INTRODUCTION

Hepatocellular carcinoma is the 5th commonest cancer world over and 3rd leading cause of cancer deaths.\(^1\) According to WHO, incidence of liver cancer is likely to increase persistently till 2030 and this tumour has second highest increase in mortality rate over last decade.\(^2\)

Cirrhosis is the leading cause of HCC as process of necrosis and regeneration seen in cirrhosis predisposes hepatocytes to the development of neoplasia and dysplasia.\(^3\) Viral hepatitis is the dominant etiological factor for cirrhosis patients with chronic hepatitis B and C are at increased risk of developing liver cancer with advancing disease.\(^4,5\) Other risk factors for HCC include alcoholic liver disease, haemochromatosis, aflatoxin exposure, autoimmune hepatitis etc.

Since there is a high risk of developing HCC in cirrhosis, it is recommended that all patients with cirrhosis be screened for liver cancer.\(^6\) HCC detected after the onset of symptoms has a dismal prognosis with only (0% – 10%) 5 years survival. In contrast, small HCC detected by surveillance can be curable.\(^7\) As potentially curative treatment modalities are available i.e. resection, liver transplantation, radiofrequency ablation with excellent outcome in patients with small size tumours, identification of HCC at early stage with a good screening test is, therefore, imperative for favourable outcome.

A screening diagnostic test should be cost effective, easily available and should have good sensitivity for diagnosis. According to Asia Pacific Association for study of Liver Diseases (APASL) guidelines, every patient with cirrhosis should have both alpha-fetoprotein (AFP) and ultrasound (US) examination for screening of HCC.\(^6\) A number of recent studies have questioned the role of AFP as a screening test. Daniele et al. noted 60% sensitivity of AFP with 20 ng/ml as cutoff value with positive predictive value (PPV) of only 50%, for diagnosis of HCC.\(^8\) American Association for Study of Liver Diseases (AASLD) guidelines only recommend ultrasound examination for screening of HCC and have noted sub-optimal sensitivity of AFP for screening of HCC.\(^9\)

AFP does have the edge in terms of being ubiquitous and economical, whereas in patients with infiltrative type
of HCC, tumour may not be picked on ultrasonography alone. Use of other imaging modalities like biphasic CT or MRI for screening of HCC in such scenario adds to the cost of screening. Pakistan is a developing country with limited resources, therefore, AFP level determination can be a useful adjunct to ultrasound examination for screening of HCC. It is imperative, therefore, to study the validity of AFP level in our patients with HCC before discarding it as screening test, as is being recommended in most of western guidelines now. There are a few small scale studies from this region regarding validity of AFP. Thus, the objective of this study was to determine the accuracy of alpha-fetoprotein for diagnosis of hepatocellular carcinoma.

**METHODOLOGY**

This study was conducted at the Department of Medicine, The King Edward Medical University, Lahore, from November 2007 to August 2011. Consecutive patients with HCC presenting the study centre were enrolled. Diagnosis of HCC was made in accordance with AASLD guidelines. Patients with suspicion of ovarian or testicular malignancy on examination or diagnostic workup were excluded.

Controls included were, 102 consecutive patients with cirrhosis without evidence of HCC. Diagnosis of cirrhosis was based on combination of physical findings i.e. palmar erythema, spider nevi, gynaecomastia, splenomegaly, ascites, impaired liver function tests, deranged clotting profile, low serum albumin and irregular liver surface, detected on ultrasound, with ratio of transverse caudate lobe to transverse right lobe width > 0.65. All patients with serum alpha-fetoprotein and abdominal US to exclude HCC. Patients with an elevated AFP (> 20 ng/ml) at enrollment were required to have a CT or MRI showing no lesion suggestive of HCC. Cirrhotic patients with nodules larger than 1 cm on US underwent biphasic CT abdomen or dynamic contrast enhanced MRI. If the appearance was typical of HCC i.e. hypervascular in arterial phase with washout in the portal venous phase, lesion was regarded as hepatocellular carcinoma. But if the findings were not characteristic or the vascular profile was not typical, a second contrast enhanced study with other imaging technique was performed or the lesion was biopsyed. Those with lesion less than 1 cm were not included and were advised follow-up with repeat ultrasonography after 6 months.

Variables obtained for all patients were age, gender, medical history, etiology of liver disease, co-morbid conditions and presence or absence of complications related to liver cirrhosis till inclusion in the study. Laboratory data included complete blood count, clotting profile, liver function tests, blood urea nitrogen and serum creatinine. Alpha-fetoprotein was determined in all patients via ELISA serum assay. All patients included, had ultrasound examination on which liver size and texture, number and maximum dimensions of space occupying lesions (SOL) wherein, presence or absence of portal vein thrombosis, lymph nodes and ascites were recorded. Contrast enhanced biphasic CT abdomen or dynamic contrast enhanced MRI were carried out in patients with HCC and those with no space occupying lesion on ultrasound but serum AFP > 20 ng/ml. In case of atypical SOL second imaging study was performed. Biopsy of lesion was only performed when tumour size was more than 1 cm with non-conclusive MRI and CT imaging. Liver cirrhosis was staged using Child Turcotte Pugh (CTP) score.

Classic contrast pattern on CT/MRI or biopsy were used as gold standard for diagnosis of HCC. Data were analyzed using PASW statistics 18. Chi-square test was used to compare the distribution of demographic and clinical variables among patients with and without HCC for discrete variables and t-test for continuous variables. P-value of ≤ 0.05 will be considered significant. Receiver curve was used to determine area under curve (AUC) and cutoff value of AFP with best possible sensitivity and specificity. We used cut off values of 20, 50, 100, 200 and 400 ng/ml for diagnosis of HCC, as mentioned in previous studies to determine sensitivity, specificity, PPV, NPV and accuracy of AFP for diagnosis of hepatocellular carcinoma.

**RESULTS**

One hundred and seventy three cases of HCC were included in this study whereas, 102 patients with cirrhosis, without evidence of HCC, were enrolled as control group. In the study group with HCC, male to female ratio was 2.5:1 (124/49) and mean age was 58.1 ± 10.12 years. Predominant etiology of liver disease was hepatitis C; 139 (80.3%), hepatitis B was diagnosed in 19 (11%), 2 (1.2%) had both hepatitis B and C, 12 patients were hepatitis B and C negative. HCC was diagnosed with typical appearance on contrast study in CT scan, in 153 (88.4%) patients, 15 (8.7%) patients had dynamic MRI for confirmation of diagnosis due to equivocal CT study and 5 (2.9%) patients had biopsy of lesion for confirmation of diagnosis. Majority of patients had a single space occupying lesion 96 (55.4%) while 12 (6.9%) had 2 lesions. Three or more lesions were diagnosed in 56 (32.3%) patients, while 8 (4.8%) patients had infiltrative type of HCC diagnosed on CT scan. Size of the largest lesion was less than 3 cm in 18 (10.4%) patients, 40 (23.1%) had 3 – 5 cm size, 82 (47.3%) had 5 -10 cm and in 25 (14.5%) patients size of HCC was more than 10 cm. Eight had infiltrative type of HCC. Portal venous infiltration was noted in 56 (32.4%) patients and 27 (15.6%) had abdominal lymphadenopathy on ultrasound examination.
In the control group of 102 patients, mean age was 54.24 ± 8.74 years and male to female ratio was 1.12 (54/48). Eighty six (84.3%) patients were hepatitis C positive, hepatitis B was diagnosed in 10 (9.8%) patients, 2 patients had both hepatitis B and C and 4 (3.9%) patients were negative for both viruses. Sixty six (64.7%) patients had decompensated liver disease, 50 (49.1%) of which had ascites, 26 (25.5%) had variceal bleeding and 16 (15.7%) patients had experienced portosystemic encephalopathy during the course of illness. Patients with and without HCC were comparable as far as stage of liver disease was concerned with non-significant difference in serum albumin, prothrombin time and Child Turcotte Pugh (CTP) scores (Table I).

Median value of AFP was 120 ng/ml in the study patients with HCC and 5.5 ng/ml in the control group. When alpha-fetoprotein in all patients with cirrhosis was evaluated for diagnosis of HCC using ROC curve, area under curve was 0.85 (95%; CI: 0.80 - 0.90) as shown in Figure 1. AFP level with best possible sensitivity and specificity for diagnosing HCC was determined using ROC curve and it was 20.85 ng/ml with sensitivity of 72% and specificity of 86.3%.

The performance of commonly used cutoff values of AFP for the diagnosis of hepatocellular carcinoma is shown in Table II.

### Table I: Comparison of patients of cirrhosis with and without hepatocellular carcinoma.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients with HCC (n=173)</th>
<th>Patient without HCC (n=102)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median duration of hep C (months)</td>
<td>24 (1-180)</td>
<td>23 (2-142)</td>
<td>0.08</td>
</tr>
<tr>
<td>Patients with bilirubin ≥ 3 mg/dl n (%)</td>
<td>39 (22.5%)</td>
<td>22 (21.5%)</td>
<td>0.85</td>
</tr>
<tr>
<td>Patients with ALT ≥ 40 IU/L n (%)</td>
<td>135 (79.8%)</td>
<td>68 (66.6%)</td>
<td>0.015</td>
</tr>
<tr>
<td>Patients with AST ≥ 40 IU/L n (%)</td>
<td>148 (87.5%)</td>
<td>86 (84.3%)</td>
<td>0.44</td>
</tr>
<tr>
<td>Patients with ALP ≥ 150 IU/L n (%)</td>
<td>100 (73.5%)</td>
<td>54 (52.9%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Patients with BUN ≥ 25 mg/dl n (%)</td>
<td>68 (43.5%)</td>
<td>26 (26.5%)</td>
<td>0.006</td>
</tr>
<tr>
<td>Patients with plat &lt; 100,000/mm³ n (%)</td>
<td>76 (46.1%)</td>
<td>42 (42%)</td>
<td>0.508</td>
</tr>
</tbody>
</table>

### Table II: Diagnostic value of serum alpha fetoprotein as biomarker for HCC.

<table>
<thead>
<tr>
<th>Cut-off value (ng/ml)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>79.1</td>
<td>70.5</td>
<td>82.03</td>
<td>66.6</td>
<td>76</td>
</tr>
<tr>
<td>20</td>
<td>72.2</td>
<td>86.2</td>
<td>89.9</td>
<td>64.7</td>
<td>77.45</td>
</tr>
<tr>
<td>50</td>
<td>59.5</td>
<td>98.03</td>
<td>98.09</td>
<td>58.82</td>
<td>73.8</td>
</tr>
<tr>
<td>100</td>
<td>50.2</td>
<td>100</td>
<td>100</td>
<td>54.25</td>
<td>68.7</td>
</tr>
<tr>
<td>200</td>
<td>45.6</td>
<td>100</td>
<td>100</td>
<td>52.04</td>
<td>65.8</td>
</tr>
<tr>
<td>400</td>
<td>42.7</td>
<td>100</td>
<td>100</td>
<td>50.7</td>
<td>64</td>
</tr>
</tbody>
</table>

**PPV = Positive predictive value; NPV = Negative predictive value.**

**Figure 1:** ROC curve of alpha fetoprotein for diagnosis of hepatocellular carcinoma.

**DISCUSSION**

Management of complications related to liver cirrhosis has improved resulting in longer survival time, thus; we are facing an increasing burden of patients with HCC. Identification of a sensitive screening test is imperative to diagnose HCC at treatable, early stage. It is pertinent to note that 66.4% of these patient had HCC diagnosed at a stage where no presently available therapy in Pakistan, can be offered with a tumour size > 5 cm.

Value of AFP as a screening test for HCC is now increasingly being questioned. This study has shown that AFP can best be used for screening of HCC with 72% sensitivity and 86% specificity, at cut off value of 20.86 ng/ml. If cut off limit was reduced to 10 ng/ml, it will compromise specificity so that the number of patients without HCC will be subjected to costly investigations and if the limit of AFP for diagnosis was increased, sensitivity of test dropped drastically as seen in Table II. This will result in missing number of patients with HCC, not acceptable for a screening test. It is this sub-optimal sensitivity of this test which puts its value as screening tool in doubt.

Marrero et al. showed that AFP had a sensitivity of 66% and specificity of 81%, at a cut off value 10.9 ng/ml.
Validity of alpha fetoprotein for diagnosis of hepatocellular carcinoma in cirrhosis

They found AFP most useful for early stage liver cancers. They also compared AFP with other new biomarkers like des-gamma carboxyprothrombin (DCP) and lectin-bound alpha fetoprotein (AFP-L3) and concluded that AFP is still a better serum biomarker.12 Stefaniuk et al. noted that AFP has high specificity but with cut off value > 100 ng/ml, sensitivity is only 20 – 30% which means 70 – 80% patients with HCC will not receive treatment when it was needed.13 In another study, it was noted that AFP at cut off value 20 ng/ml, has 60% sensitivity and positive predictive value range from 9 – 50%, depending on etiology of disease.14 Pervaiz et al. found AFP 72% sensitive and 89% specific for diagnosis of HCC.15 This limitation of AFP is most likely due to the fact that a number of small tumours may not secrete AFP resulting in normal levels despite the presence of HCC.16 Gupta concluded in his review that AFP has limited utility in identifying hepatocellular carcinoma in patients with hepatitis C.17

Sub-optimal performance of AFP has lead to introduction of number of new markers for diagnosis in the form of DCP, AFP-L3, alpha fucosidase, squamous cell carcinoma antigen (SCCA) and glypican 3 (GCP-3). All these new markers also have their own limitations for diagnosing HCC and particularly have low predictive value for small size tumours.13 Availability of these markers in cost effective manner to patients at large, is another limiting factor.

Limitations in the sensitivity of AFP in surveillance of high-risk populations have led to the use of ultrasonography (US) as an additional method for the detection of HCC. US has 78 – 90% sensitivity and 93% specific for detection of HCC, making it a useful adjunct to AFP for diagnosis.18 As per EASL guidelines 2012, use of serum AFP in addition to ultrasound adds 6 – 8% to sensitivity for HCC detection but also increases the cost of screening.19 Economic analysis by Coon et al. has found use of both AFP and abdominal ultrasound for screening in patients of cirrhosis most cost effective.20 Baig et al. have found AFP a useful and cost effective tool for screening of patients with HCC in patients of the region.21 According to latest APASL guidelines, both ultrasonography and serum alpha fetoprotein should be used for screening of HCC.6 EASL guidelines have suggested that persistently elevated AFP levels are a risk factor for HCC development and can be used to help define at-risk populations.19

CONCLUSION

Despite sub-optimal sensitivity, AFP is still the best available screening serological test for HCC and can be used along with ultrasonography for early detection of liver cancer.

REFERENCES


