

## ALPHA-FETOPROTEIN IN HEPATOCELLULAR CARCINOMA: A SEROLOGICAL AND HISTOCHEMICAL STUDY IN MALAYSIAN PATIENTS

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### Summary

Serum alpha-fetoprotein (AFP) levels and its expression in liver tissue was studied in 50 cases of histologically confirmed hepatocellular carcinoma (HCC). Serum AFP levels were elevated ( $>20$ iu/ml) in 35/50 (70%) of the cases, 28 of whom had levels greater than 500 iu/ml, which is highly suggestive of HCC. These results indicate that serum AFP, by itself, is a relatively insensitive diagnostic test for HCC. Although elevated levels in high risk patients provide a specific clue, a negative result does not exclude the diagnosis of HCC. Expression of AFP by tumour cells paralleled that of serum in the majority of cases. However, tissue AFP was negative in 7 patients who had markedly elevated serum AFP. This observation may be a reflection of preferential excretion of the tumour antigen or differential expression of the antigen by the tumour cells. None of the patients with normal serum AFP demonstrated a reaction for tissue AFP. There was no correlation between AFP production and tumour differentiation.

Keywords: Alpha-fetoprotein, hepatocellular carcinoma, serum, tissue.

### INTRODUCTION

Alpha fetoprotein (AFP) is a glycoprotein normally produced in large quantities during embryonic life in the foetal yolk sac and liver. In the human embryo, serum AFP reaches a peak of about  $3 \times 10^6$  iu/ml at 12-13 weeks. Thereafter, its level declines, reaching a concentration of about  $5 \times 10^4$  iu/ml at birth and adult levels ( $<20$ iu/ml) within 3-4 weeks. Synthesis of AFP increases in a variety of diseases, markedly so in malignant tumours of childhood such as hepatoblastoma, in hepatocellular carcinoma (HCC) and in certain germ cell tumours.

Hepatocellular carcinoma is one of the commonest malignancies among males in Malaysia. Diagnosis is usually based on clinical findings and radiological and biochemical investigations. Among the various biochemical markers employed, AFP is the most widely used. The sensitivity of AFP in the early diagnosis of HCC varies with different reports and appears to be related mainly to the level chosen as the cut-off value.<sup>2-6</sup> To evaluate the usefulness of serum AFP as a tumour marker in our HCC patients, we measured serum AFP in 50 cases of histologically confirmed HCCs. The tumour tissues from these patients were stained for AFP and the histological grade determined to assess the relationship between serum AFP and the presence of tissue AFP as well as tumour differentiation.

### MATERIALS AND METHODS

Fifty consecutive cases of histologically confirmed HCC admitted to the University of Malaya Teaching Hospital between 1982-1986 were studied. Their serum were analysed for AFP levels using radioimmunoassay (Abbott Laboratories, Chicago Ill) at the time of diagnosis. AFP levels below 20iu/ml were classified as normal and those above 500iu/ml were arbitrarily taken to be diagnostic of HCC.<sup>3,7</sup> Liver biopsies obtained from these patients were examined for the presence of AFP immunohistochemically by the peroxidase-anti-peroxidase (PAP) technique using anti-AFP antisera purchased from Dakopatts. The histological type and differentiation was classified based on WHO recommended criteria.

### RESULTS

Figure 1 shows the distribution of serum AFP levels in the HCC patients studied. AFP levels were elevated in 35/50 (70%) of the patients, 28 of whom had levels ranging from 500 to over 10,000 iu/ml.

Tissue AFP was demonstrable in the tumour cells of 19 (38%) of the biopsies examined. In all instances, the AFP was located in the cell cytoplasm. All cases who showed presence of tissue AFP had markedly raised levels of serum AFP and all except one had moderately or well differentiated tumours.

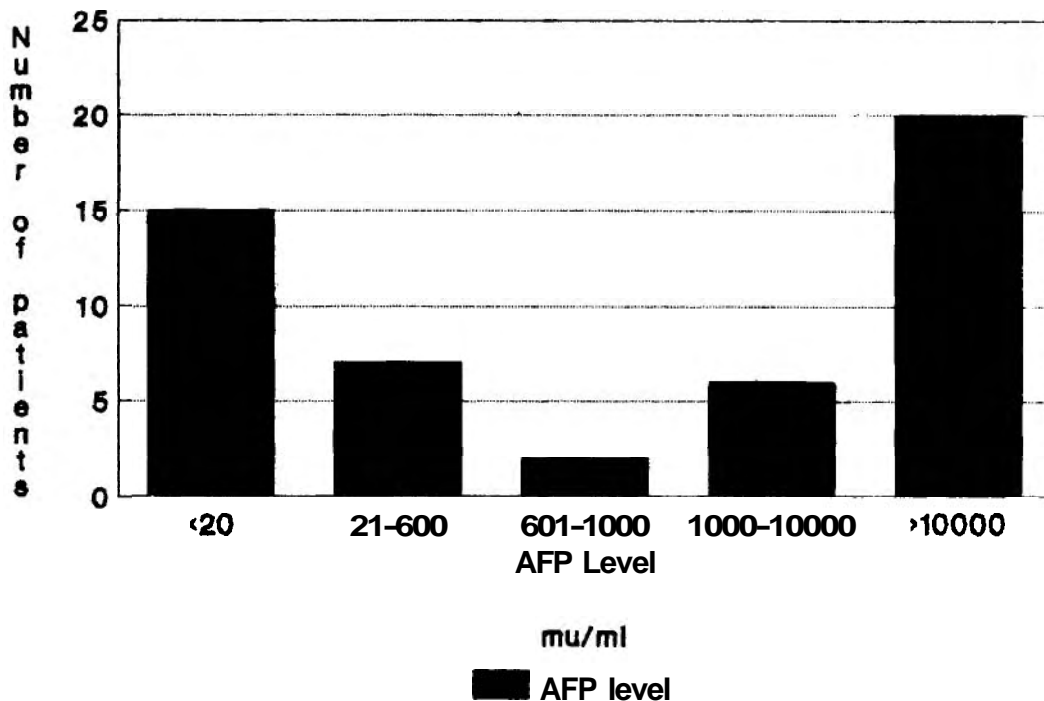


FIG. 1 : Serum AFP levels in HCC patients

None of the patients with serum AFP levels below 1000 iu/ml expressed AFP in their tissues. However, 4 cases with AFP levels between 1000 and 10,000 iu/ml and another 3 cases with levels above 10,000 iu/ml were also non reactive for tissue AFP.

Table 1 summarises the relationship between serum AFP and histological differentiation of the HCC. There was no correlation between the histological type of HCC, tumour differentiation and AFP production.

**DISCUSSION**

Although AFP is widely used as a biochemical marker of liver cell cancer, its usefulness in the early detection and diagnosis of this malignancy is limited by several factors. While markedly elevated levels are diagnostic of HCC, a significant proportion of patients

with HCC either do not have raised levels or have only mildly raised levels.<sup>8</sup> Serum AFP levels below 500 iu/ml are commonly seen in patients with liver diseases other than HCC, particularly in acute and chronic viral hepatitis.<sup>9,10</sup> Spontaneous reductions of serum AFP levels have also been reported in the early stages of malignant disease."

Our data shows that just over half (56%) of the HCC patients had levels of AFP diagnostic of the lesion (>500iu/ml). An appreciable number (30%) of patients had AFP levels within the normal range (<20iu/ml), while the remaining 14% of patients had levels that are often seen in benign liver disorders as well as in metastatic tumours in the liver. To improve the diagnostic value of biochemical markers in the latter two categories of patients, it is necessary for other tests to be used in addition to serum AFP.

**TABLE 1**  
**SERUM AFP LEVELS AND TUMOUR DIFFERENTIATION**

AFP level	Tumour grade			Total
	Well differentiated	Moderately differentiated	Poorly differentiated	
<20	4 (27)	10 (67)	1 (7)	15
21-500	3 (43)	3 (43)	1 (14)	7
>500	3 (11)	23 (82)	2 (7)	28

( ) = %

Various approaches have been suggested for improving the specificity and sensitivity of biochemical tests for the detection of HCC. These include (1) the use of a combination of biochemical markers in addition to AFP, such as des-gamma-carboxyprothrombin<sup>12,13</sup> ferritin,<sup>14,15</sup> gamma glutamyl-transpeptidase isoenzymes<sup>16,17</sup> and variant alkaline phosphatase<sup>16,18</sup> and (2) the measurement of fucosylated AFP, a molecular variant of AFP.<sup>7,19,20</sup> Combined assays of serum alpha-1 antitrypsin and alpha-1 antichymotrypsin have also been reported to have a complementary value in the diagnosis of HCC, particularly in patients without increased serum AFP levels.<sup>21</sup> More recently, the potential use of antibodies to the Hepatitis B virus X-gene product (anti-HBx) has been raised based on an observed association between the detection of this antibody and the presence of an HCC.<sup>22,23</sup> However, the relative usefulness of these strategies remains to be confirmed.

Production of AFP by liver cancer cells can be demonstrated by using the peroxidase antiperoxidase technique. The reported incidence of detection of this oncofetal protein in tissues appear to parallel that in serum<sup>24,25</sup> In our study, tissue AFP was demonstrated in 38% of the patients, all of whom had markedly elevated levels of serum AFP. All but one of these patients had well-differentiated or moderately-well differentiated carcinomas. However, the majority of tumours with negative tissue reaction also belong to these histological grades indicating a lack of correlation between expression of AFP and the degree of differentiation of the tumour. Failure to demonstrate tissue AFP in patients whose serum AFP levels were less than 1000 iu/ml is not surprising and is a reflection of the lack of sensitivity of the immunohistochemical technique used. However, a negative reaction for AFP in the seven subjects who had markedly raised levels of AFP is interesting. There may be various reasons for this observation. It has been suggested that some tumours preferentially secrete or retain the tumour antigen.<sup>24</sup> In the former instance, a high serum AFP may be associated with low tissue levels. Differential levels of production by different tumour cell populations within the same patient is another possibility to be considered. None of the patients with normal serum AFP levels had demonstrable tissue AFP suggesting that tissue retention of AFP did not occur in these subjects.

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