]. ICC accounts for 10-20% of the primary malignancies of the liver [8]. According to statistics in Vietnam, cholangiocarcinoma accounts for 6% of all surgical diseases of the biliary tract [1]. According to the definition by the World Health Organization, IHC is an intrahepatic malignancy with biliary differentiation [2]. It is difficult to give a clinical diagnosis of primary intrahepatic tumors, especially cholangiocarcinoma because the clinical symptoms are poor and non-specific. Moreover, it is also very difficult to distinguish ICC from other tumors with liver metastases. In terms of diagnostic imaging, it is difficult to determine the location of the tumor. Therefore, the disease is usually diagnosed in the late stage with a poor prognosis. The research of histopathology and immunohistochemistry ICC could identify cholangiocarcinoma and help the clinical making of the treatment methods. The study aims *to describe histopathological and immunohistochemistry characteristics in identifying ICC.*

2. Subject and method

2.1. Subject

A total of 52 patients with ICC between June 2018 and March 2020 were consecutive in the study.

***Received**: 9 October 2022, **Accepted**: 02 December 2022*
***Correspondence to:** Ngo Thi Minh Hanh, Department of Pathology, 108 Military Central Hospital*
***Email:** ngominhhanh108@gmail.com*

2.2. Method

A cross-sectional study.

58 patients were diagnosed by histopathology and immunohistochemistry with the most appropriate diagnosis being ICC. All these patients were fully collected clinical information, biochemical indicators, and immunological markers of cancer such as AFP, CA19-9, CEA and CA125...for diagnostic imaging comparison (ultrasound), CT, MRI, PET/CT), treatment following, and periodic check-ups as prescribed by the oncologist.

58 cases are entirely excluded from the cases of liver cancer, combined liver and biliary tract cancer, and tumor from other remaining sites with liver metastases. The last diagnosis of 52 cases is ICC.

All the patients were biopsied with a 16G core biopsy needle, and the biopsy specimen should measure at least 1.5cm; then the specimens were fixed immediately with 10% neutral buffered formalin (NBF).

All fixed liver specimens were processed according to standard histologic methods with HE staining.

Histopathological characteristics performed according to WHO 2019 classification. The degree of fibrosis was qualitatively assessed from nonfibrous to high-fibrous levels (the high-fibrous level means the glands sparsely separated by thick connective tissue, conversely, the nonfibrous level when the clusters of tumor cell are compact. The degree of inflammation was assessed when inflammatory cells infiltrated into connective tissue ranged from non-inflammatory (lymphocytes scattered in the matrix) to severe inflammation (inflammatory cells concentrated in clusters).

Immunohistochemical staining: Histological slides from the formalin-fixed, paraffin-embedded (FFPE) tissue that was previously HE-stained run on Ventana's automated Benchmark Ultra machine with CK7, CK19, Hepar1, CK20, TTF1, CD34. .. and other markers for differential diagnosis based on clinical and CT imaging features. There are internal negative and positive controls in each immunohistochemistry staining. Positive assessment when expressing yellow-brown color in cytoplasm and membrane or cell nucleus with levels: strong positive (+++) > 25%: 11%-25% moderately positive (++), 1-10%: weak positive (+), < 1%: negative. Moderate and strong positive were considered diffuse positive, and positive (+) was focally positive.

2.3. Statistical analysis

Using statistical software SPSS, version 20.0.

3. Result

Table 1. Clinical features and diagnostic imaging

The mean age (year) **The oldest, the youngest years old (39-84)**	**61 ± 10.3**
Gender Male	31 (59.6%)
Tumor size (cm) Biggest, smallest (1.3-13.6)	6.6 ± 3.5
Tumor site	
Perihilar CC or Klatskin's tumor (PHC)	15 (28.8%)
Peripheral CC	37 (71.2%)
Number of tumors	
1 tumor	23 (44.2%)
1 tumor and satellite nodules	11 (21.1%)
2 tumors	2 (3.8%)
Multiple tumors	16 (30.8%)
Metastasis (lymph nodes, distant metastases)	17 (32.7%)
Portal vein thrombosis	4 (7.7%)

Cholangiocarcinoma was more common in elderly patients (61 ± 10.3 years old), and men (59.6%); tumor sites in the peripheral liver accounted for 71.2%, lymph node metastasis, distant metastases accounted for 32.7%, and rarely in portal vein thrombosis (7.7%).

Table 2. Histopathology characteristics

Histopathology type	n (%)
Well-differentiated adenocarcinoma	21 (40.4)
Moderately differentiated adenocarcinoma	6 (11.5)
Poorly differentiated adenocarcinoma	14 (26.9)
Cords, bands, discreteness	5 (9.6)
Hepatocyte-like solid form	6 (11.5)
Fibrosis	
No fibrosis	5 (9.6)
Mild fibrosis	15 (28.8)
Severe fibrosis	32 (61.5)
Inflammation	
No inflammation	11 (21.2)
Mild inflammation	25 (48.1)
Severe inflammation	16 (30.8)
Necrosis	
No necrosis	44 (84.6)
Necrosis	8 (15.4)

ICC was mainly well-differentiated adenocarcinoma of 40.4%; fibrous stroma tissue accounted for 61.5%, and tumor necrosis accounted for only 15.4%, often accompanied by mild inflammation (48.1%).

Table 3. Immunohistochemical characteristics

Average numbers of immunohistochemistry makers: 5.5 ± 0.96 (4-8).

Marker	Positive		Negative		No stain	
	n	%	n	%	n	%
CK7	52	100				
Diffuse	51	99.1				
CK19	51	98.1	1	1.9		
Diffuse	50	90.0				
CK20	7	13.5	45	86.5		
Diffuse	3	42.9				
CDX2	5	9.6	16	30.8	31	59.6
Diffuse	3	60.0				
P40	1	1.9			51	98.1
Hepar-1	8	18.2	36	81.8	8	15.4
Diffuse	1	12.5				
AFP			10	19.2	42	80.8
TTF1	1	12.5	7	87.5	44	84.6
CEA			1	1.9	51	98.1

Marker	Positive		Negative		No stain	
	n	%	n	%	n	%
PSA			1	1.9	51	98.1
CA125			1	1.9	51	98.1
WT1			1	1.9	51	98.1
Napsin A			2	3.8	50	96.2
CD 34			1	1.9	51	98.1

CK7 and CK19 were mainly positive 100%, and 98.1% respectively, and most of them were diffuse. CK20 was positive 13.5% but mostly foci (58.1%), Hepar-1 was positive 18.2% and mostly foci (87.5%), and TTF1 was positive 12.5%.

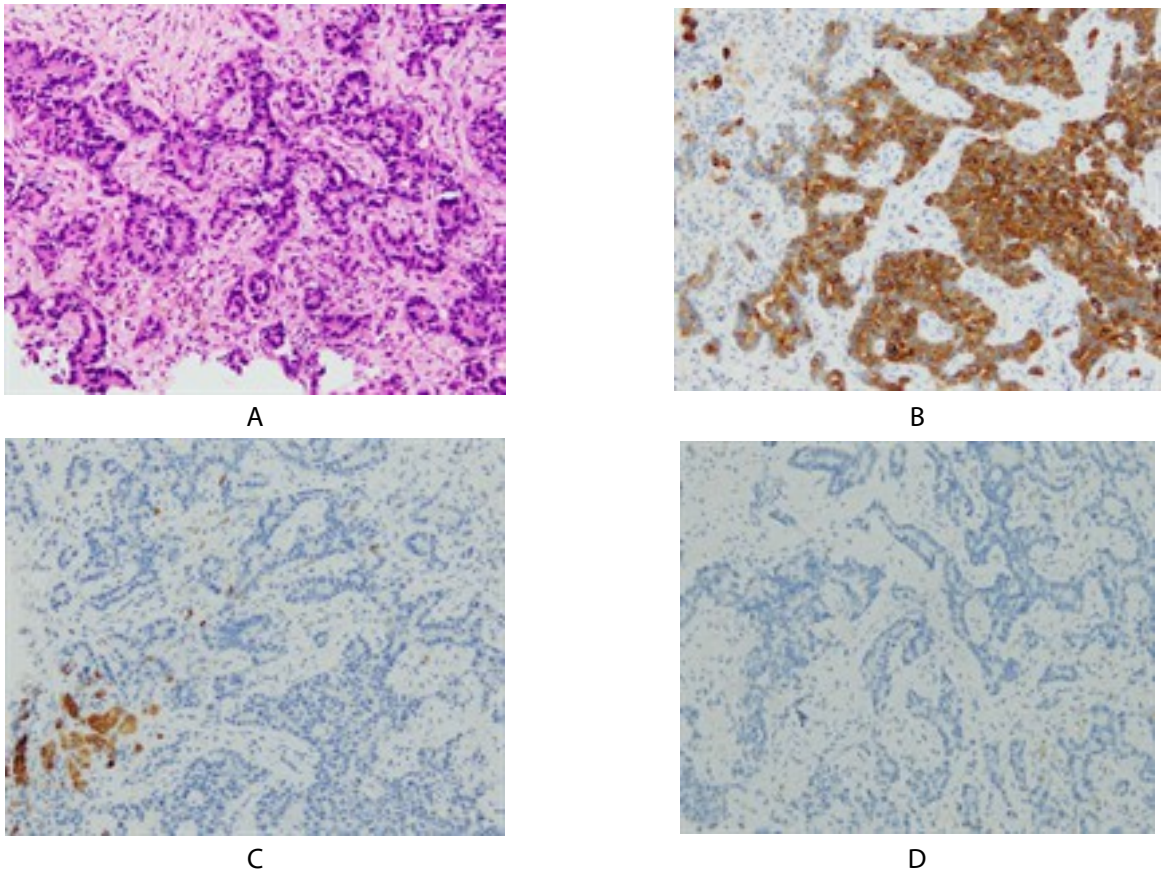

Figure. Histopathology and immunohistochemistry of ICC. Tumor cells are arranged in glands with enriched fibrous stromal tissue (A). Tumor cells were strongly positive for CK7 (B), negative for Hepar-1 (C) and CK20 (E) (x 200) (Vu Van Th, 66 years, male, G2742).

4. Discussion

4.1. Clinical features

ICC is diagnosed at a mean age of 61 ± 10.3 (39-84) years. According to studies, the average age is diagnosed when > 50 years old, occurring most frequently between the 5th and 7th decades. It is more common in men than in women [1], [2]. In our study, we found that the rate of men accounting for 59.6% is higher than one of women (Table 1). It is classified depending on its location, tumors of the biliary tree are divided into three clinically distinct types of cancers, intrahepatic, perihilar, and distal cholangiocarcinoma. In this study, Klaskin tumors

accounted for 28.8%, and the remaining tumors were located on the liver's periphery (Table 1). ICC extends from the periphery of the liver to the secondary bile duct bifurcation. Therefore, it is difficult to distinguish central cholangiocarcinoma from Klaskin tumors, especially infiltration of the common hepatic duct [5].

4.2. Histopathological features

Anatomically, cholangiocarcinoma has two main types: large duct and small duct. The large ductal type occurs in the intrahepatic bile ducts near the hilum (close to the right and left hepatic ducts). The small ductal type occurs mainly in the liver's periphery. The results from the study showed that ICC was mainly glandular with the well-differentiated adenocarcinoma accounting for 40.4%, and poorly differentiated adenocarcinoma accounting for 26.9% (Table 2). All studies have shown that ICCs are mostly adenocarcinoma, dependent on the degrees of differentiation from low to high grade [2]. Other rare variants in ICC could be squamous cell carcinoma, mucinous carcinoma, ring cell carcinoma... or sarcoma. In the study, there were 5 cases (9.6%) with histopathological images in which the tumor cells were arranged in cords, strings, and few cells spreading in the connective stroma (Table 2). Sometimes, the morphology of tumor cells is polyhedral, eosinophilic, and very similar to malignant hepatocytes. In our study, 11.5% of cases could not differentiate ICC from hepatocellular carcinoma on conventional HE slides. These cases must rely on the expression of immunohistochemistry markers.

Malignant tumors often have a stromal reactive component around tumor cells. Based on qualitative estimation as mentioned in the study methods, there were 61.5% of cholangiocarcinomas with severe fibrosis; just 9.6% of cases did not see fibrosis in the tumor tissue, which is common in those cases (Table 2). It is the fibrous characteristics and the morphology of indistinct nuclei and non-bile-forming tumor cells that distinguish it from hepatocellular carcinoma [9], but it is sometimes difficult to distinguish from adenocarcinoma metastasis from other origin. These cases must be based on immunohistochemical staining. Inflammation is also a common phenomenon in tumor stromal reactions, in general, and ICC with a server degree of inflammation accounting for 30.8%. The remaining cases had little inflammatory infiltrating, even if no inflammatory cells were visible on biopsy slides. Tumor necrosis is one of the criteria to evaluate the malignancy of some tumors generally. In ICC showing on biopsies, tumor necrosis was not popular, accounting for only 15.4%.

4.3. Immunohistochemical features

The unclear clinical features, non-specific diagnostic imaging and histopathological features are mainly glandular morphology, so ICC is sometimes difficult to distinguish between liver metastasis and primary liver cancer. Through the study, the ICC had a nearly 100% positive rate of CK7 and CK19, mostly in diffuse form (Table 3). However, these markers are still not specific, they could be expressed in many other carcinomas. Therefore, it is difficult to distinguish from adenocarcinoma metastasis into the liver. It is necessary to combine other markers to exclude liver metastasis from gastrointestinal tract, ovarian, or lung cancer... There is a range of literature on the CK7/CK20 pair that is useful for distinguishing biliary origin and liver metastasis. CK7 negative and CK20 positive help to differentiate colorectal cancer from liver metastasis whereas CK7 positive and CK20 negative could differentiate intrahepatic cholangiocarcinoma.

In this study, we found that CK20 was positive for 13.5% (Table 3). Cabbie's study also shows that CK7/CK20 is not only valuable in diagnosing bile duct cancer and liver metastases, but also helps to differentiate ICC (CK7+/CK20-) and extrahepatic cholangiocarcinoma (CK7+/CK20+). However, there are exceptions for CK20-positive ICC [3]. In our study, 13.5% of cases were positive for CK20 (Table 3). These positive cases are usually related to intestinal metaplasia changes and in these cases, the tumor was located near the center of the liver. CDX2 is valuable to differentiate from colorectal cancer [2],

[4] but generally, in the gastrointestinal tract, such as the biliary tract, CDX2 remains positive. In this study, we found CDX2 positive at 9.6% (Table 3). In addition, markers such as TTF1 that help in differential diagnosis with some lung adenocarcinomas or thyroid cancers [7] were also positive in ICC but as focal (12.5%).

5. Conclusion

Histopathological features and strongly and diffusely positive expression of CK7, CK19, and positive Hepar-1 are very useful immunohistochemistry makers for diagnosis of intrahepatic cholangiocarcinoma. However, TTF1 and CK20 also are positive expression at few rates.

References

1. Trần Đình Tho, Nguyễn Tiến Quyết, Đoàn Thanh Tùng, Nguyễn Hải Nam (2005) *Một số đặc điểm dịch tễ học của ung thư đường mật*. Y học Việt Nam 310: 26-33.
2. Bosman FT, Carneiro F, Hruban RH et al (2019) *Tumors of the liver and intrahepatic bile ducts in WHO classification of tumors of the digestive system,* vol 3, 5th Lyon: International Agency for Research on Cancer.
3. Cabibi D, Licata A, Barresi E et al (2003) *Expression of cytokeratin 7 and 20 in pathological conditions of the bile tract, Pathol*. Res. Pract 199: 65-70.
4. Chen ZI, Lin F (2015) *Application of immunohistochemistry in gastrointestinal and liver neoplasms: new markers and evolving practice*. Arch Pathol Lab Med: 11-23.
5. Mahul BA (2017) *Intrahepatic bile ducts*. AJCC Cancer Staging Manual, 8th Edition: 295-302.
6. McGlynn KA, Tarone RE, El-Serag HB (2006) *A comparison of trends in the incidence of hepatocellular carcinoma and ICC in the United States*. Cancer epidemiology, biomarkers & prevention: A publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 15(6): 1198-1203.
7. Moldvay J, Jackel M, Bogos K et al (2004) *The role of TTF-1 in differentiating primary and metastatic lung adenocarcinomas*. Pathology & Oncology Research: 85-88.
8. El Rassi ZE, Partensky C, Scoazec JY, Henry L, Lombard-Bohas C, Maddern G (1999) *Peripheral cholangiocarcinoma: presentation, diagnosis, pathology and management.* Eur J Surg Oncol 25(4): 375-380. doi: 10.1053/ejso.1999.0660.
9. Vijgen S, Terris B, Rubbia-Brandt L et al (2017) *Pathology of ICC.* HepatoBiliary Surg Nutr 6(1): 22-34.