ORIGINAL RESEARCH PAPER

Pathology

DIAGNOSTIC UTILITY OF CD34 MARKER IN DISTINGUISHING PRIMARY VERSUS SECONDARY HEPATIC CARCINOMAS: A RETROSPECTIVE STUDY IN A TERTIARY CARE HOSPITAL.

KEY WORDS: malignant liver tumours, core biopsy, hepatocellular carcinoma, metastatic carcinoma, CD34, immunohistochemistry.

Dr. Rohini Dhanya C S Post Graduate, Department Of Pathology, Yenepoya Medical College, Deralakatte, Mangalore- 575018

Dr. Anuradha C K Rao* Professor, Department Of Pathology, Yenepoya Medical College, Deralakatte, Mangalore-575018 *Corresponding Author

ABSTRACT

Distinguishing hepatocellular carcinoma (HCC) from metastatic carcinomas in malignant liver lesions is a well recognized problem in cytology and histopathology. Pathologic evaluation of biopsy samples plays a key role in establishing an accurate diagnosis for patient management. Morphologic features and a wide variety of immunohistochemistry (IHC) studies are used to solve multiple diagnostic dilemmas. In this study, we assess the role of CD34 in thin core biopsy material in malignant liver lesions for differentiating primary from secondary malignancies of liver. OBJECTIVE: The objective of the study was to assess the diagnostic utility of CD34 IHC stain on liver biopsies of clinicoradiologically/cytologically suspected malignant liver tumours to differentiate primary from metastatic malignancies. MATERIAL AND METHODS: Formalin fixed, paraffin embedded tissue sections of thin core biopsies were assessed from 26. cases of malignant hepatocytic lesions. HCC were scored for nuclear grade. Sections were immunostained using monoclonal antibody against CD34 antigen and scored semi-quantitatively. RESULTS: A higher percentage of HCC were CD34 positive (92%) as compared to metastatic carcinomas (75%); predominantly showing complete staining (54%) with endothelial rimming (92.3%). Cholangiocarcinoma showed no reaction to CD34 while metastatic carcinomas showed mostly incomplete positivity (50%). In contrast to metastatic carcinomas, 8(53.8%) cases of HCC showed grade 3 and 4, 4(30.8%) cases grade 1 and 2. CONCLUSION: CD34 as an IHC marker for endothelial staining is not specific for HCC, but the rimming pattern of staining seen in HCC is absent in metastasis and can be considered to be diagnostic.

INTRODUCTION

According to the International Agency for Research on Cancer (IARC), in year 2012 the most common causes of cancer death were lung cancer (1.6 million deaths), followed by liver cancer (745,000 deaths). [1] [2] Malignancies of the liver are the fifth most common cancer in men (554,000 cases, 7.5% of the total) and the ninth in women (228,000 cases, 3.4%) [2] Malignant liver tumors can be primary or secondary (metastasis).The registry data by ICMR and the report published by International Agency for Research on Cancer (WHO) shows the age adjusted incidence rate of hepatocellular carcinoma (HCC) in India for men in a range from 0.7 to 7.5 and for women 0.2 to 2.2 per 100,000 population per year. The male:female ratio for HCC in India is 4:1. [3] HCC is the most frequent cause of all liver cancers and represents 90% of cancers of liver globally. [4] It is a major global health problem. Liver being one of the most common sites for metastatic disease, it accounts to 25% of all metastases to solid organs [5] Most common primary sources are colon, breast, lung, pancreas, etc for metastases in liver. [6] There is a need for differentiation of HCC from metastasis as they have different treatment protocol. Morphologic features and immunohistochemistry (IHC) can accurately classify most tumors. [5]

The purpose of this retrospective single center study is to assess the impact of histopathological findings and diagnostic utility of CD34 immunohistochemical marker on tissue sections and thin core biopsies for differentiating HCC of different etiologies from secondary malignancies of the liver in a regional scenario.

MATERIAL AND METHODS

All the tissue sections of thin core liver biopsy of malignant liver tumours were retrieved from the department of pathology of Yenepoya Medical College and Hospital, Mangalore during the two year period. Two hematoxylin and eosin stained sections of paraffin embedded tissues of biopsy proven HCC and other types of malignancy were evaluated and assessed for adequacy for IHC studies. Clinical follow-up data, including serum a-fetoprotein levels and other biochemical findings, serology, abdominal ultrasound examination, and enhanced computed tomography or magnetic resonance imaging were obtained from patient records. For IHC studies, multiple 4 µm-thin sections from the selected paraffin-embedded blocks were used. The sections were mounted on coated slides and dried for 1 hr at 60°C. IHC staining using monoclonal CD34 antibody of PathnSitu was used and the procedure was performed according to the manufacturer's instructions.

The tumour growth patterns, classification of HCC into well-differentiated, moderately differentiated and poorly differentiated histologic patterns were performed according to the criteria by Edmondson and Steiner. [7][8][9] The CD34 stained sections were evaluated for the presence of brown coloured staining patterns of the microvascular endothelial cells rimming the tumor cell groups and were compared in various malignant liver tumor. CD34 positive staining was taken as any cell that stained brown with a dotty, linear, semicircular, or circular pattern and was clearly separate from an adjacent one. [10]

The average value of the counting was carried out in 10 high-power fields, CD34 immunostaining was graded as follows: grade 0 (no staining), grade 1 (<25% staining of endothelial cells), grade 2 (25-50% staining of endothelial cells), grade 3 (50-75% staining of endothelial cells) and grade 4 (>75% staining of endothelial cells). [10]

Complete CD34 immunoreactivity was defined as massive staining of the liver sinus endothelium, and incomplete CD34 immunoreactivity as partial staining of the liver sinus endothelium (including staining of the adjacent sinus endothelium in the portal area). Exclusive staining of the vascular endothelium in the portal area and no staining of the adjacent liver sinus endothelium was considered as the negative staining. [11][12] The data obtained was analysed utilizing descriptive statistics for CD34 (frequency, %) expression in malignant liver tumours.

RESULTS

In this study, we received 26 cases of malignant liver tumours. The age of the patients ranged from 36 to 86 years of age, with the mean of 61 years. Most of the cases belonged to category of 61-70 age group followed by 41-50 age groups with a male preponderance. Most of the women were under category 41-50 age group. The commonest clinical presentation was pain abdomen, followed by loss of weight, and appetite, yellowish discolouration of skin or sclera, nausea, vomiting; very few had fever and lump. The biochemical parameters analyzed included serum bilirubin, AST/ALT and alkaline phosphate (ALP) levels. These levels were significantly increased in HCC compared to metastatic carcinomas. AFP level detected using ELISA was

elevated in 10/26 cases of HCC cases only. Tumour markers assessed in cases suspicious of metastases in liver including, CEA, CA19-9, CA-125 etc were elevated. Positivity for HBsAg was found in 6/13 cases of HCC. Of the 26 hepatic malignancies, 13 were HCC, one was cholangiocarcinoma and 12 metastatic carcinomas. The case of cholangiocarcinoma showed elevated values of AST, ALT, ALP, total and direct bilirubin. The clinical parameters of the HCC and metastatic carcinoma cases in our study are listed in Table 1.

Table 1: Clinical parameters: HCC versus metastasis (n= 25)

CLINICAL PARAMETERS	HCC (n= 13)	METASTATIC CARCINOMA (n=12)
Age range (yrs)	36-85	38-86
Gender (M:F)	13:0	5:7
Elevated TB	4	1
Elevated DB	7	3
Elevated IB	2	2
Elevated AST	6	2
Elevated ALT	3	1
Elevated ALP	5	2
Elevated AFP	10	0
Elevated CEA	3	3
HBsAg positivity	6	0

Abbreviations: AFP, α-fetoprotein; ALP, Alkaline phosphate; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; CEA, Carcinoembryonic antigen; DB, Direct bilirubin; HBsAg, Hepatitis B surface antigen; HCC, Hepatocellular carcinoma; IB, Indirect bilirubin; TB, Total bilirubin.

Based on arrangement of tumour cells and pleomorphism, 13 cases of HCC were classified into three groups: i) well differentiated: 7(53.8%) cases, ii) moderate differentiated: 4(30.8%) cases and iii) poorly differentiated: 2(15.4%) cases. Microscopically, a predominant trabecular pattern was noted, followed by pseudoglandular pattern; cords with adjoining steatotic and cirrhotic features were noted in 7(27%) cases. Most of the cases showed hyperchromatic nuclei with 5 cases showing intranuclear inclusions; granular eosinophilic cytoplasm. Metastatic carcinoma showed gland formation, vague clusters, rosette-like pattern adjacent to the benign hepatocytes and bile ducts. Tumor cells showed vesicular to stippled chromatin, scant to moderate cytoplasm; one case showed PAS positive intracytoplasmic mucin. 22 cases showed significant fibrosis of which 13 cases belonged to HCC. Necrosis was noted in only 10 cases, mostly metastatic carcinomas.

Table 2: CD34 staining pattern and grading for HCC.

Cd34 STAINING PATTERN	WDHCC (n= 7)	MDHCC (n= 4)	PDHCC (n= 2)
Complete positive	4	2	1
Incomplete positive	3	2	0
Negative	0	0	1
GRADING OF CD34 STAINING			
Grade 0	0	0	1
Grade 1	1	1	0
Grade 2	2	0	0
Grade 3	2	2	0
Grade 4	2	1	1

Abbreviations: WDHCC, well differentiated HCCs; MDHCC, moderately differentiated HCCs; PDHCC, poorly differentiated HCCs.

The CD34 staining pattern also varied among the well, moderately, and poorly differentiated HCCs. Most of the well differentiated and moderately differentiated HCCs showed grade 3 and 4. One of the poorly differentiated HCCs showed grade 4 and other showed grade 0. (Figure:1) (Figure:2) (Figure:3)

Table 3: Patterns of immunostaining and grading of CD34: HCC versus other malignancies.

CD34 STAINING PATTERN	HCC (n=13)	Cholangiocarcinoma (n=1)	Metastatic Carcinoma (n=12)
Complete positive	7	-	3
Incomplete positive	5	-	6
Negative	1	1	3
GRADING OF CD34 STAINING			
Grade 0	1	1	3
Grade 1	2	-	3
Grade 2	2	-	2
Grade 3	4	-	4
Grade 4	4	-	-

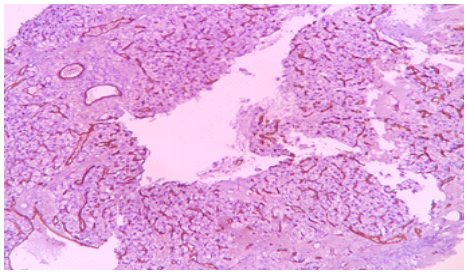

Figure 1: IHC x4. CD34 immunostaining pattern: Completely positive (endothelial rimming) and grade 4 expression in HCC.

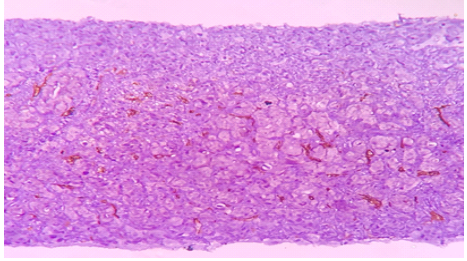

Figure 2: IHC x4. CD34 immunostaining pattern: Incompletely positive expression and grade 3 in HCC

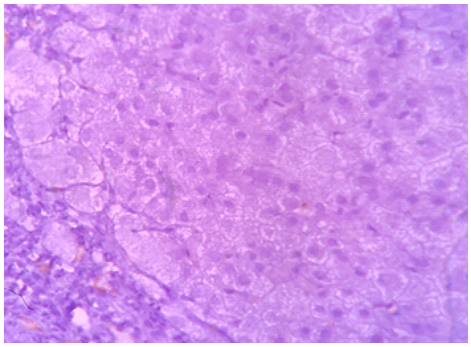

Figure 3: IHC x10. CD34 immunostaining pattern: Negative staining in HCC.

A higher percentage of HCC were positive (92%) as compared to metastatic carcinomas (75%). Complete staining (54%) was seen in slightly more HCC cases than incompletе positive CD34 staining patterns (38%). Cholangiocarcinoma showed no reaction to CD34 while incomplete positivity was seen in most of the metastatic carcinomas (50%). Considering the grading of CD34 staining, 8(53.8%) cases of HCC showed grade 3 and 4, 4(30.8%) cases grade 1 and 2. In contrast to this, metastatic carcinomas showed 5(41.7%) cases of grade 1 and 2, 4(33.3%) grade 3 only (Figure:4). Foci of cirrhosis and the adjoining normal liver showed

only portal tract staining (negative) (19%) or focal positivity (12%). 6(60%) cases with elevated AFP levels showed grade 4 and 3 predominantly. Rimming of endothelium by CD34 was seen in 92.3% HCC and was absent in metastasis and cholangiocarcinoma.

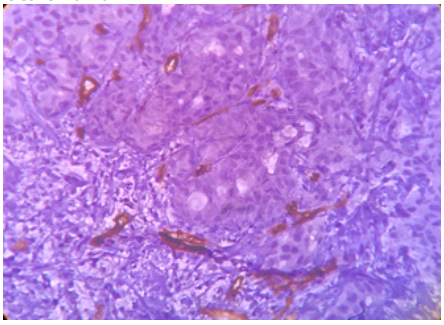

Figure 4: IHC x10. CD34 immunostaining pattern: Incompletely positive and grade 3 expression in metastasis.

DISCUSSION

It is very challenging to distinguish HCC from other hepatocellular proliferations, such as focal nodular hyperplasia (FNH), hepatocellular adenoma (HCA), and dysplastic nodules, particularly in small samples (e.g., needle biopsy). Other primary tumors (e.g., cholangiocarcinoma) and metastases may also enter the differential depending on morphology and history. [13] Assessment of liver lesions is best accomplished by combining fine-needle aspiration and needle core biopsy. Many malignancies have distinct morphologic and IHC patterns and can be correctly subclassified. Distinguishing HCC from metastatic carcinoma in biopsy specimens of liver lesions can occasionally be a diagnostic challenge. [14] Pathologic evaluation of biopsy samples plays a key role in establishing an accurate diagnosis for patient management. [5] Most metastatic malignancies in the liver may be correctly diagnosed using standard morphology and immunohistochemical techniques. [5] Currently IHC markers like Hep Par1, MOC31, CK7, CK19, Glypican3, CD10 etc have been used by various workers to differentiate HCC from secondary malignancies. [6]

HCC develops especially in the context of chronic liver disease and cirrhosis of any etiology. Many risk factors have been identified, but hepatitis B virus, hepatitis C and alcohol are the most prevalent in the world. Only 15% occur in non-cirrhotic livers. [15]

CD34 is a 110-kDa transmembrane glycoprotein present on leukaemic cells, endothelial cells and stem cells. Benign hepatic sinusoids do not express endothelial cell markers such as Ulex europaeus binding, von-Willebrand factor, CD31 or CD34. In HCC, the sinusoidal cells undergo "capillarization," where there is loss of the fenestrae, deposition of basement membrane, and express diffuse staining for CD34. [16][17] Being one of the marker for neovascularization, the expression of CD34 positive endothelial cells has been used in several studies recently to help in understanding the process of angiogenesis in cirrhosis, HCC and metastasis and have emphasized on controlling the tumor growth by suppressing their blood supply for therapeutic benefits. It also helps in the assessment of prognosis in HCC. [11] Hence, the aim of this study is to evaluate the process of angiogenesis using CD34 as an endothelial cell marker in HCC and liver metastasis and its usefulness in distinguishing these hepatocytic tumours in histological specimens. [10]

In this study we retrieved 26 cases along with clinical and/or histologic confirmation. Only male population showed HCC whereas women were predominant in the metastatic carcinomas of liver. The clinical presentations and biochemical parameters reflected the type of liver carcinomas similar to the study conducted by Ahuja et al. [18] 6 (100%) cases with hepatitis B positivity and 10 (100%) cases with elevated AFP levels showed development of hepatocellular carcinoma. Our study compared favourably with other studies done in India. [18] [19] 13 HCC, one cholangiocarcinoma and and 12 metastatic carcinomas were diagnosed in this study. Microscopic predominant trabecular patterns was noted in HCCs followed by pseudoglandular pattern. Trabecular pattern, thus was most diagnostic of HCC, in this study similar to the study by Coston et al. Cords with adjoining steatotic and cirrhotic features were seen in 7(27%) cases. Metastatic carcinoma showed gland formation, vague clusters, rosette-like pattern adjacent to the benign hepatocytes and bile ducts; with cells showing vesicular to stippled chromatin, scant to moderate cytoplasm; one case showed PAS positive intracytoplasmic mucin. Our study showed that complete and incomplete positive reaction to CD34 marker in liver tumour mass supports a diagnosis of HCC. CD34 positivity (complete > incomplete) in sinusoid-like blood vessels was found in 92.3% of the HCC cases and was consistent with the results by Wang et al. [11] Of the two cases diagnosed of poorly differentiated HCC, one showed complete positivity and the other negative staining. CD34 was negative or only focally positive in normal liver tissue and cirrhosis. The CD34 staining pattern also varied among the well, moderately, and poorly differentiated HCCs. None of the well differentiated and moderately differentiated HCCs showed negative reaction to CD34 marker. Most of the well differentiated HCCs showed grade 3 and 4; and extremes of grade 4 and grade 0 was shown by poorly differentiated HCCs. Similar findings were also found in studies by Amarapurkar et al, Cui et al, De Boer et al.[10][20][21] Endothelial rimming staining within and around the border of tumor aggregates, seen in 12/13 cases of HCC in our study, was very specific similar to the study by Saad et al. [22] One of the two poorly differentiated HCC that was confirmed with HepPar 1 positivity had shown CD34 negativity in this study similar to other studies [22][20] S. Cui et al also highlighted that the negative staining cannot exclude HCC. [20] The diagnosis of HCC with CD34 staining pattern was useful when considered in conjunction with other clinical, biochemical and histopathological information. [16] These results reflect the heterogeneity of some lesions and the problem of representative sampling of mass lesions in thin core biopsy material. Similar findings were encountered by Boer de et al. [21] However, positive staining is not specific, since 9(75%) cases of metastatic carcinoma also showed complete/ incomplete positive staining.

One case of cholangiocarcinoma showed CD34 negativity in contrast to 6% CD34 positivity in study conducted by Haas et al. [23] Our study also showed 9/12(75%) cases CD34 positivity (incomplete > complete) in metastatic carcinomas in contrast to 6/30 (20%) cases in the study by Saad et al. [22] However both studies showed similar pattern of diffuse staining of endothelial cells in tumor tissue, without showing the characteristic rimming pattern around the tumor cell aggregates. [22] Summary of CD34 expression in HCC, cholangiocarcinoma and metastatic carcinoma by immunohistochemistry is shown in table 4.

Table 4: Comparison of studies of CD34 expression in HCC, cholangiocarcinoma and metastatic carcinoma by immunohistochemistry.

Cd34 Positive Staining	HCC (N,%)	Cholangiocarcinoma (n,%)	Metastatic Carcinoma (n,%)
Saad et al [21]	27/30, 90%	-	6/30, 20%
Zimmerman et al [13]	48/51, 94%	0%	0%
Current study	12/13, 92.3%	0/1, 0%	9/12, 75%

Abbreviation: N, no.of cases.

To our knowledge, there have been only few studies [22][14] of CD34 staining in malignant liver specimens till date. Haas et al compared CD34 marker between HCC and cholangiocarcinoma only. [23]

Agrin is a proteoglycan component of vascular and bile duct basement membranes in the liver. Study conducted by Tátrai et al reported that agrin expression was more specific than CD34 expression in HCC vascular staining. [24] But CD34 is still widely used as a vascular endothelium marker for tumor angiogenesis, microvascular density and prognosis in HCC. [11]

Our study showed that CD34 positive staining in liver biopsy specimens in conjunction with other clinical and pathological information especially raised AFP levels and a trabecular morphological pattern supports in the diagnosis of HCC. Complete and incomplete positive staining in metastatic carcinoma has shown that CD34 marker is not specific. However, the rimming pattern of endothelial staining around the border of tumor and within the tumour aggregates seen in 92.3% of HCC, was very specific whereas rimming staining of CD34 marker was not seen in metastatic carcinoma.

In this study, we have also evaluated the CD34 expression for angiogenesis in well to moderately differentiated HCC and probable gradual loss of expression with dedifferentiation in poorly differentiated HCC which is insufficient to opine in our study in owing to small sample size. However, further studies on a larger sample size may help validate/ substantiate these findings.

CONCLUSION

Diagnosing malignant liver tumors especially on needle biopsy specimens can be challenging. Accuracy in interpretation of diagnosis is essential, as some lesions require only regular follow up, whereas others may be advised for resection, chemotherapy, or transplantation. The treatment and prognosis of HCC and metastatic carcinoma are significantly different; hence it becomes imperative and clinically important to distinguish primary from metastatic malignancy. A highly elevated serum AFP levels and trabecular morphological pattern can be considered as diagnostic . In the absence of this, immunohistochemical markers can be of considerable help. CD34 as an IHC marker for endothelial staining is not specific for HCC, but the rimming pattern of staining seen in HCC is absent in metastasis.

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