

Differentiation of Hepatocellular Carcinoma from Metastatic Carcinoma of the Liver - Clinical and Cytological Features

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Abstract

Differentiation of hepatocellular carcinoma (HCC) from metastatic carcinoma in liver may be difficult on fine needle aspiration cytology (FNAC), when both appear as moderate to poorly differentiated tumours. The present study was done to assess clinical, serological, biochemical, radiological and detailed cytomorphological features to distinguish HCC from metastatic carcinoma in FNAC of the liver masses. The individual cytomorphological features which helped in differentiating HCC from metastatic carcinoma were: hepatocytic appearance of cells (92%), trabecular pattern (92%), naked nuclei (76%), intranuclear inclusions (52%) and bile (40%). The most common clinical presentation in HCC cases was pain abdomen (40%). Positivity for HBsAg was found in 7 (33.3%) cases while anti HCV antibody was detected in 4 (19%) cases. The level of serum alpha fetoprotein (AFP) was elevated in 88.9% cases, but 40% cases showed mild elevation of AFP level. 17/25 cases of HCC had solitary space occupying lesion (SOL) and 8 cases had multiple SOLs. The present study reveals that most useful cytomorphological features in the distinction of HCC from metastatic carcinoma include trabecular pattern, hepatocytic cells, bile pigment, intranuclear inclusions and atypical stripped nuclei in HCC. Viral markers and alpha-fetoprotein estimation can supplement the results.

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Key Words : FNAC, hepatocellular carcinoma, metastatic carcinoma.

Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver in adults. On the other hand, liver is one of the most common sites for metastatic disease accounting for 25% of all metastasis to solid organ.

Most valuable for small tumours are radiological studies e.g. ultrasonography (USG), computed tomography (CT), magnetic resonance imaging (MRI) and hepatic angiography.¹ Fine needle aspiration cytology (FNAC) of the liver under USG or CT guidance has become a popular procedure to establish a

diagnosis for liver masses.² An accurate diagnosis particularly of poorly differentiated HCC requires differentiation from cholangiocarcinoma and other primary malignant tumours and more common metastatic carcinoma.³ The present study was done to analyse clinical, biochemical and radiological parameters along with detailed cytomorphological features to distinguish cases of HCC from metastatic carcinoma liver.

Materials and Methods

This was a prospective study over a period of two years (from May 2004 to April 2006) comprising of a

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total of fifty cases with liver space occupying lesions (SOLs), clinically or radiologically suspicious for malignancy and referred to the Department of Cytopathology, PGIMER, Chandigarh for FNAC under radiological guidance.

Clinical details studied in all cases included:

- History of pre-existing liver disease or malignancy, alcohol consumption and other relevant details.
- Biochemical data: The results of liver function tests including serum bilirubin, liver enzymes; serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT) and alkaline phosphatase (ALP).
- Serological data: Serological markers including serum alpha fetoprotein (AFP), Australia antigen (HBsAg), anti HCV (hepatitis C virus) and other tumour markers like CEA (carcinoembryonic antigen) or CA125, whenever available were recorded.
- Ultrasonography (USG) was carried out in all cases and liver SOL was categorized as single or multiple in number, size and involvement/invasion of adjacent structures as well as presence of associated lesions particularly cirrhosis was documented.

FNAC was performed under ultrasound guidance, using a 21-23 gauge lumbar puncture needle or Chiba needle, depending on the depth of lesion, fitted to a 20-ml disposable syringe attached to a metallic syringe holder. 1-3 passes were made to get adequate aspirates. Direct air dried smears were prepared for routine May-Grünwald-Giemsa (MGG) and few smears were immediately fixed in 95% alcohol for Haematoxylin and eosin stain (H&E). Special cytochemical stains, such as Periodic acid Schiff (PAS), were performed as and when required.

The MGG and H&E stained smears were seen for a detailed cytomorphological analysis. The cases were diagnosed by FNAC using established criteria and 25 cases each of HCC and metastatic carcinoma were diagnosed. Follow-up histopathology was available in three patients, who underwent a hepatic lobectomy and the diagnosis of HCC was confirmed.

The metastatic group included: 17 cases of adenocarcinoma, 2 cases of poorly differentiated carcinoma, 2 cases of neuroendocrine carcinoma, 1 case each of small cell carcinoma and renal cell carcinoma and 2 cases of carcinoma, not otherwise specified (NOS).

Statistical analysis was done to compare various

parameters in the groups of HCC and metastatic carcinoma, using the Chi square test and Fisher's exact test. A probability value of 0.05 or less was considered significant.

Observations

Age and sex distribution: The age of the patients with HCC ranged from 35 to 82 years, with a mean of 58.84 ± 11.93 years. In the metastatic carcinoma group, the age ranged from 22 to 72 years with a mean age of 50.28 ± 14.88 years. The male to female ratio was 22:3 in HCC group and 18:7 in metastatic group.

Clinical characteristics: The most common clinical presentation in HCC cases was pain abdomen (40%), followed by loss of weight and anorexia (24%), jaundice (12%) fever (8%) and lump (4%).

Biochemical parameters (Liver function tests): The biochemical parameters analyzed included serum bilirubin, SGOT/SGPT levels and alkaline phosphate (ALP) levels. The serum bilirubin was elevated in 8/25 (32%) cases. 5/25 (20%) cases had elevated SGOT levels while 4/25 cases (16%) had elevated SGPT levels. ALP was elevated in 4/25 (16%) cases. Prothrombin time index (PTI) was more than 75% in all patients allowing the invasive procedure (FNAC) to be performed.

Viral markers: Hepatitis B surface antigen (HBsAg) and anti HCV antibody serology was available in 21 patients. Positivity for HBsAg was found in 7(33.3%) cases while anti HCV antibody was detected in 4(19%) cases.

Serum alpha fetoprotein (AFP): AFP level elevation was detected using ELISA in 18/25 cases of HCC. The level of serum AFP was elevated in 16(88.9%) cases. 10(40%) cases showed mild elevation of AFP level (<100 ng/ml), 4(22.2%) cases showed moderate elevation (100-500 ng/ml), 2(11.7%) cases showed marked elevation (>500 ng/ml) of AFP levels, while 2(11%) cases showed AFP in normal range (<20ng/ml).

With regards to degree of differentiation, raised AFP levels were recorded in 7/8 cases of well differentiated HCC. 9/10 (90%) cases of moderate to poorly differentiated HCC showed elevation of AFP. However the level of serum AFP did not correlate significantly with the degree of differentiation in HCC (Fisher's exact test, $p = 0.14$).

Imaging studies in HCC: The radiological studies included USG, CT and MRI. 17/ 25 cases had solitary SOL and 8 cases had multiple SOLs. The size of the

lesions varied from 2.5 cm to 12 cm. None of the lesions in the HCC group was less than 2 cm in size. Cirrhosis of the liver was radiologically documented in 10/25 patients (40%). Portal vein thrombosis was detected in 12% of patients and portal hypertension detected in 20% cases.

The metastatic carcinoma group showed a solitary SOL in 8/25 patients and multiple lesions in 17 patients. This finding was reverse of that seen in HCC cases. The size of lesions was 0.5 to as large as 10 cm. None of the metastatic case showed cirrhosis.

Cytomorphology: The cytomorphological analysis encompassed study of cellularity, pattern of arrangement, cytoplasmic and nuclear details and many additional features (Fig.1). Based on these observations, the cases of HCC were classified into well and moderate to poorly differentiated carcinomas. A comparative analysis of the cytomorphological features of HCC with metastatic carcinoma of the liver is presented (Table 1). It was seen that the useful cytological features in diagnosis of HCC include trabecular pattern with small capillaries transgressing clusters of tumours cells, hepatocytic cells, bile pigment, intranuclear inclusions and atypical stripped nuclei.

Benign hepatocytes were present in 4(16%) cases of HCC. Necrosis was seen in 2(8%) and mitosis was noted in 2(8%) cases. Cholangiolar epithelium was seen in 2(8%) cases. 5(20%) cases showed glycogenated background while inflammatory background was noted in 2(8%) cases of HCC. None

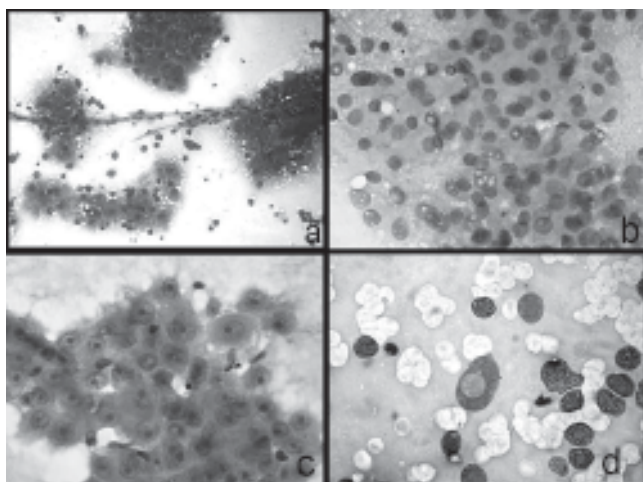


Fig. 1 : Cytomorphology of Hepatocellular carcinoma: (a) showing predominantly trabecular pattern with many naked nuclei (MGG, x 110); (b) showing prominent cytoplasmic and nuclear vacuolation and intracytoplasmic bile pigment (H&E, x 512); (c) showing prominent central nucleoli (H&E, x 512); (d) showing intranuclear inclusion in a case of hepatocellular carcinoma (H&E, x 1156).

Table 1 : Showing comparative analysis of the cytological features of HCC with metastatic carcinoma of the liver

	HCC (n=25)	Metastatic carcinomas (n=25)
CELLULARITY		
High	13	10
Moderate	10	10
Low/scanty	2	5
PATTERN OF ARRANGEMENT		
Monolayered cell clusters	0	0
Mixed monolayered cell clusters and trabecular pattern with small capillaries transgressing clusters of tumours cells	12	1
Predominantly trabecular with small capillaries transgressing clusters of tumours cells (dissociated cells <20%)	11	1
Trabecular + dissociated (dissociated cells 20-50 %)	2	10
Predominantly dissociated (dissociated cells > 50 %)	0	0
Multilayered cell clusters with irregular branching	0	13
CELLULAR DETAILS		
Cell appearance		
Hepatocyte like	23	1
Variable	2	24
Size		
Small	0	3
Medium	10	11
Large	15	11
Cytoplasmic vacuoles	22	10
Bile	10	0
NUCLEAR DETAILS		
Bi/ Multinucleation	16	6
NC ratio		
High	19	6
Normal	19	6
Location		
Central	22	22
Eccentric	3	3
Anisonucleosis		
Mild	7	7
Moderate	15	16
Severe	3	2
Nucleoli		
Inconspicuous	0	6
Visible	1	3
Single	9	10
Prominent	12	6
Intranuclear Inclusions	13	0
Nuclear vacuoles	12	4
Naked nuclei	19	5

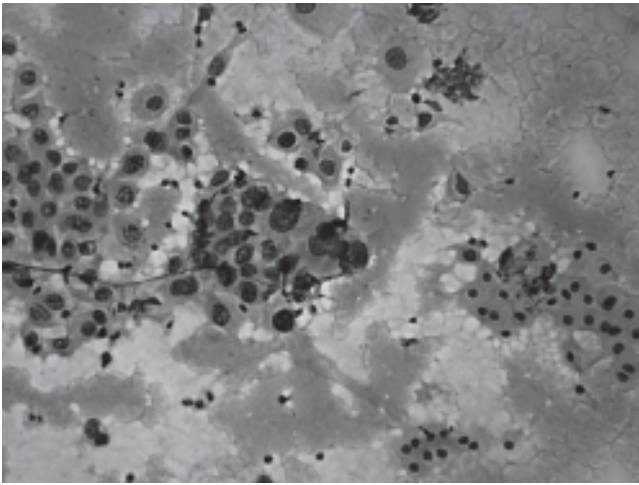


Fig. 2 : Microphotograph showing a cluster of malignant cells admixed with clusters of benign hepatocytes in a case of metastatic adenocarcinoma liver (H&E, x 256).

of the HCCs showed mucin.

The metastatic group showed admixture with benign hepatocytes in 14(56%) cases (Fig.2). Necrosis was seen in 11(44%) cases and prominent mitosis was present in 4(16%) cases. 3(12%) cases showed mucinous background and 3(12%) cases showed inflammatory background. None of the cases showed a glycogenated background.

The final diagnosis was arrived at by combining the clinical, serological, biochemical, radiological and cytomorphological features.

Based on pattern of arrangement and nuclear features, the cases of HCC were classified in two groups: i) well differentiated, 10(40%) cases and ii) moderate to poorly differentiated, 15(60%) cases.

Discussion

The role of FNAC in the diagnosis of liver SOL is well established. Although 80% of malignant lesions of the liver can be correctly diagnosed through cytomorphological analysis and good clinical correlation, around 20% can pose differential diagnostic problems. The distinction of moderately to poorly differentiated hepatocellular carcinoma (HCC) from metastatic carcinoma can pose a major problem to cytologists and this distinction is clinically important.⁴

The commonest clinical presentation is pain abdomen followed by loss of weight and anorexia (24%), jaundice (12%), fever (8%) and lump (4%). HBsAg positivity in case of HCC in our study (33.3%) was lower than expected, which is between 50-70% as reported from other centres in India⁵ as well as from our own Institute previously.⁶

Hepatitis C virus is the other major etiologic factor for HCC in Mediterranean region and in South East Asia. In India, the prevalence of HCV antibodies in patients with HCC in previous studies was 4-10%.⁶ 19% patients of HCC evaluated in this study showed presence of HCV antibody. Recent studies from Hyderabad confirm our results, showing an increase in HCV prevalence in cases of HCC.⁵

Alpha-fetoprotein (AFP) estimation has long been regarded as a tumour marker for HCC. 88.9% HCC cases showed elevation of AFP levels in serum. Our study compares favourably with previously reported cases from India.⁶

On radiological examination, HCC and metastatic carcinoma to the liver may show overlapping features. In our study, 68% cases of HCC and 32% metastatic cases showed a solitary lesion on ultrasound examination. Associated cirrhosis was documented in 40% HCC cases, the findings are almost the same as reported by Wee et al.⁷

The cytological features of HCC are well documented by reports from our Institute⁸ as well as others.⁹ In a large series of HCC analyzed by stepwise logistic regression analysis, Cohen et al⁹ put forward that high N/C ratio, trabecular pattern of arrangement and atypical naked hepatocytic nuclei were the three primary criteria for diagnosis of HCC. In the present study, high N/C ratio was found in 76% cases. Predominantly, trabecular pattern was seen in 44% of cases and a trabecular and monolayered pattern was seen in 48%. Cohen et al⁹ described trabecular pattern in 63% of their cases. Green¹⁰ and Suen¹¹ also found the trabecular pattern as the most common form of arrangement of neoplastic hepatocytes. Atypical hepatocytic naked nuclei in variable numbers were documented in 76% (19/25) of our cases. Their presence has been reported in 73% cases by Cohen et al⁹ and 90% of HCCs by Pedio et al.¹² Cohen et al⁹ reported slightly higher values with findings of prominent nucleoli in 60% and multiple nucleoli in 54% of their cases. Intracytoplasmic bile pigment was detected in 40% (10/25) cases in the present study. The frequency of bile in liver aspirates in previous studies of HCC ranged from 17 to 68%.^{6,8}

Differentiation of poorly differentiated HCC from a poorly differentiated metastatic tumour is a diagnostic difficulty. In our study, there were 25 cases of metastatic tumours of the liver. The male predominance was less evident in the metastatic group. Cytologically, moderate to high cellularity was seen in 20(80%) cases. The pattern of arrangement

was predominantly in irregular clusters and mixture of trabecular and dissociated cells with focal acinar formation. The cells were of variable sizes and shapes with high N/C ratio, exhibiting varying grades of anisonucleosis. Admixture with clusters of benign hepatocytes was seen in 56% cases and with cholangiolar epithelium in 2 cases. Necrosis (in 44% cases) and mucinous background (in 12% cases) are the other important soft points to suggest metastatic tumour. The differential diagnostic points between HCC and metastatic carcinomas have been described earlier.⁴ In the present study, the serum AFP levels were not elevated in metastatic carcinoma cases.

Conclusion

The most useful cytomorphological features in the distinction of HCC from metastatic carcinoma include trabecular pattern with small capillaries transgressing clusters of tumours cells, hepatocytic cells, bile pigment, intranuclear inclusions and atypical stripped nuclei in HCC whereas absence of above features and presence of cohesive three dimensional groups and individually dispersed cells with attempted gland formation, presence of benign hepatocytes, cholangiolar epithelium, and mucin along with a variable cytomorphology depending upon the primary site favour metastatic carcinoma. In doubtful cases, viral markers and alpha-fetoprotein estimation can supplement the results.

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