Clinical Usefulness of Alpha-1-Antitrypsin in the Diagnosis of Hepatocellular Carcinoma

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Serum levels of alpha-1-Antitrypsin(AAT) were determined in 42 patients with hepatocellular carcinoma(HCC), 5 patients with metastatic liver cancer from stomach adenocarcinoma, 10 patients with liver cirrhosis, 10 patients with chronic hepatitis, and 66 controls by rocket immunoelectrophoresis using rabbit antiserum. The mean level of serum AAT was 225.5 ± 73.0 mg/dl in 66 controls. The serum AAT in patients with HCC was 428.7 ± 123.3 mg/dl, which was significantly higher than those in the controls and in patients with liver cirrhosis or chronic hepatitis($p < 0.02$). The level of AAT in metastatic liver cancer was similar to that in HCC. The positive cut-off value for elevation of serum AAT in this study was determined as above 445 mg/dl, the mean plus 3 standard deviations in the controls. Elevations of serum AAT were observed in 54.8%, 60.0%, and 10.0% of patients with HCC, metastatic liver cancer, and liver cirrhosis, respectively, while none of the patients with chronic hepatitis or the controls was positive. The serum AAT levels in 42 patients with HCC were analyzed with regard to sex, age, serum albumin, HBsAg, alpha-fetoprotein(AFP), and diameter of HCC, with no significant differences being observed between these factors and the serum AAT levels except for the diameter of the HCC. The positive rate in the HCC with a diameter of 10 cm or more was 74.1%, which was a significantly higher rate compared with 20.0% in the HCC with diameters less than 10 cm. The positive rate of AFP for HCC was 61.9%, when 500 ng/ml of AFP was used as the cut-off value. The positive rate of serum AAT in HCC patients whose serum AFP was below 500 ng/ml was 43.8%, suggesting that the diagnostic sensitivity for HCC be increased by the simultaneous measurement of AAT with AFP to 78.6%. In this study, the sensitivity, specificity, false-positive, and false-negative results of serum AAT for the diagnosis of HCC were 54.8%, 95.0%, 5.0%, and 45.2%, respectively. These results suggest that the measurement of serum AAT is a useful tumor marker for diagnosis of the development of HCC, particularly in follow-up patients with chronic liver diseases.

Key Words : Serum alpha-1-antitrypsin, Tumor marker, Hepatocellular carcinoma

INTRODUCTION

Hepatocellular carcinoma(HCC) is the second most common cause of cancer death next to stomach cancer in the Korean male population(Ministry of Health and Social Affairs, 1989). The natural course of untreated HCC is very poor, with a mean survival time being reported to be about 4 months after onset of the initial symptoms and 2 months after admission(Nagasue et al., 1984). In recent years, the survival rate has been increasing because of the development of therapeutic modalities, such as hepatic resection(Nagasue and Yukaya, 1989), hepatic artery infusion of chemotherapeutic agents(Miura, 1989), and transcatheter arterial chemoembolization(Yamada et al., 1983), and increased detection of small-sized HCC(Okuda, 1986).

Numerous previous reports have clearly demonstrated that liver cirrhosis(LC) and chronic hepatitis(CH) are closely related to the development of HCC in Korea: LC was found in 70 to 8 % of HCC, and CH was found in about 10%(Chung et al., 1983; Jung and Kim, 1988). Most of these 2 benign liver diseases developed following the infection of hepatitis B virus in Korea(Suh, 1982), suggesting that careful and frequent follow-up of patients with LC and CH may increase the detection rate of small HCCs.

Imaging techniques, such as ultrasonography and computed tomography, have recently been playing a major role in the detection of HCC. However, it has often been difficult to carry out ultrasonography or computed tomography regularly. Consequently, much attention has been paid to biochemical tumor markers, because tumor markers are much easier to determine frequently and regularly compared with imaging tests.

Alpha-fetoprotein(AFP) has been one of the most widely used tumor markers for the

diagnosis of HCC. Although AFP has been proven to have a considerably high sensitivity and specificity for HCC, the clinical usefulness of AFP has been limited by the presence of AFP non-producing HCC(Waldmann and McIntire, 1974). Furthermore, the existence of small potentially curable HCCs has not been frequently suspected by the measurement of serum AFP alone(Okuda, 1986). Therefore, it seems imperative to develop new tumor markers with high sensitivity and specificity for HCC to improve the detection of HCC in the early stages especially in high-risk patients.

Alpha-1-antitrypsin(AAT) is one of the potent inhibitors of proteolytic enzymes naturally existing in serum and considered to be a potentially useful tumor marker for HCC(Chio and Oon, 1979; Koh et al., 1988). The clinical usefulness of AAT, however, in predicting the existence of HCC has not been fully established. The main purpose of this study is to investigate whether or not the measurement of serum AAT levels is clinically useful in detecting the development of HCC in patients with chronic liver disease. In this study, we have demonstrated that serum AAT is a useful tumor marker particularly in detecting low AFP-producing HCC.

MATERIALS AND METHODS

Entered into this study and admitted to the Korea Cancer Center Hospital between June 1990 and October 1990 were 42 patients with previously untreated HCC(31 males and 11 females) and 5 with metastatic liver cancer(3 males and 2 females), along with 10 patients with LC(7 males and 3 females), 10 patients with CH(8 males and 2 females), and 66 healthy persons(40 males and 26 females). The median ages(range) of the patients with HCC, metastatic liver cancer, LC, CH, and controls were 52(25-75), 61(32-70), 48(40-55), 48(21-61), and 51(21-67) years, respectively.

Diagnosis of HCC, LC, and CH was made on the basis of clinical and cytologic findings. Patients having a liver mass in computed tomography and 500 ng/ml or more of serum AFP were diagnosed as HCC. HCC was con-

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firmed by percutaneous aspiration cytology when the patient had a liver mass in Computed tomography with less than 500 ng/ml of serum AFP. LC was diagnosed when the patient with abnormal liver function had evidence of considerable portal hypertension, such as ascites, esophageal varix, and splenomegaly, etc. Controls were selected from healthy persons who had undergone a routine medical examination in the Korea Cancer Center Hospital during the study period. All 5 patients with metastatic liver cancer were metastasized from adenocarcinoma of the stomach.

Sera were obtained before the initiation of chemotherapy or surgery in patients with HCC and metastatic liver cancer. The assay system used in the present study was essentially the one described by Laurell(1972), but with some modifications. Serum levels of AAT were determined by rocket immunoelectrophoresis using antiserum prepared by the R & D Center, Kukje Pharma. Ind. Co. Ltd. Antiserum to human AAT, purified from pooled human blood, was obtained from rabbits immunized with AAT. Gel plates for rocket immunoelectrophoresis were made by mixing purified AAT, at a concentration of 1.5%, with 40 mM of tris-acetate, 2mM of EDTA, and 1% of agarose. After adding 5 μ l of the patient's serum and 3 standard solutions of AAT to each well of the gel plate, electrophoresis was performed at 15 volt/cm at room tem-

perature for 2 hours. The level of AAT(mg/dl) was calculated in the standard curve with the height of the precipitate formed in the gel plate. AFP and HBsAg were determined by radioimmunoassay using GammaDab[¹²⁵I] alpha-fetoprotein radioimmunoassay kit(Baxter & Clinical Assay, USA) and Ausria II-125 kit (Abott, USA). Student's t-test and Chi-square test were used to evaluate the significance of the difference between the results.

RESULTS

THE level of serum AAT was 225.5 $\pm$ 73.0 mg/dl(mean $\pm$ SD) in 66 healthy controls(Table 1). There was no significant difference in serum AAT levels between male and female. The level of AAT in the age group of 40-49 was higher compared with those of 20-39 and 50-59(p<0.01). In this study, the value above the mean plus 3 standard deviations in the controls(445 mg/ml) was considered as positive cut-off value for the elevation of serum AAT.

The level of serum AAT in patients with HCC was 428.7 $\pm$ 123.3 mg/dl(mean $\pm$ SD), which was significantly higher than those in the controls and in patients with LC or CH(p<0.02), while the serum AAT level in metastatic liver cancer was similar to that in HCC(Table 2). Of 42 patients with HCC, 23(54.8%) were positive for serum AAT, while only one(10.0%) out of 10 with LC and none(0%) out of 10 with CH was positive (Fig. 1). In 5 patients with metastatic liver can-

Table 1. Serum levels of alpha-1-antitrypsin in controls

	No. of Patients	Age (years)	AAT (mg/dl)	No. Positive	% Positive
Total	66	46.6 $\pm$ 11.6*	225.5 $\pm$ 73.0*	0	0
Male	40	49.0 $\pm$ 11.2	225.9 $\pm$ 76.0	0	0
Female	26	43.0 $\pm$ 11.1	225.0 $\pm$ 68.0	0	0
Age (years)					
20-39	19	-	199.5 $\pm$ 40.5	0	0
40-49	13	-	280.0 $\pm$ 58.5+	0	0
50-59	27	-	208.2 $\pm$ 79.8	0	0
60-69	7	-	262.1 $\pm$ 70.9	0	0

*: Mean $\pm$ SD

+: Serum AAT levels in the age group of 40-49 was higher than those of 20-39 and 50-59(p<0.01).

Table 2. Serum levels of alpha-1-antitrypsin in patients with hepatocellular carcinoma, liver cirrhosis, chronic hepatitis, and metastatic liver cancer

	No. of Patients	Age (years)	AFP (ng/ml)	AAT (mg/dl)	No. Positive	% Positive
HCC	42	51.8 ± 10.2*	352 ± 210*	428.7 ± 123.3*	23	54.8
LC	10	47.7 ± 4.7	—	322.5 ± 107.4 ⁺	1	10.0 ⁺
CH	10	45.8 ± 12.1	—	288.0 ± 87.1 ⁺⁺	0	0.0 ⁺⁺
Met Ca	5	54.2 ± 14.1	—	470.0 ± 144.9	3	60.0

HCC : hepatocellular carcinoma, LC : liver cirrhosis, CH : chronic hepatitis, Met Ca : metastatic liver cancer from stomach adenocarcinoma

* : Mean ± SD
+ : Significantly different from the HCC : p<0.05
++ : Significantly different from the HCC : p<0.01

Fig. 1. Serum levels of alpha-1-antitrypsin in 66 controls, 10 chronic hepatitis(CH), 10 liver cirrhosis(LS), 42 hepatocellular carcinoma(HCC), and 5 metastatic liver cancer(Met Ca) patients. Levels greater than 445 mg/dl(mean + 3 SD of control level) were considered positive.

Fig. 2. Serum levels of alpha-1-antitrypsin according to the diameter of tumor mass in computed tomography (A) and serum alpha-fetoprotein (B) in 42 patients with hepatocellular carcinoma. Levels greater than 445 mg/dl (mean + 3 SD of control level) were considered positive.

cer, the mean AAT level was 470.0 ± 144.9 mg/dl, which was significantly higher compared with that in the controls(p<0.01), and the positive result was found in 3 patients(60.0%) (Table 2). The serum AAT levels in 42 patients with HCC were analyzed with regard to sex, age, serum albumin, HBsAg, AFP, and diameter of HCC, no significant difference being observed between these factors and serum AAT levels except for the diameter of the HCC(Table 3). The positive rate of AAT in HCC with diameters greater than 10 cm was 74.1%, which was significantly higher than those with diameters less than 10 cm(p<0.01) (Fig. 2).

The specificity of AAT for HCC was 95.0%,

with the false-positive and false-negative results being observed in 5.0% and 45.2%, respectively. The sensitivities for the diagnosis of HCC of AFP, when 200 and 500 ng/ml of AFP were used as the cut-off values, were 69.0% and 61.9% in 42 HCC patients: 60.0% and 40.0% in 5 patients with tumor size less than 5 cm: 70.0% and 60.0% in 10 patients with tumor size of 5.0-9.9 cm: and 74.1% and 66.7% in 27 patients with tumor size of 10 cm or larger. The positive rates of serum AAT in HCC patients whose serum AFPs were less than 200 and 500 ng/ml were 46.2% and 43.8%, respectively. These results suggest that by the simultaneous measurement of AAT and AFP, the diagnostic sensi-

Table 3. Effects of sex, age, albumin, HBsAg, alpha-fetoprotein, and diameter of tumor mass on serum levels of alpha-1-antitrypsin in patients with hepatocellular carcinoma

	No. of Patients	Age (years)	Diameter (cm)	AFP (ng/ml)	AAT (mg/dl)	No. Positive	% Positive
HCC	42	51.8±10.2*	9.3±4.1*	352±210*	428.7±123.3*	23	54.8
Sex							
male	31	50.7± 9.0	9.7±4.5	361±210	446.6±117.4	19	61.3
female	11	55.2±12.4	8.3±2.3	328±211	378.2±125.3	4	36.4
Age							
20-39	6	—	8.0±3.1	400±179	402.5±93.8	3	50.0
40-49	10	—	8.8±2.0	411±168	471.5±109.6	7	70.7
50-59	16	—	9.3±4.9	355±220	418.8±137.0	7	43.8
60-79	10	—	10.5±4.2	260±218	417.5±118.1	6	60.0
Albumin							
≥3.5	30	51.4±11.0	8.5±2.5	349±214	415.3±124.9	15	50.0
<3.5	12	52.8± 7.8	11.4±5.9	359±202	462.1±112.4	8	66.7
HBsAg							
(+)	29	51.2± 8.9	9.2±4.7	403±181	431.4±123.7	16	55.2
(-)	13	53.2±12.5	9.5±2.0	238±226	422.7±122.0	7	53.8
AFP(ng/ml)							
≥500	26	50.9± 8.7	9.5±3.3	—	435.0±115.8	16	61.5
200-499	3	62.0±10.8	8.3±2.4	—	435.0±74.3	1	33.3
<200	13	51.5±11.5	9.1±5.4	—	414.6±143.9	6	46.2
Diameter of Mass (cm)							
<5.0	5	52.2±10.0	—	296±213	373.0±144.3	1	20.0
5.0-9.9	10	52.1± 7.9	—	349±223	354.0±95.0	2	20.0
≥10	27	51.7±11.0	—	363±203	466.7±111.1	20	74.1 ⁺

* : Mean ± SD

+ : The positive rate was higher in patients with HCC greater than 10 cm in diameter compared to those with less than 10 cm ($p<0.01$).

tivity for HCC can be increased to 83.0% and 78.6%, when 200 and 500 ng/ml of AFP were used as the cut-off values, respectively.

DISCUSSION

Although AFP has been widely used as a tumor marker for HCC, the positive rate of AFP in Korean patients with HCC has been reported to be about 50-80%(Chung et al., 1983; Jung and Kim, 1988), suggesting that, in the remaining 20-50% of patients, the development of HCC cannot be deduced by the measurement of serum AFP. The elevation of serum AFP level is highly associated with the type of tumor cells, AFP-producing clones, consisting of HCC and some associated with the characteristics of

cells including poor differentiation and fast growth(Okuda, 1986).

Clinical application of AFP has clearly contributed to the early detection of HCC. However, many patients have still not been cured mainly because the majority of HCCs have been detected in the advanced stage. In addition to the presence of AFP non-producing HCC, AFP is not specific for HCC(Bates and Longo, 1985). Serum AFP was reported to be at the levels of above 40 ng/ml in about 75% of patients with testicular teratocarcinoma or embryonal cell cancer. The level of AFP was also reported to be increased in sera of some patients with stomach cancer, pancreatic cancer, colon cancer and lung cancer(Waldmann and McIntire, 1974). Furthermore, serum AFP was elevated moderately in 10-25% of benign liver diseases,

such as LC and acute and chronic hepatitis (Bates and Longo, 1985; Di Bisceglie and Hoofnagle, 1989). Therefore, if a tumor marker indicating the existence of HCC with a high sensitivity and specificity is identified, it will be much easier to detect HCCs in the early stages, especially in follow-up patients with high-risk diseases such as LC and CH.

AAT, named for its identification as an alpha-1 globulin and a potent inhibitor of trypsin, is a serum glycoprotein with 394 amino acids and a molecular weight of about 54,000 (Chan and Rees, 1975; Carrell et al., 1982). Serum AAT is synthesized and cleared by catabolization mainly in the liver cells with a half-life only about a week in serum (Laurell et al., 1977). About 90% of trypsin activity is inactivated or neutralized by AAT (Kahn et al., 1977). AAT is also an inhibitor of several serine proteolytic enzymes, such as chymotrypsin, elastase, thrombin, plasmin, collagenase, renin, and urokinase (Morse, 1978), which are the enzymes responsible for blood coagulation, fibrinolysis, metabolism of connective tissue, and processes of inflammation and immunologic reactions (Travis and Salvesen, 1983). AAT was reported to be elevated in the serum (Chio and Oon, 1979; Koh et al., 1988) and the tumor tissue of patients with HCC (Yoon et al., 1982; Chedid et al., 1990). The diagnostic significance of serum AAT as a tumor marker for HCC, however, is still not established but under investigation. In this study, the absolute value and positive rate of AAT were significantly higher in HCC, compared with those in patients with LC and CH.

The exact mechanism and role of increased AAT in HCC is unclear at the present time. Elevation of AAT is caused by increased production mainly in the liver cells (Weissmann et al., 1970) and partly in the surrounding mononuclear cells infiltrating the tumor cells (Ikuta et al., 1982), although there is also some evidence that tumor cells produce AAT (Reintoft and Hagerstrand, 1979). There has been considerable evidence that the lysosomes of tumor cells and inflammatory cells surrounding tumor cells produce the proteases, resulting in the subsequent enhanced synthesis of AAT in the liver cells to compensate for the activity of proteases (Weissmann et al., 1970). On the other hand, serum AAT may be elevated as a kind

of non-specific acute phase reactant responding to inflammation accompanying the progression of the tumor (Bates and Longo, 1985). In view of this, the enhanced production of AAT is generally regarded as one of the defense mechanisms of the body against cancer growth.

Elevated levels of AAT are not specific for HCC. It has been reported that AAT is significantly elevated in the sera of patients with a variety of malignant diseases, such as stomach cancer, colon cancer, pancreatic cancer, breast cancer, and lung cancer (Bernacka et al., 1988; Tountas et al., 1985; Roberts et al., 1975; Harris et al., 1974). We have also demonstrated that the mean levels of serum AAT were significantly elevated in patients with HCC, pancreatic cancer, cervical cancer, ovarian cancer, breast cancer, lung cancer, head and neck cancer, esophageal cancer, stomach cancer, and colon cancer. However, the diagnostic value of serum AAT as a clinically useful tumor marker was validated only in patients with HCC and pancreatic cancer (Hong et al., 1991). The elevation of serum AAT is also not specific for malignancies. Elevation of serum AAT was reported in certain inflammatory diseases (Wendt et al., 1984; Plusa and Tchorzewski, 1985) and in pregnancy (Kim, 1981).

These findings suggest that the measurement of AAT lacks satisfactory sensitivity and specificity to differentiate HCC from other kinds of diseases. Serum AAT is, however, thought to be a useful marker to diagnose the development of HCC in patients with LC and CH, because the positive rate was very low in patients with LC and CH, and 7 patients (43.8%) were positive in 16 HCC patients with low AFP level. In this study, the sensitivity of AFP was 61.9%, when the level of above 500 ng/ml was taken as the positive cut-off value. The sensitivity of AAT in the diagnosis of HCC was 54.8% and the diagnostic sensitivity can be increased to 78.6% by simultaneous determination of AFP in addition to AAT.

Serum AAT levels may be influenced by many factors. In an attempt to investigate the factors affecting AAT levels, we analyzed serum AAT levels with regard to sex,

age, serum albumin, HBsAg, AFP, and diameter of HCC. No significant correlations between these factors and serum AAT were found except for the diameter of the tumor. In this study, patients with an HCC less than 10 cm in diameter had a lower positive rate (20.0%) for AAT compared to those with more than 10 cm in diameter (74.1%). To draw a definitive conclusion about factors, serum AAT should be analyzed in a large number of patients.

Although the diagnostic application of serum AAT to small HCC is limited as AFP, the measurement of serum AAT is a useful tool for the diagnosis of HCC, especially in low-AFP producing HCC, and the combined measurement of AAT and AFP can improve the detection rate of HCC in the early stages, particularly in follow-up patients with chronic liver diseases.

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