Original Article

Immunohistochemical expression of CD34 in hepatocellular carcinoma and non-malignant hepatic nodules: experience at a tertiary care hospital in Bangladesh

Akter MM[a], Ahmed M[b], Afroze N[c], Amin M[d]

ABSTRACT

Background: *Capillarization of sinusoids occur in hepatocellular carcinoma (HCC) and several studies showed these altered sinusoids become immunoreactive for CD34. HCC and some non-malignant hepatic nodules mimic microscopically. The aims of this study were to find out the location and pattern of CD34 expression in HCC and non-malignant hepatic nodules and to see whether CD34 expression can differentiate them.*

Methods: *This cross-sectional study was conducted in the Department of Pathology, BIRDEM General Hospital, Dhaka from March 2020 to February 2022. Thirty-one cases of HCC and 31 cases of non-malignant hepatic nodules were included in this study. Detail microscopic examinations were done regarding HCC, its morphological type, grade, cirrhosis, dysplasia and of other type of non-malignant hepatic nodules. Immunohistochemical staining of CD34 was performed in all cases.*

Results: *No sinusoidal expression of CD34 was found in normal hepatic tissue. In benign hepatic nodules, sinusoids showed none to up to 20% areas of sinusoidal expression with +/++ intensity. HCC showed expression of CD34 in more than 50% areas with an intensity of +++ irrespective of grade and morphological type. Extent of CD34 expression increased significantly with the increasing grade of tumor. In cirrhosis, CD34 expression significantly increased in dysplastic than non-dysplastic cirrhotic nodules.*

Conclusion: *The extent of expression became significantly increased from low grade dysplasia and more significantly increased in HCC than non-malignant hepatic nodules.*

Key words*: hepatocellular carcinoma, CD34 expression, immunohistochemistry.*

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INTRODUCTION

Incidence of liver diseases are steadily increasing over the years.[1] The highest incidence of hepatocellular carcinoma (HCC) is in Asia accounting for about 76% of all cases worldwide.[2] It accounts for approximately 35% (2 million) deaths per year worldwide. Half of it is due to neoplastic disease and remaining half is due to non-neoplastic disease.[1] Among the neoplastic diseases, HCC is one of the leading causes of death. It is sometimes difficult to differentiate well differentiated HCC from a high grade dysplastic cirrhotic nodule.[3] Different molecular marker such as Des-gamma-

Author information

a. Mun Mun Akter, Assistant Professor, Department of Pathology, City Medical College, Gazipur, Bangladesh.

b. Mousumi Ahmed, Professor and Head, Department of Pathology, BIRDEM General Hospital, Dhaka, Bangladesh.

c. Nazma Afroze, Professor and Ex-Head, Department of Pathology, BIRDEM General Hospital, Dhaka, Bangladesh.

d. Musfequl Amin, Registrar, Jamalpur Medical College Hospital, Jamalpur, Bangladesh.

Address of correspondence: Mun Mun Akter, Assistant Professor, Department of Pathology, City Medical College, Gazipur, Bangladesh. Email: munmunpathr9@gmail.com

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carboxyprothombin (DCP), Golgi protein 73 (GP 73) and Glypican-3 (GPC 3) showed varying amounts of detectable expression in case of benign disease and non-malignant neoplastic nodules.[4] Some has lower sensitivity, some show staining of surrounding non-tumor cells and some show no difference in intensity between dysplastic cells and surrounding liver cells. Currently no single marker is sufficiently sensitive and specific for the diagnosis of HCC. There remains an urgent need to identify a panel of immunostaining marker for the accurate diagnosis of liver nodules.[4] Sinusoidal endothelial cells in liver are different from other sinusoidal endothelial cells and do not show expression of CD34. But they show phenotypic changes in the early stage of hepatocarcinogenesis. As the arterial blood supply for HCC increases, the sinusoidal endothelial cells may form basement membranes and take on the morphological appearance of capillaries. This is known as capillarization and become immunoreactive for CD34 molecules.[3] CD34 positive endothelial progenitor cells may play a role in sinusoid like vessel formation or sinusoidal capillarization during hepatocarcinogenesis. Therefore, CD34 might have a role in diagnosis of HCC and non-malignant hepatic nodules. The aim of this study was to find out the location and pattern of immunohistochemical expression of CD34 in HCC and non-malignant hepatic nodules.

METHODS

This cross-sectional study was conducted in the Department of Pathology, BIRDEM General Hospital, Dhaka, Bangladesh from March 2020 to February 2022. The study protocol was approved by Institutional Review Board (IRB) of BIRDEM Academy. Thirty-one cases of HCC and 31 cases of non-malignant hepatic nodule cases were included. Metastatic hepatic nodules, primary malignant hepatic nodules other than HCC, hepatic abscess, hemangioma or hydatid cyst which present as hepatic nodules were excluded. H&E stained slides and paraffin blocks of all cases were collected. H&E stained slides were reviewed and detail microscopic examination was done.

Periodic acid Schiff (PAS) and Periodic acid Schiff with Diastase (PASD) were done for analysis of different variants of HCC if needed. Immnohistochemical staining of CD34 was performed in all cases. Immuno-histochemical staining was performed on sections 4 micrometer thick, using mouse anti-human progenitor cell antigen–1 (CD34). Then sections were stained with mouse anti-human progenitor cell antigen–1 (CD34) (Dako Envision, Denmark) at a 1:50 dilution. For appropriate positive control, sample taken from the appendix was used.

RESULTS

In non-malignant hepatic nodules and HCC, CD34 positive staining was found in blood vessels and bile duct epithelial cells in portal tracts (Table I). However, lining epithelium of sinusoid of periportal area and peri-nodular area of cirrhotic nodule and hepatic adenoma showed sparsely staining for CD34. The extent of this staining was up to 20% of sinusoidal cells.

Table I. Location of CD34 expression in non-malignant hepatic nodule and hepatocellular carcinoma

	Sinusoidal lining epithelium in (%) (n)			Blood vessels and bile ducts in portal tracts (%) (n)
	Periportal area	Perinodular area	Sinusoidal lining in tumor or nodule	
Nonmalignant hepatic nodule (n=31)				
Cirrhosis (n=25)	80 (25)	80 (25)	80 (25)	80 (25)
Focal nodular *hyperplasia (n=1)*	-	-	-	3.2 (1)
Adenomatous *hyperplasia (n=1)*	-	-	-	3.2 (1)
Chronic *hepatitis (n=2)*	6.4(2)	-	-	6.4(2)
Hepatic *adenoma (n=2)*	6.4(2)	6.4(2)	6.4(2)	6.4(2)
HCC (n=31)	100 (31)	100 (31)	100 (31)	100 (31)

Twenty-five cases of cirrhosis was found in the study. Among these cases (13 cases, 52%) were associated with low grade dysplasia. Present study showed that extent of sinusoidal staining increased significantly in the presence of dysplasia (Table II).

Table II. Extent of CD34 staining in sinusoids within the nodule in cirrhotic patients (n=25)

Cirrhosis	Extent of CD34 staining (n=25), % (n)			P value*
	<10%	10-20%	>20%	
Without dysplasia	36(9)	12(3)	0	0.001*
Low grade dysplasia	0(0)	52(13)	0	
High grade dysplasia	0	0	0	

*Chi-square test was carried out to measure the level of significance
[s]= significant

Grading and morphological type of HCC was done according to WHO 2021 guideline. Extent of CD34 staining increased significantly with the increasing grade of tumor (Table III). Thirty-one cases of HCC were included in this study. Macrotrabecular type of HCC showed 50-90% extent of sinusoidal cell expression of CD34. Clear cell type showed 50-90% of sinusoidal staining. However, only Schirrous type showed more sinusoidal expression of CD34 (>90%). No significant difference of its expression was found in different morphological types.

In non-malignant cases, some sinusoidal lining cells within the nodule showed sparse staining for CD34. However, the extent of staining was within 20% of sinusoidal cells. Whereas, in HCC, sinusoids within the tumor showed more than 50% of staining. The intensity of staining was graded from negative (-), (+), (++) or (+++) as per Cui et al. 1996. The intensity of sinusoidal CD34 staining in non-malignant hepatic lesion was +/++. No sinusoids of normal hepatic tissue showed staining for CD34. In all malignant lesion the intensity of staining was +++ (Table IV).

Table III. Extent of CD34 staining in sinusoidal cells in tumor of different grades and morphological types of HCC (n=31)

Grade	Number of case % (n)	Extent of CD34 staining in Sinusoidal lining epithelium in (%) (n) Tumor (n=31)% (n)				P value*
		50-60%	60-80%	80-90%	>90%	
Well differentiated		16.5 (5)	9.7 (3)	6.5 (2)	-	<0.001*
Moderately differentiated		-	3.2 (1)	61.3(19)	-	
Poorly differentiated		-	-	-	3.2(1)	
HCC type						
Clear Cell	6.5(2)	3.2(1)	_	3.2(1)	-	0.148
Macro-trabecular massive	58.1(18)	6.5 (2)	12.9 (4)	38.7 (12)	-	
Scirrhous	9.6(3)	-	-	6.5 (2)	3.2 (1)	
Steatohepatitic	25.8(8)	6.5(2)	-	19.4 (6)	-	

*Chi-square test was carried out to measure the level of significance
[s]= significant

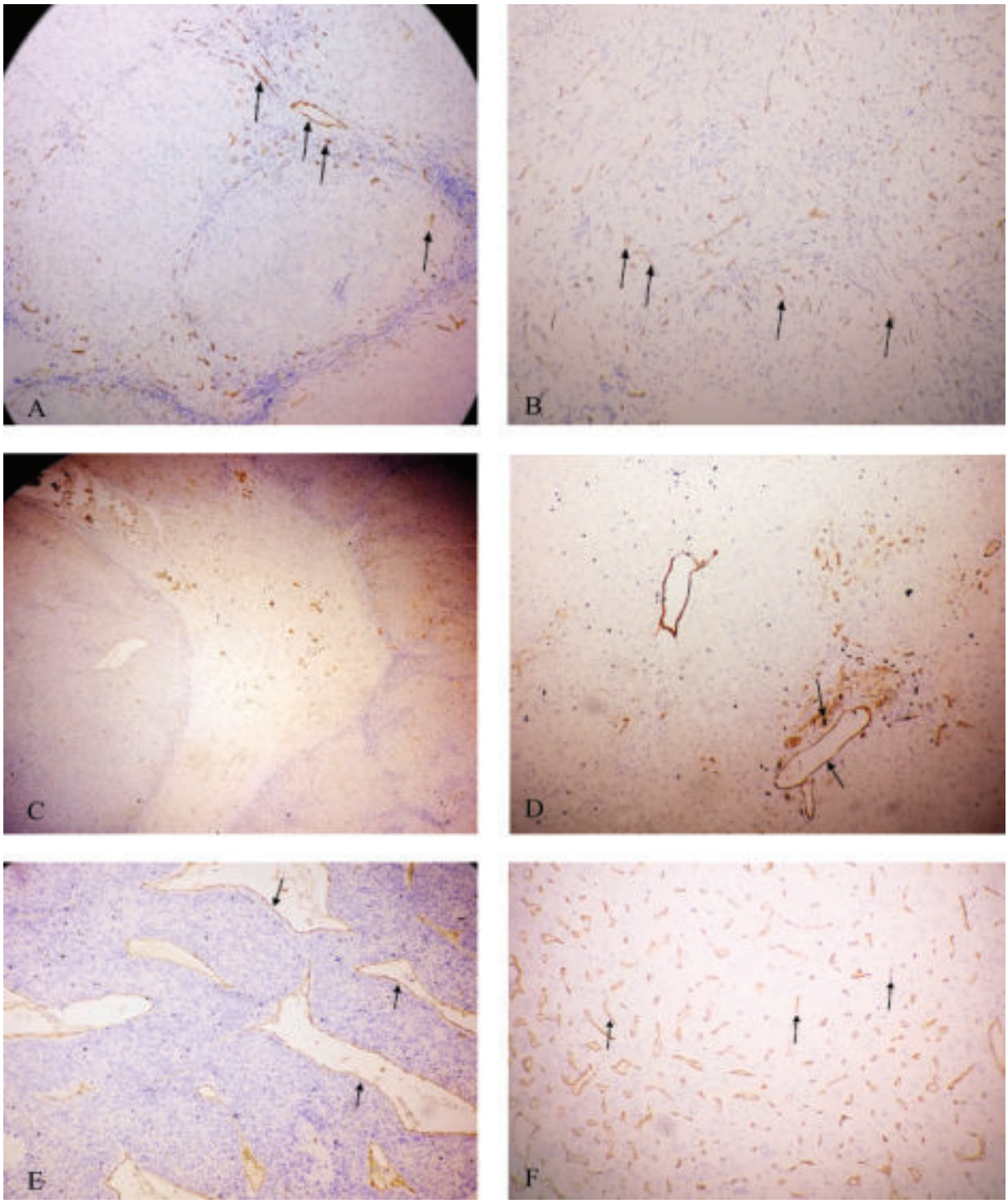

Figure 1. CD34 expression. (A) Cirrhosis without dysplasia. Low power view shows expression marked by arrow with extent less than 10%. (B) Cirrhosis with dysplasia. Low power view shows expression marked by arrow with extent 20%. (C) Focal nodular hyperplasia. Low power view shows expression marked by arrow with extent less than 10%. (D) Adenomatous hyperplasia. Low power view shows expression marked by arrow with extent less than 10%. (E) Macrotrabecular HCC. High power view shows expression marked by arrow with extent more than 50%. (F) Steatohepatitis HCC. High power view shows expression marked by arrow with extent more than 50%.

Table IV. Extent and intensity of sinusoidal CD34 expression in nonmalignant hepatic nodule and different grades of HCC

Diagnosis (No of case)	*Staining Intensity of CD34 in sinusoids (No. Positive Cases)	Extent (%) of sinusoidal CD34 staining
Nonmalignant hepatic nodule		
Cirrhosis (25)	+/++(25)	<10%
		10-20%
Chronic Hepatitis (2)	+(2)	<10%
Hepatic Adenoma (2)	++(2)	10-20%
Focal Nodular Hyperplasia (1)	-(1)	<10%
Adenomatous Hyperplasia (1)		
HCC		
Well Differentiated (10)	+++(10)	50-90%
Moderately Differentiated (20)	+++(20)	60-90%
Poorly Differentiated (1)	+++(1)	>90%

*-Negative Staining, +Weak Staining, ++ Moderate Staining, +++ Strong Staining

DISCUSSION

In non-malignant hepatic nodules, CD34 positive staining was found in blood vessels and bile duct epithelial cells in portal tracts. However, lining epithelium of sinusoid of periportal area and perinodular area of cirrhotic nodule and hepatic adenoma showed sparsely staining for CD34. The extent of this staining was up to 20% of sinusoidal cells. These findings were similar to previous study of Dhillon[6] et al. Ruck[7] et al. Cui[3] et al, Maedea[8] et al. and Boer[9] et al. Intensity of CD34 immunostaining varied from + to ++ which showed similar results found by Cui[3] et al.

Macrotrabecular type of HCC showed 50-90% extent of sinusoidal cell expression of CD34. Clear cell type showed 50-90% of sinusoidal staining. However, Schirrous type showed more sinusoidal expression of CD34 (>90%). Variation of its expression was not statistically significant in different morphological types of HCC. Grading of all 31 cases of HCC was done as per WHO criteria. In this study, HCC was graded into 3 grades according to WHO 2021 guideline. Moderately differentiated was the commonest grade of HCC (20 cases, 64.5%). CD34 stain was a more in extent with the increasing grade of tumor, which was statistically significant. Intensity of immunostaining of HCC were examined which revealed strong (+++) staining irrespective of grading of HCC. It was similar with the results found by Cui[3] et al.

In non-malignant hepatic nodule cases some sinusoidal lining cells within the nodule, showed sparse staining for CD34. However, the extent of staining was up to 20% of sinusoidal cells. Whereas, in HCC sinusoids within the tumor shows more than 50% of staining. The intensity of non-malignant hepatic lesion was +/++ . In all malignant lesion the intensity of staining was +++. Study done by Cui[3] et al. found that the positive staining of CD34 in sinusoid-like vessels was useful for diagnosis of HCC and the sinusoid like vessels expressed CD34 step by step from early stage of HCC to advanced stage. A study done by Yao[4] et al. found that, in normal and non-malignant hepatic nodule, there was negative CD 34 immunoreaction (in which only blood vessels and bile ducts in portal tracts and /or rare sinusoidal space near portal tracts were positive) but diffuse CD34 expression was positive in most sinusoidal space throughout the mass in most HCC tissues (62 of 65, 95.4 %). These findings were similar to present study. In the present study, an increased immunoreactivity was observed in the periportal sinusoids of the cirrhotic nodules and diffuse and strong staining was observed in the overall HCC. Similar findings also found in study by Carlo[10] et al. 2002.

Conclusion

CD34 expression was found in more than 50% of sinusoidal cells in HCC. In non-malignant hepatic nodules including cirrhosis, some sinusoidal expression of CD34 was observed but extent of expression was up to 20%. In dysplastic cirrhotic nodules expression of CD34 increased significantly than non-dysplastic cirrhotic nodule. In HCC, intensity of immunostaining was increased than non-malignant hepatic nodule. So, CD34 may have a role to differentiate HCC from non-malignant hepatic nodules.

Authors' contribution: MMA designed, prepared and reviewed the study, performed sample collection, data collection and procedures. MA did literature search and helped in data analysis. MA and NA helped in patient selection, sample collection, data analysis, reviewing manuscript and monitoring procedures. All authors read and approved final manuscript to be submitted.

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