

# Hepatitis B- and Hepatitis C- Associated Hepatocellular Carcinoma: Evaluation of Alpha-Fetoprotein as a Diagnostic Marker

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Hepatocellular carcinoma (HCC), a tumour originated from hepatocytes, is one of the most common cancers worldwide.<sup>1</sup> High prevalence of HCC occurs in Southeast Asia, the Western Pacific and sub-Saharan Africa.<sup>2</sup> In a large scale prospective study, HCC accounts for at least 20% of cancer deaths in male patients admitted to hospital, and 25.4% of males in an autopsy series.<sup>3</sup> The age-adjusted death rate of liver cancer is 25 to 100 per 100,000 population.<sup>4</sup> The prognosis of patients with HCC is very poor, not only because of the high malignancy of the tumours but also the late onset of the presenting symptoms which coincides with the advanced stage of the disease. Surgical removal is difficult and chemotherapy is relatively ineffective.<sup>5</sup>

The identification of hepatitis B surface antigen (HBsAg) by Blumberg in 1965 has led to the understanding of biology of hepatitis B virus (HBV) and its clinical significance. Epidemiological and clinical evidence suggests that there is a very close association between HBV infection and hepatocellular carcinoma.<sup>6,7</sup> Liver cancer is usually developed 8-30 years following the

**SUMMARY** Hepatocellular carcinoma (HCC) is one of the most common cancers, especially in Asia and Africa. The prognosis of HCC is very poor because of the high malignancy and the failure of early diagnosis which is mainly dependent on the late onset of clinical symptoms. Chronic infection with hepatitis B virus (HBV) and/or hepatitis C virus (HCV) is the most commonly known risk factor for developing HCC. Mass screening and monitoring of general population or of high-risk population, by measurement of serum alpha-fetoprotein (AFP), have been implemented in several countries. However, the use of AFP as a diagnostic marker for HCC is questionable due to its limited sensitivity and specificity. This article analyzed the serum level of AFP in 72 histopathologically confirmed hepatocellular carcinoma cases in Thailand. Elevation of serum AFP was detected in 75.8%, 88.9%, 79.2% and 80.0% of patients with HBsAg, anti-HCV antibody, HBV DNA, and HCV RNA, respectively. However, only 58.8% of HCC patients without any of the four markers had elevation of serum AFP. AFP is thus not a sensitive screening marker for HCC in general population, especially in those not associated with HBV or HCV. However, since elevated serum AFP was found in most patients with evidence of HBV or HCV infection, the monitoring of serum AFP level in those high-risk patients can be valuable for screening and monitoring of hepatocellular carcinoma.

infection with HBV. Immunological evidence of previous infection with the virus was found in the majority of patients with HCC. Chronic carriers of HBV are 390 times more prone to develop liver cancer than noncarriers.<sup>2</sup> The causal relationship has been strengthened by the finding that HBV DNA is integrated into the host cellular genome in a majority of liver cancers.<sup>8</sup> Since the recent discovery of hepatitis C virus (HCV) in 1989, several lines of evidence indicated that this

virus also plays a very important role in the development of hepatocellular carcinoma, especially in the area that HBV is not endemic.<sup>9,10</sup> Concurrent HBV and HCV infec-

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tion determines the highest risk of developing hepatocellular carcinoma.<sup>11</sup> However, despite many attempts to relate HBV and HCV genes and their products to neoplastic transformation, the relationship of HBV and HCV and the transformation of hepatocytes at the molecular level is still not clearly understood.

Screening of populations at risk of developing hepatocellular carcinoma, especially those with chronic hepatitis B, is the most cost-effective approach in the early detection of HCC. The current screening method used in several countries employs both abdominal ultrasonography and measuring serum alpha-fetoprotein (AFP).<sup>12</sup> AFP, a single-chain glycoprotein (alpha-globulin) with a molecular size of 68 kDa, is a major serum protein in human fetus and reaches a peak level at 3 months of gestation. After 1 year of age, AFP concentration declines to the level considered normal throughout the remainder of life.<sup>13</sup> Serum level of AFP can be significantly elevated in patients with HCC, germ cell tumours and occasionally gastric and pancreatic cancers. Elevated serum AFP is a useful indicator of advanced liver tumour or of recurrence of this tumour after treatment.<sup>14</sup> The diagnostic value of AFP as a tumour marker is however limited by the fact that elevated serum levels are also found in other non-cancerous liver diseases.

Liver cancer is the most common malignancy in Thailand, with an estimated incidence of 40.5 per 100,000 in males and 16.3 per 100,000 in females.<sup>15</sup> It can be estimated that more than 11,000 new cases of liver cancer are diagnosed each year. The majority of liver cancer patients in Thailand were diagnosed at the advanced, inoperable stage of the disease. Appropriate screening and diagnostic tools will certainly improve the survival rate of these

patients. In the endemic area of both HBV and HCV such as in Thailand, the value of alpha-fetoprotein as a diagnostic marker for detecting hepatocellular carcinoma in patients with HBV and HCV has not been evaluated.

## MATERIALS AND METHODS

### Specimens

Serum samples from 72 patients with histopathologically confirmed diagnosis of hepatocellular carcinoma, attending a large medical centre, Siriraj Hospital, between 1994-1995, were used in this study. The sera were collected from the patients prior to surgical treatment or chemotherapy and were stored at -20°C until use.

### Hepatitis B surface antigen

Assay for hepatitis B surface antigen (HBsAg) was carried out using an HBsAg ELISA kit (Genelabs Diagnostics, USA), following the manufacturer's instruction.

### Antibody to hepatitis C virus

Antibody to HCV was detected using a third-generation anti-HCV ELISA (Murex Diagnostics, UK) composed of antigens from both the structural and nonstructural regions and was performed according to the manufacturer's protocol.

### Detection of HCV RNA and HBV DNA

Detection of HCV RNA was performed using a nested reverse transcription polymerase chain reaction (RT-PCR) as previously described<sup>16</sup> with some modifications. Briefly, total RNA was extracted from 100 µl of serum using a modified acid guanidinium thiocyanate-phenol-chloroform method, and was reverse transcribed into cDNA. Two pairs of oligonucleotide primers derived from conserved sequences in the 5'-noncoding region of HCV genome, which can amplify all the genotypes and subgenotypes of HCV known to date, were used in

the nested PCR reaction, each round comprised of 35 cycles. The PCR products after 2 rounds of amplification were 300 base-pairs in length.

Assays for HBV DNA were carried out using a single-step polymerase chain reaction approach. DNA was extracted from 20 µl of serum samples from HCC patients using a modified proteinase K digestion technique.<sup>17</sup> The viral DNA was subjected to 30 cycles of amplification using a pair of primers derived from the conserved region of the nucleocapsid gene of HBV genome. The PCR-amplified product was 257 base-pairs in length.

### Alpha-fetoprotein

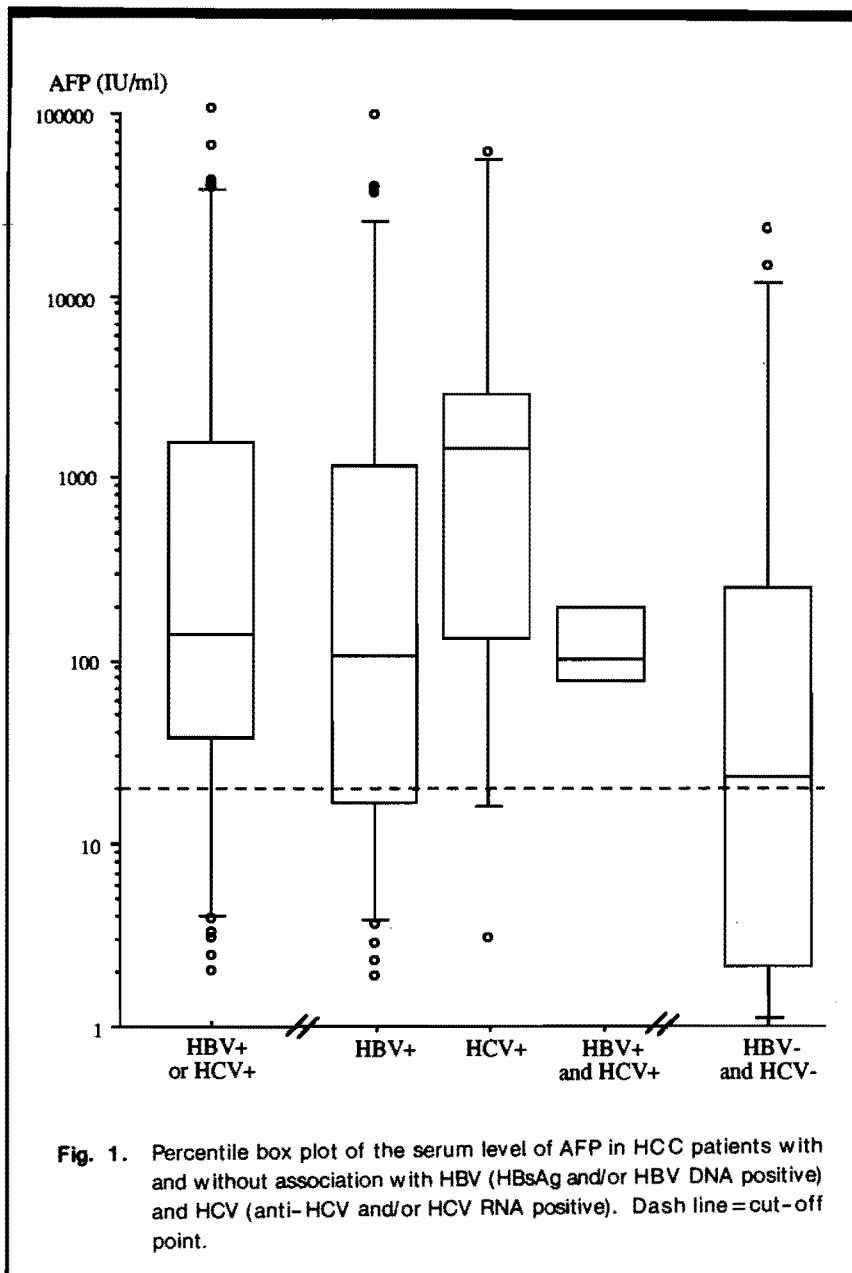
Serum alpha-fetoprotein was measured using an automated Cobas Core AFP EIA system employing a sandwich-type, solid-phase enzyme immunoassay (Roche Diagnostics, Switzerland). Elevated serum AFP was defined as serum AFP level higher than 200 IU/ml (equivalent to 25 ng/ml).

## RESULTS

### Markers for hepatitis B and hepatitis C infection

Of 72 patients with histopathologically confirmed hepatocellular carcinoma, 45 patients had HBsAg and 9 had antibody to hepatitis C virus. Three patients also had both HBsAg and anti-HCV in their sera. Twenty-one HCC patients had neither HBsAg nor anti-HCV. HBV DNA was found in 24 of 45 patients (53.3%) with HBsAg, and HCV RNA was found in 6 of 9 patients (66.7%) with anti-HCV. Interestingly, 4 of 21 patients with neither HBsAg nor anti-HCV had HCV RNA in their sera.

A total of 45 patients had either HBsAg or HBV DNA or both. Thirteen patients had either anti-HCV antibodies or HCV RNA or both, and 17 patients had neither HBsAg, HBV DNA, anti-HCV antibody nor HCV RNA (Table 1).



### Serum alpha-fetoprotein level

Level of serum AFP in HCC patients ranged from 0.70 to 99,292.60 international units (IU)/ml (Fig. 1). Elevation of AFP above the cut-off limit of 20 IU/ml was found in only 50 of 72 patients with hepatocellular carcinoma (69.4%). 75.6% of HCC patients with HBsAg had elevated level of AFP, compared to 88.9% of patients with anti-HCV (no statistical difference). Similarly, elevated AFP was found in 79.2% of patients with HBV DNA and in 80.0% of those with HCV RNA. All 3 patients with both HBsAg and anti-HCV antibody had elevated serum AFP. However, only 58.8% of patients with neither HBsAg, HBV DNA, anti-HCV nor HCV RNA had elevated serum AFP. The results are summarized in Table 2. The elevation of serum AFP was observed more commonly in HBV- and HCV-associated liver cancer patients than in those not associated with the two viruses ( $p < 0.05$ ).

### DISCUSSION

Although the mechanism of hepatocarcinogenesis is not well understood, hepatitis B and hepatitis C viruses are well regarded as the major causative agents of hepatocellular carcinoma. The importance of HBV or HCV involvement in the development of primary liver cancer however varies in different geographical regions. In areas where HBV

**Table 1.** Summary of HCC patients with HBV, HCV or neither marker.

	HBsAg and/or HBV DNA-positive	anti-HCV and/or HCV RNA- positive	Negative for HBsAg, HBV DNA, anti-HCV and HCV RNA
Number of patients	45	13	17
Median age (years)	49	56	54
Age range (years)	27-70	34-69	4-74
Sex ratio (male : female)	5.4 : 1	12 : 1	3.2 : 1

**Table 2.** Serum AFP in patients with HBV-associated and HCV-associated hepatocellular carcinoma.

	Patients with elevated AFP	
	Number of patients (positive/tested)	Percent
HCC patients, all groups	50/72	69.4
HBsAg-positive	34/45	75.6
anti-HCV-positive	8/9	88.9
HBV DNA-positive	19/24	79.2
HCV RNA positive	8/10	80.0
Negative for HBsAg, HBV DNA, anti-HCV and HCV RNA	10/17	58.8

is non-endemic, such as in Japan or Western Europe, HCV infection is more significant than HBV infection in causing primary cancer.<sup>18,19</sup> In the contrary, in areas endemic for both HBV and HCV, patients with HCC usually have markers for HBV infection and not those for HCV.<sup>20</sup> The study presented herein demonstrated that previous infection with HBV is more important than that with HCV in Thai patients with primary hepatocellular carcinoma. HBsAg was found in 62.5% of HCC patients whereas anti-HCV antibody was detected in 12.5% of these patients. It is not known whether the concurrent infection with both HBV and HCV had higher risk of HCC compared to other groups since only 3 of 72 patients (4.2%) had both HBsAg and anti-HCV antibody.

Recent studies have recommended the use of AFP as a tumour marker for screening of liver cancer cases.<sup>12</sup> However, the cost-effectiveness of the mass screening program for AFP level in general population should be carefully analysed since this tumour marker, like others, can also be elevated in non-neoplastic diseases.<sup>21</sup> Furthermore, as shown in this study, only 69.4% of

patients with histopathologically confirmed hepatocellular carcinoma had elevated serum AFP level. Mass screening of AFP in general population will miss approximately 30% of patients with hepatocellular carcinoma.

The elevation of serum AFP was more common in HCC patients with association to either HBV or HCV (75.6 and 88.9%, respectively), when compared to those without these markers (58.8%) ( $p < 0.05$ ). No difference was found in the elevation of AFP in patients associated with HBV or HCV. Patients with HBV DNA or HCV RNA, which indicated the state of active replication of the viruses, did not have higher percentage of AFP elevation compared to those with only HBsAg or only anti-HCV antibody. This may imply that HCC can develop long after the initial viral infection and no active viral replication is required for hepatocarcinogenesis or for the elevation of serum AFP. The prevalence of elevated serum AFP level in HCC patients with antibodies to HCV in this study (88.9%) was higher than that in Europe (56.1%)<sup>9</sup>, but slightly lower than that in Taiwanese (91.5%).<sup>14</sup>

In summary, alpha-fetoprotein

is not a sensitive neoplastic marker for mass screening of HCC in the general population, especially in those with no association with HBV and HCV. However, the monitoring of serum AFP level in those patients who are considered to be at high risk of HCC as judged by evidence of HBV or HCV infection (HBsAg, HBV DNA, anti-HCV antibody and HCV RNA) may be useful for early detection of primary liver cancer.

#### ACKNOWLEDGEMENTS

This work was supported by the National Science and Technology Development Agency of Thailand, the Anandha Mahidol Foundation, China Medical Board and Mahidol University. We acknowledge Ms Montana Neelamek, Mr Somkiat Udnan, and Ms Kornwika Worawattananon for laboratory assistance.

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