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Pathology

A FINE NEEDLE ASPIRATION STUDY OF HEPATOCELLULAR CARCINOMAS AND DIFFERENTIATION FROM METASTATIC ADENOCARCINOMA

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ABSTRACT A retrospective study of USG/ CT scan guided fine needle aspiration cytology were performed on 71 cases of mass lesions in the liver. Of the 71 cases included over a period of 3 years , 31 were cases of hepatocellular carcinoma while 40 were cases of metastasis to the liver. The various cytological features studied included cell patterns, cell shape, nuclear and cytoplasmic features, bile and the presence or absence of normal hepatocytes in the background. The cytologically significant cytological criteria ($_p$ value <0.001) in diagnosis of hepatocellular carcinoma included trabecular/ arborescent pattern along with polygonal cells with central nuclei, intranuclear inclusions and granular cytoplasm. While key features for metastasis to liver include acinar or syncytial pattern, columnar/ cuboidal cells with normal hepatocytes in cords in the background. The cytopathologist when applying cytological criteria for diagnosis should take into account the clinical history, radiological studies and the biochemical reports.

KEYWORDS : Hepatocellular carcinoma, Cytopathology, liver metastasis

INTRODUCTION

Percutaneous USG guided fine deedle aspiration is a rapid, inexpensive safe and minimally invasive technique for obtaining a diagnosis in solid space occupying lesions (SOL) of the liver. [1, 2] An important function of the FNAC is to separate the primary liver tumours from metastatic adenocarcinoma .[3,4] Attempts have been made to differentiate the two entities.[1,5] Literature is replete with lists of cytological criteria for various neoplasms, but they rarely separate the key criteria from secondary criteria. Our study undertakes a step wise regression analysis to identify the key cytological criteria that distinguish hepatocellular carcinoma from metastatic neoplasms. We also undertake to grade the hepatocellular carcinomas into well, moderate and poorly differentiated carcinomas. We discuss the divergent problems in diagnosis at the two ends of the spectrum of well differentiated and poorly differentiated hepatocellular carcinomas. The value of alphafetoprotein(AFP) is well established in the diagnosis of hepatocellular carcinoma.[6] We have in this study integrated AFP, radiology and primary work up in metastatic lesions with cytological feature in arriving at the final conclusion in cases of solid mass lesions of the liver.

MATERIAL & METHODS

USG/CT guided FNA were performed on 71 cases of mass lesions in the liver, using a 5-11cm long and 22-23 guage spinal needles fitted with 10-20ml disposal syringe. Wet fixed smears were stained with Papanicolaou stain while direct air dried smears were stained with May – Grunwald – Giemsa(MGG) stain in all the cases. Of the 71 cases included over a period of 3 years, 31 were cases of hepatocellular carcinoma (HCC) while 40 were cases of metastasis to the liver. The hepatocellular carcinoma were further classified into well differentiated, moderately differentiated and poorly differentiated carcinomas. All the cases were reviewed for presence or absence of ascertained cytological features. The various cytomorphological features studied included cell patterns (trabecular, arborescent, cohesive, syncytial, acinar papillae like, dyscohesive), cell shape (polygonal, cuboidal, columnar, signet ring),bare nuclei, nuclear features (size, shape, N:C ratio, pleomorphism),nucleoli (prominence , size, shape), intranuclear inclusions, cytoplasm (well/ ill defined, amount), vacuoles and granularity, presence / absence of bile, mucin and the background for presence / absence of normal hepatocytes in cords and also necrosis. The cases with well documented clinical, along with serological, radiological or primary work up were included in the study, thus excluding the result of fine needle aspiration (FNAC) as a sole diagnostic criteria. The chi- square tests were applied to establish statistically significant cytomorphological features inorder to evaluate features which help differentiate hepatocellular carcinomas from metastatic carcinomas. The adjuctive role of radiology, serology and primary work up were also evaluated.

RESULTS

The results of the individual cytomorphological features studied are given below along with relative strength of each feature (p value) in distinguishing between hepatocellular carcinoma and metastasis to the liver.

CELL PATTERN

A trabecular pattern (figure 1a) and arborescent pattern (figure 1b) in hepatocellular carcinoma had a significant p value <0.001.

The acinar or glandular pattern had a significant p value for metastatic carcinoma (p <0.001).

CELLS

Polygonal cells with round central nuclei noted in 25 of the 31 cases of HCC (figure 1c) and in none of metastatic carcinoma (p value <0.001). Cuboidal / columnar cells with basal nuclei were present in 16 of 40 cases of metastatic carcinoma and none of the hepatocellular carcinoma (p value <0.001).

NUCLEAR FEATURES

None of the nuclear features seemed to statistically significant in differentiating between HCC and metastatic carcinoma (P value of each <0.1).

CYTOPLASMIC FEATURES

A eosinophilic granular cytoplasm of variable intensity was seen in 22 cases of HCC compared to 3 cases of metastatic adenocarcinoma (P value <0.001).

BACKGROUND FEATURES

The presence of normal hepatocytes in cords is seen attached to the clusters of tumour cells (figure 1d) in 24 cases of metastatic adenocarcinoma and in none of the HCC cases (p value <0.001).

A necrotic background is seen in 4 cases of HCC and in 18 cases of metastatic Adenocarcinoma (p<0.005).

The radiological, serological (AFP) and the primary work up to support the cytological cases were evaluated. 17 of the 31 cases of HCC had radiology and serology (AFP) while 37 of the metastatic cases had radiology with primary work up to support the cytological diagnosis. 14 cases of HCC and 3 cases of metastasis had only radiology to support the cytological diagnosis.The various sites of the primary are enumerated in table 1. The comparism between cytomorphological features of hepatocellular carcinoma and metastatic carcinoma with the chi-square analysis are shown in table 2. The grading of hepatocellular carcinoma showed 7 cases of well

differentiated, 15 moderately differentiated and 6 poorly differentiated carcinoma. The three cases – one of fibrolamellar type and 2 cases of neuroendocrine type were identified on cytomorphology. The well and moderately differentiated HCC were identifiable on the basis of the easily identifiable trabecular and arborescent pattern along with polygonal cells having granular cytoplasm. The poorly differentiated HCC however did not have discernable patterns and individual cell cytology .However in 4 of the 6 cases the AFP was markedly elevated (>400 mg/ml). Also the remaining 2 cases radiology was in favour of an HCC along with extensive work up of the patient showing no identifiable primary other than liver. The AFP values in the available cases of HCC and metastatic carcinomas are shown in table 3 along with their relative utility in table 4.

Table 1:Number of cases with Metastasis to liver and their Primary sites.

Gall bladder - 09	Ovary – 03
Stomach -06	Breast -01
Oesophagus -01	Lung-01
Periampullary -04	Prostate-02
Large intestine -06	Pancreas -06
	Occult -03

Table 2:chi – Square Test - P Value Results Of Cytomorphological Features

	HCC	Metastasis	P value	
Patterns				
Trabecular	20	0	35.42	P<0.001
Arborescent	16	0	27.74	P<0.001
Cohesive	13	27	4.62	P<0.05
Synctial	01	16	13.30	P<0.001
Acinar	03	30	29.74	P<0.001
Dyscohesion	11	11	0.56	P<0.5
Cells				
Polygonal	25	0	45.58	P<0.001
Cub/columnar	0	16	15.59	P<0.001
Signet	0	03	2.6	P<0.1
Base nuclei	13	07	5.13	P<0.02
Endothelial cells	18	0	31.12	P<0.001
Nuclei				
Nuclear pleomorphism	29	40	2.5	P<0.1
Prominent nucleoli	17	14	2.8	P<0.1
Intranuclear inclusion	14	04	11.52	P<0.005
Cytoplasm				
Granularity	22	03	22.5	P<0.001
Lipid/glycogen vacuoles	08	02	2.5	P<0.01
Bile	07	0	11.52	P<0.005
Mucin	0	03	2.6	P<0.10
Background				
Necrotic	04	18	8.41	P<0.005
Normal hepatocytes	0	24	18.02	P<0.001

Table 3: AFP (ng/ml) correlations for HCC and metastatic Adenocarcinoma

Type (Total no of cases	AFP<10ng /ml	AFP11-400ng/ml	AFP>400ng /ml	Not available
Metastasis (40)	05	-	-	35
WDHCC (07)	01	05	-	01
MD HCC (15)	-	05	03	07
PD HCC (6)	-	-	04	02
Fibrolamellar	01	-	-	01
Neuroendocrine	-	01	01	02

Table 4: Clinical – radiological and cytological correlation to aid the diagnosis

Grade	Cytology	Radiology	AFP
WD HCC	Helpful	Helpful	Doubtful
MD HCC	Very helpful	Helpful	Helpful
PD HCC	Doubtful	Helpful	Helpful

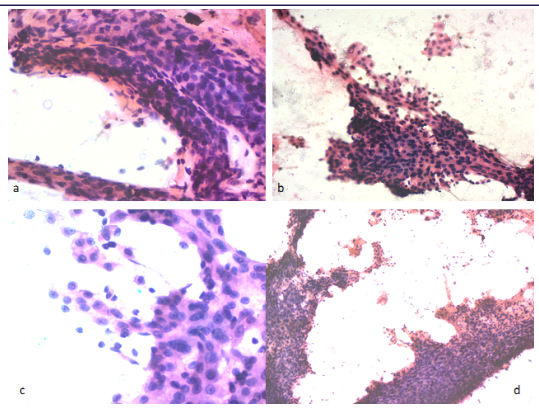

Figure 1: (a) trabecular pattern, (b) arborescent l pattern (c) polygonal cells with prominent nucleoli in hepatocellular carcinomas and (d) cords of normal hepatocytes seen attached to cluster of tumor cells in metastatic carcinoma.

DISCUSSION

The differentiation between metastatic and primary neoplasia of the liver on the basis of history, physical examination, radiological studies and serology is not without its difficulties. Metastatic tumours often are not associated with cirrhosis unlike the primary hepatocellular carcinomas. The value of testing for alpha feto protein (AFP) for the diagnosis of primary hepatocellular carcinoma is established. But its limitations in early stage HCC, well differentiated HCC, certain subcategories like small cell hepatocellular carcinoma and also a single negative value are known .

Cytodiagnosis of hepatocellular carcinomas has also not between without its peril's. About 80 % of malignant lesions of the liver can be correctly diagnosed through cytomorphologic analysis and good clinical correlation, 20 % pose differential diagnostic problems.[7] The divergent problems do exist with very well differentiated and poorly differentiated hepatocellular carcinoma .[8]

The necessity for the accurate differentiation of HCC from metastasis lies in the fact that the treatment of choice for early case of HCC is resection. The morphological diagnosis of HCC is complicated by two divergent problems. In the well differentiated HCC the resemblance to hepatocytes is obvious but proof of malignancy may be extremely difficult. There is a need to differentiate them from macroregenerative nodule in a cirrhotic background, focal nodular hyperplasia and hepatocellular adenoma. In the poorly differentiated hepatocellular carcinomas, malignancy is easily recognizable but the histogenetic orgin of the cells may not be too obvious and the need to differentiate from metastatic adenocarcinomas becomes difficult.

Numerous cytomorphological features have been reported as FNAB microscopic criteria for hepatocellular carcinoma.[9-13] But few reports have separated key cytological criteria from the secondary cytological criteria .[3,4] Therefore the practicing cytopathologist examining a liver FNAB often is unsure how much weight to give any one criteria to accept or reject a diagnosis of hepatocellular carcinoma in the context of differentiating it from metastatic adenocarcinomas. The chi-square test (p value <0.001) is employed to ascertain the strength of each the cytological characteristics studied.

The study identified the pattern (trabecular and / or arborescent), polygonal cells with granular cytoplasm and presence of endothelial cells to be key criteria to favour hepatocellular carcinoma over metastatic carcinoma (p<0.001, table 2). The presence of acinar and / or synctial pattern and cuboidal or columnar cells and presence of hepatocytes against the background of the clusters favours metastatic carcinomas. The study by Dilip Das identified features for HCC to be trabecular pattern, hepatocytic cells , eosinophilic granular cytoplasm, lipid vacuoles, bile pigments and atypical stripped nuclei(p<0.001 to <0.0001).[3] The Bottles et al study identified three key criteria for hepatocellular carcinoma were polygonal cells with central placed nuclei, trabecular pattern and bile.[4] In our study the presence of bile and intranuclear inclusions for HCC were less strong criteria (p<0.005). Even lesser criteria for HCC were lipid or glycogen vacuoles (p<0.01), bare nuclei (< 0.02) and prominent nuclei (P<0.1).

We graded the hepatocellular carcinomas in to well (7 cases) , moderate(15 cases) and poorly differentiated carcinomas (6 cases). The features for well differentiated hepatocellular carcinomas were trabecular and / or arborescent pattern, polygonal cells with round central nuclei, with slight increase in N:C ratio and well defined abundant granular cytoplasm with over all cellular monomorphism. The moderately differentiated unlike the well differentiated had higher N/C ratio, prominent nucleoli , ill defined moderate granular cytoplasm with overall moderate pleomorphism among cells. The poorly differentiated hepatocellular carcinomas showed no trabecular and /or arborescent pattern rather had clusters with dyscohesion and no resemblance to hepatocytic orgin, markedly increased N/C ratio, marked pleomorphism with scanty cytoplasm.

In our study and in our experience we do not have many cases to be included in the differential diagnosis at well differentiated end of the spectrum. We had a case of focal nodular hyperplasia. This case contained abundant normal hepatocytes and numerous epithelial cells in ductal formations, reflecting the proliferating bile ducts. The lack of these ductal cells does not allow the exclusion of FNH, but favours a liver cell adenoma.[8]

The differentiation of poorly differentiated HCC from poorly differentiated metastatic adenocarcinoma was rather difficult. In our analysis we found a diligent search for acinar pattern(30 of 40 cases) and presence of normal hepatocytes in cords at the periphery of the clusters (24 of 40 cases) favour metastatic poorly differentiated adenocarcinomas.

When we tried to integrate our cytological features in the difficult cases, with serology (AFP) and radiology , we had some significant observations(Table 4). While AFP is of doubtful significance in well differentiated carcinoma and to some extent in moderately differentiated (3 of 15 cases >400 ng/ml), it is of immense value in poorly differentiated hepatocellular carcinomas (4 of 6 cases >400ng/ml). Also a single negative value of AFP has no significance. Early stage of hepatocellular carcinoma shows lower AFP and an increase in size in associated with rising AFP. A fall if AFP in HCC reflects necrosis in AFP secreting tumour nodules.[14,15]

The clinical features and radiology are helpful in the well differentiated end of the spectrum. Focal nodular hyperplasia (FNH) has a typical radiological appearance and discernable cytological features.[16] Hepatocellular adenoma classically are single and well defined on radiology. Their clinical spectrum is very typical with solitary nodules occurring in women of reproductive age group on oral contraceptives.[17,18] Macroregenerative nodule occurring in a cirrhotic background measuring 5 to 15 mm in diameter , but rarely may be 5cm or more in diameter.[19]

We strongly feel when applying the cytological criteria, the cytopathologist should of course, take into account the clinical history, radiological studies and biochemical determinations. Our study indicates that cytomorphology is definitely useful but not unequivocal tool for establishing the diagnosis in mass lesions of the liver. The best policy for a diagnostic strategy would be require a multidiscipiliniary approach with the clinicians, radiologists and pathologists joining in the larger interests of the patient.

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