Arginase-1: A Useful Immunohistochemical Marker in Differentiating Hepatocellular Carcinomas from Non-Hepatocellular Carcinomas of Liver

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ABSTRACT

Introduction: Arginase-1 has recently been reported as a sensitive tool for the differentiation of hepatocellular carcinoma from the metastatic liver tumours. There are no reports regarding the utility of this marker from South Asian or Mediterranean regions where hepatocellular carcinoma is quite prevalent and on the rise due to many factors of which viral hepatitis C and B are noteworthy. Objectives: The objective of current study was to determine the diagnostic utility of Arginase-1 in differentiating hepatocellular carcinoma (HCC) from non-hepatocellular carcinomas (non-HCC) by calculating the sensitivity, specificity and positive and negative predictive values of Arginase-1 immunohistochemistry (IHC). Methods: The study was conducted at the Department of Pathology, Shaukat Khanum Memorial Cancer Hospital & Research Centre, Lahore. It was a descriptive, cross-sectional survey on 75 cases of diagnosed cases of liver cancers. Non-HCC included cholangiocarcinoma and metastatic carcinomas in liver. IHC was applied to check the Arginase-1 detection in paraffin embedded tissues from the tumour samples. Results: Twenty-three (31%) cases were diagnosed as HCC and 52 cases were non-HCC. Mean age of HCC and non-HCC patients was 53.61 ± 13.20 and 56.90 ± 14.06 years respectively. HCC was more common in males (61%) whereas non-HCC tumours affected both genders equally. Twenty-two out of 23 (95.65%) HCC were positive and one (4.35%) was negative for Arginase-1. Whereas, 51 out of 52 (98.08%) non-HCC were negative for Arginase-1 and only one (1.92%) was positive for this immunomarkers. The sensitivity and specificity of Arginase 1 for the differentiation of HCC from non-HCC and metastatic liver tumours were calculated to be 95.65% and 98.08% respectively, while positive predictive value (PPV) and negative predictive value (NPV) were 95.65% and 98.08% respectively. Conclusion: Arginase-1 IHC offers a fairly sensitive and specific tool for the differentiation of HCC from non-HCC of the liver.

Key words: Arginase-1, immunohistochemistry, hepatocellular carcinoma, metastatic carcinoma, cholangiocarcinoma, sensitivity, specificity

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver cancer. The annual number of new cases of HCC worldwide is over one million. Globally, it is the fifth most common cancer and the third leading cause of cancer related death, preceded only by the lung and stomach cancers1. Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) infections and alcoholism are the important etiological factors because the resulting cirrhosis from above factors may lead to HCC. HCC is the commonest hepatic tumour in Southeast Asia and Sub-Saharan Africa while metastatic tumours predominate over primary hepatic tumors in Europe.
arginase-1 (HepPar-1), polyclonal carcinoembryonic antigen (CEA) and CD10 along with alpha-fetoprotein (AFP) and glypican-3 labeling some HCCs. However, the utility of each of these markers is limited either by suboptimal sensitivity or difficulty in interpretation. For example, AFP suffers from a low sensitivity of 30% to 50%. Polyclonal CEA and CD10 can be difficult to interpret and their sensitivities can be low in poorly differentiated HCCs (25% for polyclonal CEA and 50% for CD10). Over the past decade, HepPar-1, a mitochondrial urea cycle antigen, has been increasingly used as a positive marker for hepatic differentiation. However, HepPar-1 also suffers from relatively low sensitivity in poorly differentiated hepatocellular carcinomas. Glypican-3, a heparin sulphate proteoglycan expressed at high levels in HCC, has shown high specificity, but it has suboptimal sensitivity in the diagnosis of HCC when used in isolation as it is well known to be immunoreactive in a wide variety of other tumors e.g. germ cell tumors.

Recent literature has characterized a new immunohistochemical marker ‘Arginase-1’ as a potential marker of hepatocellular differentiation in both surgical pathology and cytopathology. Arginase-1 (ARG1) is an enzyme that converts L-arginine to L-ornithine plus urea, an important step in urea cycle. In normal human liver, Arginase-1 is expressed with a high degree of specificity. Thus the diagnostic utility of ARG1 has been studied and it has been found that ARG1 has sensitivity of 96.0% and specificity of 99.6% for HCC. Even in context of large round pink cell tumours, the specificity of ARG1 was found to be 100% versus 93.9% for HepPar-1. Some recent studies have shown promising results for Arginase-1 and indicated towards its better performance in compared to HepPar-1 and Glypican-3.

The rationale of this study was to determine the diagnostic role of this novel marker ARG1 in the differentiation of HCC from non-HCC of liver in local population. This would determine its significance as a diagnostic tumour marker so that it may provide a useful diagnostic adjunct in surgical pathology practice.

MATERIAL AND METHODS:

This descriptive, cross sectional study was conducted at Department of Pathology, Shaukat Khanum Memorial Cancer Hospital & Research Centre - Lahore (SKMCH&RC) in 2013-2014 after approval by Institutional Review Board (IRB) of the hospital. Seventy-five cases of diagnosed liver cancers reported from January 2013 to October 2013 were included. Formalin fixed paraffin embedded (FFPE) tissue blocks were retrieved. Patients with incomplete information and poorly processed/preserved samples were excluded. The data on age, gender and site of involvement was extracted from the clinical histories. IHC staining (expression of ARG1) was performed using an avidin-biotin-peroxidase complex method as per the specifications given by the manufacturer. A rabbit monoclonal antibody for ARG1 (Product name: ab133543/EPR6672) by Abcam was used. IHC staining was assessed independently and ARG1 staining was recorded on the proforma in terms of ARG1 positive and ARG1 negative cases. Cytoplasmic staining for ARG1 was taken as positive staining and the positivity was compared with that of positive control. The gender and age of the patients were presented as frequencies and percentages. True and false positive/ negative results for ARG1 IHC staining were obtained and a 2x2 table was made. Sensitivity, specificity, positive predictive value and negative predictive value were calculated.
RESULTS

Age was normally distributed (Shapiro-Wilk p value = 0.185) in study population (n=75). Mean ± S.D age was 55.89 ± 13.80 years. Overall males (39, 52%) were slightly more in number than females (36, 48%). The age distribution is shown in Figure 1.

Only 23 (30.7%) cases were found to have hepatocellular carcinoma (HCC). Mean ± SD age of patients having HCC was 53.61 ± 13.20 years. Minimum age at the time of diagnosis of HCC was 30 years whereas maximum age was 75 years. In the HCC cases, well and moderately differentiated HCC were more common histological grades (each grade having 9 cases, 39.1%) whereas only 5 (21.7%) cases were diagnosed as poorly differentiated HCC.

On the other hand, fifty-two (69.3%) cases were diagnosed to have non-HCC. Mean ± SD age of non-HCC patients was 56.90±14.06 years and the age range was 20 – 90 years. In the non-HCC group of patients, 7 cases (9.3% of the study population) were found to have cholangiocarcinoma and 45 (60% of the study population) cases were found to have metastatic (secondary) malignancy. Colorectal carcinoma was the most common known primary site of metastatic malignancy with 8 (15.4%) cases followed by gall bladder and pancreaticobiliary tumours. The detailed list of non-HCC cases is given in Table 1.

Arginase-1 immunohistochemistry

When immunostaining was performed with ARG1, out of total 75 cases, 23 (30.67%) were positive and remaining 69.33% were negative for ARG1. However, 22 out of 23 (95.65%) hepatocellular carcinomas were positive and one (4.35%) were negative for this immunomarker. The ARG1 positivity was seen in all different grades of HCC (Fig. 2). Whereas, 51 out of 52 (98.08%) non-HCC cases were negative for ARG1 (Fig. 3) and only one (1.92%) was positive for this immunomarkers. The 2 x 2 table is given in Table 2.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholangiocarcinoma</td>
<td>7</td>
<td>13.5</td>
</tr>
<tr>
<td>Colorectal carcinoma</td>
<td>8</td>
<td>15.4</td>
</tr>
<tr>
<td>Carcinoma of gall bladder</td>
<td>7</td>
<td>13.5</td>
</tr>
<tr>
<td>Pancreatobiliary malignancy</td>
<td>5</td>
<td>9.6</td>
</tr>
<tr>
<td>Carcinoma of lungs</td>
<td>2</td>
<td>3.8</td>
</tr>
<tr>
<td>Upper GIT cancer</td>
<td>2</td>
<td>3.8</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>1</td>
<td>1.9</td>
</tr>
<tr>
<td>Female genital tract malignancy</td>
<td>1</td>
<td>1.9</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>1</td>
<td>1.9</td>
</tr>
<tr>
<td>Stomach cancer</td>
<td>1</td>
<td>1.9</td>
</tr>
<tr>
<td>Urinary bladder cancer</td>
<td>1</td>
<td>1.9</td>
</tr>
<tr>
<td>CA-Unknown primary</td>
<td>16</td>
<td>30.8</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>100.0</td>
</tr>
</tbody>
</table>

It was found that the sensitivity of ARG1 for the differentiation of HCC from non-HCC is...
95.65% (95% CI: 79.01 – 99.23). The specificity for the same is 98.08% (95% CI: 89.88 – 99.66%). Similarly, the positive predictive value (PPV) and negative predictive value (NPV) of ARG1 for the differentiation of HCC from non-HCC are 95.65% (95% CI: 79.01 – 99.23) and 98.08% (95% CI: 89.88 – 99.66%) respectively.

**DISCUSSION**

The most frequently met differential diagnostic challenge in the liver is HCC versus intrahepatic cholangiocarcinoma or metastatic adenocarcinoma. Complicating the diagnostic process is that pathologists are frequently asked to handle and diagnose tiny liver needle core biopsies with various biopsy artifacts.

In our study, the mean age of liver cancer cases was 55 years and males were slightly more in number than females. These results are comparable to similar researches conducted for ascertaining epidemiology of HCC.

Only 30% cases in our study were found to have hepatocellular carcinoma (HCC). Minimum age at the time of diagnosis of HCC was 30 years whereas maximum age was 75 years. This age pattern is in accordance with the available literature.

In our studies, 70% cases were diagnosed to have non-HCC with the mean age of 56 years. These results contrast with the study conducted by Abdullah et al. They found out that HCC was more common than non-HCC. Moreover, mean age at diagnosis in their study was 63 years which is significantly higher than that in our study. This difference can be explained based on geographical variation in incidence of HCC.

Out of 52 cases of non-HCC, histologically 13% tumours proved to be cholangiocarcinoma. Colorectal carcinoma was the most common known site of metastatic malignancy with 8 (15.4%) cases followed by gall bladder and pancreaticobiliary tumours. In our studies, 30% cases were of unknown origin based on available information and histology. These results are in accordance with the results of other studies.

In current study, 22 out of 23 cases of HCC were found to be positive for ARG1 immunostaining. Thus, the sensitivity of this marker has been calculated as 95.65%. Whereas, 51 out of 52 non-HCC cases were negative for ARG1 and the specificity of ARG1 has been calculated as 98.08%.

Some recent studies have also explored the diagnostic utility of ARG1. Radwan et al. studied immunoreactivity of HCC with Arginase-1 and reported that the sensitivity of Arginase-1 for HCC diagnosis was 84% and the specificity was 96%. Timek and colleagues studied 47 cases of HCC and 1250 cases of metastatic tumours infiltrating the liver and compared the diagnostic accuracy of various immunomarkers. They reported that Arginase-1 carries 85% sensitivity and 100% specificity to diagnose primary hepatocellular malignancy.

Sang et al. demonstrated the sensitivity, specificity, positive predictive value and negative predictive value of Arg-1 in distinguishing HCC from metastatic tumors to be 96.2%, 100%, 100% and 90.6%, respectively. A recent report by Fatima and coworkers reported that Arginase-1 was positive.
positive in 75 out of 85 and negative in all 46 cases of metastatic tumours that means 88% sensitivity and 100% specificity. Yan et al. used immunohistochemistry to demonstrate the sensitivity of Arg-1 in 151 HCCs and found that its overall sensitivity of was 96.0% and the sensitivities of Arg-1 in well, moderately, and poorly differentiated HCCs were 100%, 96.2%, and 85.7%, respectively. McKnight and coworkers also demonstrated Arg-1 positivity in 37 of 44 (84.1%) cases of HCC, compared with 32 of 44 cases (72.7%) and 25 of 44 cases (56.8%) for HepPar-1 and Glypican-3, respectively. They concluded that arginase-1 had superior sensitivity compared with Glypican-3 and HepPar-1 in the diagnosis of HCC and its specificity was also very high.

The observations of our study are comparable with the results of most of the studies mentioned above especially in regards to the specificity of Arg-1 expression. However, current study has found higher sensitivity for HCC as compared to the previous investigators. This may be explainable due to small sample size of the current study.

CONCLUSION

ARG1 immunostaining offers a sensitive and specific tool for the differentiation of HCC from non-HCC of the liver.

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