Prediction of the Development of Hepato-Cellular-Carcinoma in Patients with Liver Cirrhosis by the Serial Determinations of Serum Alpha-L-fucosidase Activity

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Objective Evaluation of the usefulness of the serial determinations of serum alpha-L-fucosidase (AFU) activity for prediction of the development of hepatocellular carcinoma (HCC) was performed. **Methods and Patients** Serum AFU activity was determined monthly for 42 months in 73 patients with liver cirrhosis (LC). **Results** HCC was diagnosed in 27 patients by means of ultrasonography during this observation period. In 23 (85%) of the 27 patients, serum AFU activity was found to exceed 700 nmole/ml/h during the LC stage. HCC developed within a few years in 23 (82%) of 28 LC patients with AFU activity exceeding 700 nmole/ml/h, in contrast, it developed in only 4 (9%) of 45 LC patients with AFU activity below 700 nmole/ml/h. AFU activity was already elevated in 23 (85%) of 27 patients at least 6 months before the detection of HCC by ultrasonography. **Conclusion** It is conceivable that the development of HCC can be predicted by means of serial determinations of serum AFU activity in patients with LC. (Internal Medicine 38: 927-931, 1999)

Key words: exoglucosidase, tumor marker, early diagnosis of HCC

Introduction

Elevated levels of alpha-L-fucosidase (AFU) have been reported in patients with hepatocellular carcinoma (HCC) (1-8). We have reported the usefulness of the determination of serum AFU activity as a new tumor marker in the diagnosis of HCC (9). Previously, patients with an AFU activity exceeding 700 nmole/ml/h who had not developed HCC were regarded as false-positives; it later became evident that HCC developed at a high incidence in these patients. Therefore, it is conceivable that such cases are not false-positives but actually are in the very early stages of HCC. To substantiate this assumption, a prospective study is needed. The present study therefore, was conducted to evaluate the usefulness of serial determinations of serum AFU activity in LC patients for the prognostication of developing HCC. We attempted to clarify whether the development of HCC can be predicted from the dynamics of serum AFU activities in patients with LC, and if so, whether prediction by this method can be made earlier than the detection of HCC with ultrasonography.

Subjects and Methods

Serum AFU activities were determined at monthly intervals in 73 LC patients (HCC was not yet detectable by ultrasonographies performed by 2 physicians with at least 10 years of experience with abdominal ultrasonographical diagnostic techniques). Determinations were performed in the Clinic of Internal Medicine of Surugadai Nihon University Hospital, Tokyo, from April 1994 to September 1997.

These patients were followed by ultrasonography (Aloca) every 3 months for from 8 months to 3.5 years. Subjects consisted of 58 patients with C type LC, 12 with B type LC, and 3 with non-B non-C type LC. Serum AFU activity was assayed by a modified method of Zielke et al (10). In brief, 10 µl of serum was added to 100 µl of 1 mmole/L p-nitrophenyl-α-L-fucopyranoside dissolved in 150 mmole/l citrate-phosphate buffer (pH 5) containing 0.05 mg/ml BSA. This solution was incubated at 37°C for 1 hour, and the reaction was stopped by the addition of 100 µl of 0.2 N NaOH. The absorbance of p-nitrophenol was read at 405 nm, and the enzyme activity was expressed as nano-moles of p-nitrophenyl-α-L-fuco-pyrano-

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side cleaved per milliliter per hour at 37°C.

Statistical analysis

Data were analyzed by Student's unpaired t-test and differences were considered significant at $p < 0.05$.

Results

During the observation period, HCC developed in 27 out of 73 patients with LC.

In 23 (85%) of those 27 cases, AFU activities were already elevated up to 700 nmole/ml/h, during the LC stage. Though the incidence of HCC was very high in LC patients with an AFU activity exceeding 700 nmole/ml/h (23 (82%) of 28 patients), HCC developed in only 4 (9%) of 45 LC patients with AFU activity below 700 nmole/ml/h (Fig. 1).

Figure 2 shows the chronological changes in AFU activity in patients with LC who developed HCC, during the 42-month observation period. In 23 (85%) of 27 LC patients, AFU activity increased to 700 nmole/ml/h at least 6 months before HCC

was detected by ultrasonography. In the remaining 4 patients, AFU activity did not rise until HCC was detected. Elevation of AFU activity occurred 3 years or more before the detection of HCC in 4 patients, 2 years or more before in 7 patients, 1 year or more before in 17 patients, and 6 months or more before in 23 patients. AFU activity stayed at high levels after the initial elevation in 17 of 23 patients, but in the remaining 6 cases fell to less than 700 nmole/ml/h, and then rose again before the detection of HCC. In 3 of 4 patients who maintained low levels of AFU activity and accompanying HCC, AFU activity was below 700 nmole/ml/h, when the HCC was detected.

The dynamics of AFU activity in the LC patients who did not develop HCC during the observation period are shown in Fig. 3. Except for 3 of the 45 cases (indicated by ●—●), the AFU activities were maintained at low levels below 700 nmole/ml/h. Figure 4 shows the ultrasonographic images of HCC which had high levels of AFU before being diagnosed as HCC. Eleven (48%) of the 23 HCCs were solitary and less than 20 mm in diameter.

Discussion

AFU is an exoglucosidase which exists in the liver, kidney, and epididymis, of mammalian species and catabolizes the fucose-containing sugar proteins (11).

The mechanism of the elevation of serum AFU activity in patients with HCC is still obscure. One possibility is an increase in protein synthesis by the neoplasm with a consequent increase in fucose turnover (1). This possibility, however, is not supported by recent reports showing significantly low AFU levels in HCC tissue compared with nontumoral liver tissue (5), and similar levels of AFU activity in the culture supernatants of HCC cell lines and Chang liver cells (8).

It is well known that sugar chains of sugar proteins vary in the course of carcinogenesis. In hepatocarcinogenesis, for example, fucosylation of some sugar proteins increases, and this increase of fucosylation may lead to the elevated alpha-L-fucosidase activity.

Whether this speculation is true or not, serum AFU activity is significantly higher in patients with HCC than in those with LC and chronic hepatitis, and we have reported the sensitivity of serum AFU activity in the detection of HCC and its specificity of 96%, and 75% (cut-off level=700 nmole/ml/h). (unpublished personal data), this sensitivity is not sufficiently high to serve as a marker for HCC, however, HCC did develop in LC patients with a high AFU activity at a high incidence rate following the above report. These findings led us to add a prospective study for clarifying the relationship between serum AFU activity and the development of HCC in LC patients.

One of the purposes of this study was to detect when serum AFU activity starts to become elevated in patients with LC, a high risk group for HCC. Computed tomography, magnetic resonance imaging and ultrasonography have been used to detect HCC, with ultrasonography being the most practical and reliable technique to detect early HCC. However, it is still difficult to detect small isoechogenic HCC (12–15). Although al-

Figure 1. Relation between the incidence of development of HCC and AFU activity during the LC stage. The incidence of development of HCC was significantly higher in LC patients with an AFU activity exceeding 700 nmole/ml/h than in those with an AFU activity below 700 nmole/ml/h. ●: Accompanying HCC, ○: No accompanying HCC. AFU: alpha-L-fucosidase, HCC: hepatocellular carcinoma, LC: liver cirrhosis *: $p < 0.01$

Figure 2. Dynamics of AFU activities of LC patients who developed HCC. AFU activities increased up to 700 nmole/ml/h at least temporarily until HCC was detected in 23 of 27 LC patients who developed HCC. These elevations of AFU activities occurred at least 6 months before the detection of HCC. ●: The point of the initial elevation of AFU activity up to 700 nmole/ml/h, ★: The point of re-elevation of AFU activity up to 700 nmole/ml/h, ▲-▲: Cases in which HCC developed without elevation of AFU activity up to 700 nmole/ml/h. ○: The point of the initial detection of HCC by ultrasonography, -: before AFU: alpha-L-fucosidase, HCC: hepatocellular carcinoma, LC: liver cirrhosis, M: month(s), Y: year(s).

pha-fetoprotein (AFP) is the most useful marker for HCC, this marker is not very sensitive for the detection of early HCC (16, 17). It has been reported that there are some isoforms of AFP differentiated by its difference of sugar chains (18–20). It was recently reported that the AFP isoform, as analyzed by lectin affinity immunoblotting, is useful for preclinical diagnosis of HCC (21, 22). An AFP level exceeding 100 ng/ml is required to detect AFP isoforms, but AFP is usually not too highly elevated in the early stages of HCC.

In contrast, AFU activity is not related to the tumor size (8). Further, in this study, AFU activity rose before the HCC was detectable by ultrasonography in about 85% of the patients with LC associated HCC. LC patients with AFU activity exceeding 700 nmole/ml/h tended to develop HCC at a high incidence and in a short interval. Conversely, patients with LC who maintained low levels of AFU activity infrequently developed HCC.

It is unclear why AFU activity remained low in 3 cases of HCC. It may be due to changes of sugar chain of sugar protein

accompanying the development of HCC not occurring in only fucose.

The fact that 48% of the HCCs which were discovered by elevation of AFU in the LC stage were the so-called small hepatic cancer, strongly suggests the usefulness of monitoring AFU levels in LC patients in order to establish an early diagnosis of HCC.

In conclusion, serial determinations of serum AFU activity in patients with LC were helpful to predict the development of HCC. Physicians should recognize that patients with LC with high AFU activities are a super-high risk group for HCC and should perform diagnostic imaging studies at least once a month. If HCC is suspected in these patients, further examination (i.e. hepatic angiography, tumor biopsy etc.) must be performed. By doing so, HCC is likely to be detected at a very early stage, and it would be expected that the prognosis of HCC would improve.

Figure 3. Dynamics of AFU activities in LC patients who did not develop HCC. AFU activity levels remained at low levels during the observation period in all but 3 patients indicated by ●—●. 0: The point at the end of the observation period. —: before. AFU: alpha-L-fucosidase, HCC: hepatocellular carcinoma, LC: liver cirrhosis, M: month(s), Y: year(s).

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Figure 4. Ultrasonographic figures of HCC discovered by the elevation of AFU activity in the stage of LC. Eleven of the 23 HCCs were solitary and less than 20 mm in diameter, the so-called small hepatic cancer.

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