INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common histological type of primary liver carcinoma. HCC is a major health problem worldwide due to its high incidence and high rates of mortality.¹ In Pakistan, recent studies have shown strong association between HCV infection with genotype 3a and HCC.²,³ Liver is a common site of metastasis from many primary sites due to rich portal and systemic venous supply. Thus, metastatic cancer is the most common malignant tumour in adult liver.⁴ The distinction of liver metastatic tumour from HCC may present a diagnostic challenge that carries impact on subsequent prognostication and therapeutic management.

In differential diagnosis between HCC and metastatic carcinoma, clinical information regarding serum tumour marker levels and radiological findings are helpful. A tissue diagnosis is mandatory to make a definitive diagnosis. The pathologist uses morphology to establish a differential diagnosis and then uses histochemical and immunohistochemical studies to refine the diagnosis. Immunohistochemistry is helpful when morphology and identification of secretory substances fail. In this regard, most commonly used markers include antibodies directed against alpha fetoprotein (AFP), polyclonal carcinoembryonic antigen (PCEA), various cytokeratins, factor XIII, CD34 and CD10. All these antibodies are not a good choice because of their low sensitivity. Among immunohistochemical markers, Hep par-1 has been reported as most sensitive and specific immunohistochemical marker for HCC.⁵-⁸

The rationale of study was to establish the diagnostic utility of Hep par-1 in difficult cases when differential diagnosis lies between poorly differentiated HCC and metastatic carcinoma of liver. The objective of this study was to evaluate the diagnostic utility of Hep par-1 in differentiating hepatocellular carcinoma from metastatic carcinoma taking histo-pathology as a gold standard.

METHODOLOGY

This comparative cross-sectional study was carried out at the Pathology Department of Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore.
Cancer Hospital and Research Centre, Lahore, from April 2007 to February 2008. Sixty cases of liver carcinomas comprising 30 cases each of HCC and metastatic carcinoma were included in the study. Patients aged 40 years or above with primary and metastatic liver tumours were included as per inclusion criteria. Cases in which specimens have poor fixation and cases of cholangiocarcinomas were excluded. Non-probability convenience sampling was applied.

The study was started after approval by Institutional Review Board (IRB) of Shaukat Khanum Memorial Cancer Hospital and Research Centre. Haematoxylin and eosin stained slides were reviewed in all cases to verify the histological findings. Representative formalin fixed tissue blocks were selected for Hep par-1 staining. A 4 µm section was obtained from each block. Sections were deparaffinized in xylene and rehydrated and immunohistochemical method was carried out for Hep par-1 detection. The specificity control tests were carried out.

The equipments used were grossing laboratory, histoprocessor, microtome, frosted glass slides, H and E stainer, Dako immunohistochemical stain, and Hep par-1 antibody.

The staining results of tumour were expressed as the product of brown cytoplasmic granular staining intensity and percentage of positive cells. The average intensity of the staining corresponds to the presence of negative, weak, moderate, and strong staining. The percentage of tumour cells is scored as follows:

0 = no reactivity; 1 = less than 5% of cancer cells positive; 2 = 5 - 25% positive; 3 = 25 - 50% positive, 4 = 50 - 75% positive; 5 = 75 - 90% positive; and 6 > 90% of tumour cells positive.

Haematoxylin and eosin stained slides were seen for tumour morphology and grade of the tumour. The hepatocellular carcinomas were graded into well, moderately, and poorly differentiated depending on histological differentiation. Metastatic carcinomas were also typed according to their histology. Hep par-1 immunohistochemical stain was performed on 30 cases of hepatocellular and 30 cases of metastatic carcinoma. The results of Hep par-1 staining and all other information were collected on a proforma.

The gender, age of the patient, positive symptoms, and investigations performed were presented as proportions, frequencies and percentages. Age was presented as mean and standard deviation. The results of H & E and IHC stain Hep par-1 were compared. True and false positive/ negative results were obtained and a 2 x 2 table was made (Table I). Sensitivity, specificity, positive, and negative predictive values and accuracy were calculated using the formulas.

### RESULTS

The study comprised of 60 cases of liver carcinoma. The ages of the patients were between 40 - 76 years with a median age of 56 years. Among all 60 patients, 41 (68.3%) were male and 19 (31.7%) were female patients.

The main presenting complaint was right hypochondrium pain seen in 33 patients (68.3%), followed by palpable right hypochondrium mass (23.3%) and jaundice (8.4%).

Ultrasound and computed tomography (CT) revealed multifocal liver lesions in 34 patients (56.7%) and unifocal lesion in 26 patients (43.3%).

Among 30 patients of hepatocellular carcinoma, 9 were hepatitis C positive (30%) and 8 were hepatitis B positive (26.7%). In 6 patients (20%), viral serology was unavailable and in 7 patients (23.3%), it was not performed. Among 30 patients of metastatic carcinoma, 22 patients (73.3%) showed no reactivity for hepatitis B and C, one patient (3.3%) was hepatitis B positive and in remaining 7 patients (23.3%), results were unavailable.

Staining of Hep par-1 was compared between hepatocellular and metastatic carcinomas. The distinct brown cytoplasmic granular staining of benign hepatocytes was taken as positive internal controls. The staining results of Hep par-1 in HCC and metastatic carcinoma are shown in Figure 1 and 2.

Out of 30 cases of HCC, 23 cases revealed moderate to strong staining results for Hep par-1, weak staining was noted in 2 cases and 5 cases were non-reactive. The pattern of Hep par-1 staining with percentage of positive tumour cells is summarized in Table II.

Out of 30 cases of metastatic carcinoma, only one case showed staining in < 5% tumour cells and remaining 29 cases showed no reactivity. In 2 cases of metastatic carcinomas the staining was not done.

### Table I: Comparison of H&E and Hep par-1.

<table>
<thead>
<tr>
<th></th>
<th>Hepatocellular carcinoma</th>
<th>Metastatic carcinoma</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>25</td>
<td>1</td>
<td>26</td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
<td>29</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>30</td>
<td>60</td>
</tr>
</tbody>
</table>

### Table II: Pattern of Hep par-1 staining in hepatocellular carcinoma.

<table>
<thead>
<tr>
<th>Hep par-1 staining</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5%</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>5 - 25%</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>25 - 50%</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>50 - 75%</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>75% - 90%</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>&gt; 90%</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>100</td>
</tr>
</tbody>
</table>
carcinoma focally entrapped benign hepatocytes between tumour cells exhibited positivity for Hep par-1, which on review of corresponding haematoxylin and eosin sections were benign hepatocytes rather than tumour cells. This expression of Hep par-1 in benign hepatocytes which are entrapped between the tumour cells, carries no significance. It implies that staining of IHC marker should be cautiously examined, as it could mislead in interpretation of staining results. Therefore, it is important that in doubtful cases H & E stained sections should be reviewed with Hep par-1 staining results.

The validity of IHC stain Hep par-1 was calculated by sensitivity, specificity and accuracy. The sensitivity of Hep par-1 was 83.3%, specificity was 96.6%, positive and negative predictive values and accuracy were 96.5%, 85.2% and 90% respectively.

**DISCUSSION**

The distinction of poorly differentiated HCC from metastatic carcinoma to liver is a challenging dilemma for surgical pathologist, specifically when submitted biopsy is scant. The pathologist uses morphology to establish a differential diagnosis and then uses histochemical and immunohistochemical studies to refine the diagnosis but these techniques have limitations. The distinction of adenocarcinoma from HCC can be made on morphological grounds. The adenocarcinoma is characterized by formation of gland like structures and hepatocellular carcinoma shows trabecular, solid, and sinusoidal patterns. The presence of bile pigment is pathognomonic for hepatocellular differentiation while adenocarcinoma is characterized by mucin secretion; so identification of mucin by tumour cells using mucin histochemical stain can establish a carcinoma as metastatic adenocarcinoma.9 However, histochemical stains in this regard are of low sensitivity and specificity.

Among immunohistochemical markers, alpha-fetoprotein (AFP) expression in a tumour is specific for hepatocellular differentiation if germ cell tumour can be excluded.10 However, low sensitivity (30 - 50%) has rendered AFP as a less useful option for diagnosis. Polyclonal carcinoembryonic antigen (pCEA) shows a characteristic canalicular pattern in 60 - 90% cases of HCC while adenocarcinoma shows diffuse cytoplasmic expression without canalicular accentuation.11 However, the interpretation of this staining pattern can be difficult in some cases. CD10 yield canalicular pattern12 and CD34 shows sinusoidal pattern in HCC. They are poor substitutes because of low sensitivity. Albumin mRNA detected by in situ hybridization is specific for hepatocellular differentiation and has high sensitivity (90%). However, use of this test is limited by its restricted availability.13

Recently reported antibodies with good sensitivity include Glypican 3 (GPC-3), Arginase-1 (Arg-1) and Hep par-1. GPC-3 is expressed in HCC, however, it is useful in distinguishing HCC from benign hepatocellular mass lesions.14-16 The disadvantage is that it is negative in well differentiated HCC. Arginase-1 shows cytoplasmic expression with patchy nuclear reactivity. Arg-1 reactivity can also be seen in neutrophils and macrophage.17 Its reported sensitivity is also lower on FNA samples.18 Arg-1 is reported more sensitive marker than Hep par-1, however, its expression is identified in other non-hepatic tumours.19 Hep par-1 is a monoclonal antibody developed from failed liver allograft. It has emerged as a most sensitive and specific immunohistochemical marker for HCC.5-8 It yields diffuse cytoplasmic granular staining pattern in normal and neoplastic hepatocytes. It is usually negative or only focally positive in adenocarcinomas from most sites. A recent study has shown that antigen for Hep par-1 antibody is the urea cycle enzyme carbamoyl phosphate synthetase (CPSI).20

In the original study by Wennerberg et al. the sensitivity of Hep par-1 was 87%.21 Other studies have reported sensitivities ranging from 82 - 93.7%, which is comparable to our result of 83%,9,18,22,23

The present study validates the result of Zimmerman et al.22 Their study yielded 83% sensitivity. In their study, only 2 cases of metastatic carcinoma showed staining for Hep par-1 while in this study, only one case of metastatic carcinoma exhibited focal staining.

The study by Lee suggested correlation between Hep par-1 positivity and degree of hepatocytic differentiation.4 The present study validates the result of Leong et al.5 establishing that the antigen expression has no correlation to the degree of hepatocytic differentiation.
As in this study, 6 cases with 90% tumour cells staining, two were poorly differentiated HCC and among 14 cases of HCC with strong staining results, 5 cases were of poorly differentiated HCC. This confirms that no relationship exist between antigen expression and degree of hepatocytic differentiation.

One of the limitations in this study was the small sample size. The main reason for this was short period of time for collection of cases as per inclusion and exclusion criteria. The same study could lead to more accurate results, provided it was performed on a larger sample size. Secondly, in this study, expression of Hep par-1 was not compared with a panel of other IHC markers routinely used for hepatocellular neoplasms.

CONCLUSION

Hep par-1 is a reliable immunohistochemical marker for cases of hepatocellular carcinoma (HCC). It has been shown to have high specificity (96.6%) and slightly lower sensitivity (83.3%) for identification of hepatocellular phenotype. It can be used along with other markers in morphologically difficult cases when differential diagnosis lies between poorly differentiated HCC and metastatic carcinoma.

REFERENCES