



UTILITY OF IHC MARKERS: HEP PAR 1 AND MOC 31 IN DIFFERENTIATING PRIMARY AND METASTATIC MALIGNANCY OF LIVER IN FINE NEEDLE ASPIRATES

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ABSTRACT

The pathological distinction between primary hepatocellular carcinoma (HCC) and metastatic carcinoma(MC) is difficult, especially when diagnosis has to be made on small samples from fine-needle aspiration (FNA). Immunohistochemical studies have been frequently applied to differentiate between them. To evaluate the efficacy of HEP PAR 1 and MOC 31 in differentiating Hepatocellular carcinoma and Metastatic carcinoma on fine-needle aspiration(FNA) samples. 30 paraffin embedded cell blocks representing 11HCC, 19MC(6 from gall bladder, 2 pancreas, 2 lung, 1 stomach, 8 unknown) were immunostained with antibodies for HEP PAR1 and MOC 31. 3 cases were excluded because H&E recuts showed insufficient material for immunohistochemical evaluation(1 poorly differentiated HCC and 2 MC). HEP PAR 1 stained 9 out of 10 HCC cases and 3 out of 17 MC cases. The positivity was cytoplasmic, diffuse, and granular. These 3 MC cases included 2 from lung &1 from stomach. HEP PAR 1 negative HCC was poorly differentiated.MOC 31 stained positively for 15 out of 17 MC while none of HCC cases were stained positive. The positivity was membranous and (or) cytoplasmic. The two cases negative for MOC 31 were poorly differentiated metastatic adenocarcinoma (MA). The results of the current study demonstrate that HEP PAR1 and MOC 31 are effective markers to differentiate between HCC and MC.Although 3 of the MC cases in the current study were found to be positive with HEP PAR 1, with the help of clinical correlation and other immunohistochemical stains a definite diagnosis could be rendered.For MOC 31 negative cases site-specific markers can also be obtained depending on the clinical scenario.

Keywords: FNA of liver, IHC- immunohistochemistry, HEP PAR 1 AND MOC 31, Cell block.

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INTRODUCTION

FNAC has been used in the diagnosis of liver lesions. However, the differentiation of hepatocellular carcinoma (HCC) from metastatic adenocarcinoma (MA) on FNAB is sometimes a difficult. Differentiation between malignant primary or secondary tumours is extremely important from management point of view. Presence of metastases usually rules out surgery whereas; if HCC is diagnosed at an early stage, surgical resection is possible and may assure cure [1]. Although the cellular features of the specimen are still essential in the differential diagnosis between HCC and MA, application of immunomarkers has

improved the diagnostic accuracy and is especially helpful in challenging cases [2-4].

In past decades, numerous antibodies have been suggested as useful in the distinction of HCC from MA on surgical biopsy specimens and have suggested that the monoclonal antibodies MOC-31 and HepPar1 are better markers for differentiating HCC from MA[5-8]. Hep Par 1 yields a diffuse cytoplasmic granular staining pattern in normal and neoplastic hepatocytes. MOC-31, an antibody directed against a cell surface glycoprotein. It is also consistently (80%–100%) expressed in cholangiocarcinoma and metastatic adenocarcinoma from a variety of sites, such as colorectum, pancreas, stomach, lung, breast, and ovary. The application of these antibodies to FNAB cell block material, which is fixed and processed differently from tissue biopsies, has been less thoroughly tested [2, 4, 8, 9].

This study evaluates the efficacy of HEP PAR 1 and MOC 31 in differentiating HCC and MC in fine needle aspiration biopsy cell block material.

MATERIALS AND METHODS

30 paraffin embedded cell blocks representing 11 HCC and 19 MC were selected in the Department of Pathology, Silchar medical college and hospital from a period of 1 year July 2015 to June 2016.

Of the 11 HCC cases 6 were well differentiated, 3 were moderately differentiated and 2 were poorly differentiated (Figure No. 1). Of the 19 MC, 6 were from gall bladder, 2 from lung, 2 from pancreas and 1 from stomach. For remaining 8 cases primary was unknown (Table No. 1). These patients could not be followed as they were discharged before further investigations could be done to detect the primary. Of the 8 cases 7 were reported as adenocarcinoma and 1 as malignant round cell tumor.

Following smear preparations, the needles and syringes used to obtain fine-needle aspirates were rinsed in 10 ml of 50% ethanol in a specimen container. Any residual clot or tissue in the hub of needles was removed carefully in the laboratory with the aid of another needle and rinsed in 50% ethanol. The entire material was centrifuged in a 10- ml disposable centrifuge tube at 4,000 rpm for 6 minutes to create 1 or more cell pellets as required. The supernatant fluid was decanted. Then fixing in alcoholic formalin substitute solution was done. Alcoholic formalin substitute consisting of 9 parts of 100% ethanol and 1 part of 40% formaldehyde, hcoH = 30.03 is prepared and the deposit fixed in that. Recentrifugation of the fixed cell pellets, at the end of 45 minutes' fixation at 4,000 rpm for 6 minutes was done. The cell pellets were wrapped in crayon paper, placed in a cassette, and stored

in 80% ethanol until ready for processing in the automatic tissue processor using a 13- hour processing schedule. Routine H&E (Harris H&E) staining was used on all cell block sections. Adequate cellularity for malignant cases was tentatively defined as at least 3 groups of atypical epithelial cells (more than 10 cells in each group) and single atypical cells. Most cell blocks contained more than 5 groups of atypical epithelial cells or benign hepatocytes.

3 cases were excluded because H&E recuts showed insufficient material for Immunohistochemical evaluation (1 moderately differentiated HCC and 2 metastatic adenocarcinoma). Four -micron thick, deparaffinized sections were immunostained with hep par 1 and moc 31. Appropriate positive and negative controls were stained in parallel with the slides.

RESULTS

Immunoscore

For HEP PAR 1, the staining intensity (weak or strong) and the distribution of positive score as: negative [<5% of tumor cells stained], focal staining [5%-50%], and diffuse [>50%]) were noted.

The following were considered to be positive for HEP PAR1

- Diffuse (>50%) and strong,
- Focal (5-50%) and strong

For MOC 31 all positive immunoreactivity was plasmalemmal and (or) cytoplasmic staining (cell membrane-based staining).

The immunohistochemical results OF HEP PAR 1 are illustrated in TABLE 2 AND 3 and representative slides in figure no. 3.

The immunohistochemical results of MOC 31 are illustrated in Table 4 and 5 and representative slides in figure 5 and 6.

HEP PAR 1 stained 9 out of 10 HCC cases (90%) and 3 out of 17 MC cases (17.6%). These 3 MC stained were: 2 from lung and 1 from stomach. HEP PAR 1 negative HCC was poorly differentiated. MOC 31 stained none of the HCC cases and 15 out of 17 MC cases (88.2%). The two cases negative for MOC 31 were poorly differentiated MC.

The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of HEP PAR 1 are 90%, 82.3%, 75%, 93.3%, and 85.2% respectively. The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of MOC 31 are 88.2%, 100%, 100%, 83.3%, and 92.5% respectively.

Table 1. No of metastatic carcinoma cases according to primary

Site of primary	No. of cases
Gall bladder	6 (31.58%)
Pancreas	2 (10.53%)

Lung	2 (10.53%)
Stomach	1 (5.26%)
Unknown	8 (42.10%)
Total	19

Table 2. HEP PAR 1 Staining Results

HEP PAR 1 Staining	HCC(10)	MC(17)	TOTAL(27)
Positive	9	3	12
Negative	1	14	15
Total	10	17	27

Table 3. HEP PAR 1 Staining Results In HCC Cases According to Grade

HEP PAR 1 Staining	Well Differentiated	Moderately Differentiated	Poorly Differentiated
Positive	6	3	0
Negative	0	0	1
Total	6	3	1

Table 4. MOC 31 staining results in metastatic cases

MOC 31 Staining	MC(17)	HCC(10)	Total
Positive	15	0	15
Negative	2	10	12
Total	17	10	27

Table 5. MOC 31 staining results in metastatic cases according to primary

Site	Positive	Negative
GALL BLADDER(6)	5	1
STOMACH(1)	1	0
PANCREAS(2)	1	1
LUNG(2)	2	0
UNKNOWN(6)	6	0

Fig 1. Frequency of hepatocellular carcinoma cases according to grade

Distribution of HCC cases according to Grades

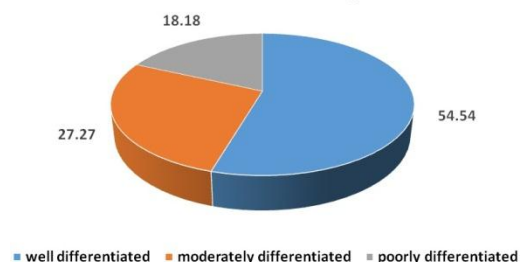


Fig 2. Frequency distribution of metastatic carcinoma cases

METASTATIC CARCINOMA CASES

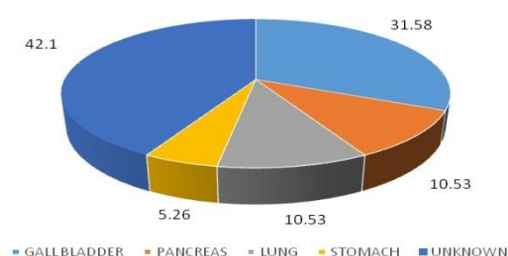


Fig 3. Cell Block Of HCC (Well Differentiated) (H&E) (10X)

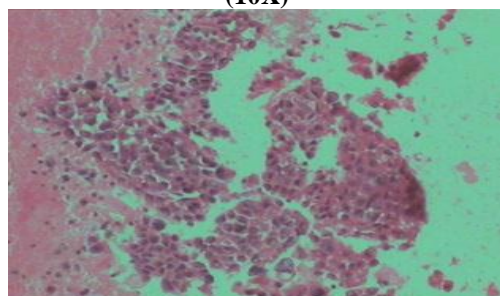


Fig 4. Well differentiated HCC showing strong positive reaction (granular cytoplasmic) with HEP PAR 1(40X)

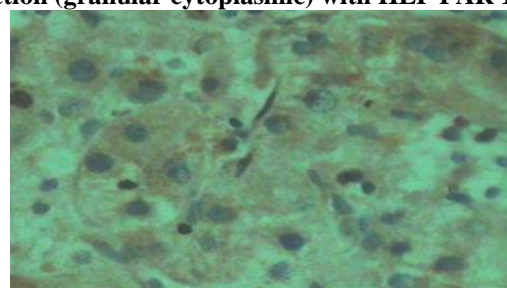


Fig 5. Cell Block of Metastatic Adeno Carcinoma Showing Columnar Cells(H&E) (40X)

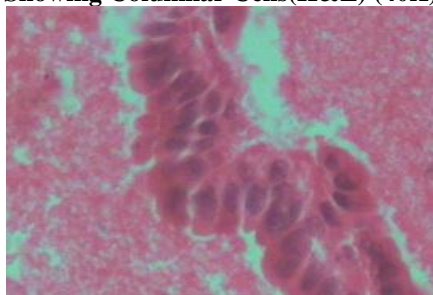
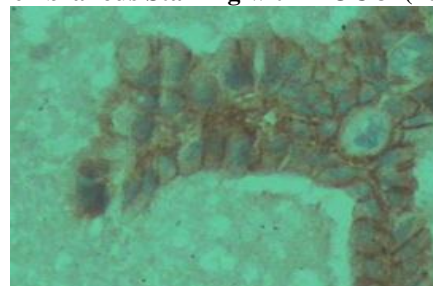


Fig 6. Metastatic Adeno Carcinoma Showing Membranous Staining with MOC 31(40X)



DISCUSSION

The separation of metastatic tumor from hepatocellular carcinoma poses a different challenge. In this setting, the cells are clearly malignant but the origin of the malignancy is uncertain. A number of criteria have been proposed for drawing this distinction. In statistically evaluating these criteria, Bottles [10], found three criteria that in combination were most useful in distinguishing hepatocellular carcinoma from metastatic neoplasms. These three key criteria include polygonal cells with centrally placed nuclei, malignant cells separated by sinusoidal capillaries, and bile. Ninety-seven percent of hepatocellular carcinomas demonstrated two or three of these criteria, but none of the metastatic tumors demonstrated more than one key criterion [10].

Despite careful examination and application of these criteria, there will be a small percentage of cases that still cannot be clearly distinguished. In this setting, ancillary studies, in particular immunohistochemical stains (cytokeratin, carcinoembryonic antigen [CEA], α -fetoprotein, α -1-antitrypsin), may be helpful [11].

MOC-31 is a monoclonal antibody raised against a small cell carcinoma cell line. The antibody reacts with a 41 Kda, membrane-based glycoprotein of unknown function [12]. A number of recent studies have demonstrated reactivity with adenocarcinomas from a variety of primary sites. Most of these studies have examined the utility of MOC-31 in separating adenocarcinoma from mesothelioma, and most demonstrate reactivity with a high percentage of adenocarcinomas (90 to 100%) and relatively few mesotheliomas (0 to 8%) [12-15].

Wennerberg originally reported the development of a new monoclonal antibody designated as hepatocyte paraffin 1 (HepPar1), which was produced in mice using tissue from a failed liver allograft. HepPar1 reacts with both normal and neoplastic hepatocytes in routine formalin fixed, paraffin embedded material, producing distinct granular, cytoplasmic staining of hepatocytes. In the original study, bile ducts and nonparenchymal liver cells were found to be negative for HepPar1 whereas 37 of 38 HCC cases, including the fibrolamellar variant, were positive [16]. Two of 35 biliary tract carcinomas

demonstrated only rare positive cells, as did 3 of 10 gastric tumors, all of which were poorly differentiated signet ring cell or mixed intestinal/signet ring cell carcinomas. Subsequently, additional reports have evaluated the role of HepPar1 in the differentiation of primary HCC from CC or MC [17-20]. Based on these studies, HepPar1 has been shown to have very high specificity (bordering on 100%) and slightly lower sensitivity (approximately 80–90%) for the identification of the hepatocellular phenotype.

In the current study we wanted to establish the efficacy of HEP PAR 1 and MOC 31 on FNA cell block material in the differential diagnosis of HCC & MC. We selected 30 cases with definitive diagnostic material on the cell block as well as with adequate clinical information regarding disease course and clinical diagnosis. The findings of the current study are in agreement with the majority of previous studies performed on histologic sections [20-21].

HepPar1 proved to be an excellent immunohistochemical marker on the FNA cell block material from the 30 cases studied. It showed 90% positivity in HCC, whereas 17.6% MC Cases. The most interesting results were noted in the MC cases. These cases were selected on the basis of common sites with metastases to the liver and included cases of primary gall bladder, gastric, pancreatic, and lung malignancies with evidence of liver metastases. HepPar1 was negative in all cases with the exception of 1 gastric metastases and 2 lung metastasis to the liver, which exhibited the characteristic diffuse, granular cytoplasmic staining. However, in the gastric carcinoma case foci suggestive of hepatoid differentiation were identified in cell block preparation.

Hepatoid adenocarcinomas of gastric primary sites are known to metastasize to the liver and can demonstrate HepPar1 reactivity [22]. This finding may have diagnostic importance for the pathologist, because the mere demonstration of HepPar1 reactivity in cell blocks from an FNA sample of a hepatic neoplasm should not be considered unequivocal evidence of HCC, but rather should be evaluated in the correct clinical setting. These metastatic gastric lesions are characteristically multiple and demonstrate central umbilication and, in

addition to hepatoid differentiation, also have foci of conventional gastric adenocarcinoma.

Although the results are generally similar to those observed in surgical specimens, there are some noticeable differences in applying this antibody to aspiration material. The most significant limitation is the quantity of material available in aspiration specimens; 5 of the 33 cases originally selected for the study could not be stained because there was insufficient diagnostic material remaining in the cell block.

CONCLUSION

In the current study, we investigated the utility of HepPar1 and MOC 31 in FNA cell block material from HCC and MC cases, and were able to demonstrate its

effectiveness in differentiating HCC from MC. A relatively small number of MC cases, (mainly those with a gastric primary tumor with possible hepatoid differentiation) also expressed immunoreactivity for HepPar1, a finding for which clinical correlation would be extremely helpful. Similar to most diagnostic studies, MOC-31 is less sensitive as it failed to stain two poorly differentiated adenocarcinoma and more specific as it stained none of the hepatocellular carcinoma.

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Nil

CONFLICT OF INTEREST

No interest

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