Decreased plasma fibronectin in liver diseases correlated to the severity of fibrotic, inflammatory and necrotic changes of liver tissue.

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Abstract

We applied a modified enzyme-linked immunosorbent assay (ELISA) for the measurement of human plasma fibronectin and determined the level of plasma fibronectin in 90 patients with various liver diseases and 10 normal subjects. Diagnoses were made by liver biopsy under peritoneoscopy. Plasma fibronectin was significantly decreased in liver cirrhosis patients, but not in acute hepatitis or chronic hepatitis patients. Decreased plasma fibronectin was correlated poorly with 18 laboratory tests, including liver function tests, and inflammatory marker determinations performed prior to peritoneoscopy. A correlation was found between the decreased plasma fibronectin and the severity of fibrotic, inflammatory and necrotic changes of the liver. These results suggested that the level of plasma fibronectin may reflect the severity of tissue injury resulting from chronic liver diseases.

KEYWORDS: plasma fibronectin, liver diseases, liver biopsy

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Decreased Plasma Fibronectin in Liver Diseases Correlated to the Severity of Fibrotic, Inflammatory and Necrotic Changes of Liver Tissue

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We applied a modified enzyme-linked immunosorbent assay (ELISA) for the measurement of human plasma fibronectin and determined the level of plasma fibronectin in 90 patients with various liver diseases and 10 normal subjects. Diagnoses were made by liver biopsy under peritoneoscopy. Plasma fibronectin was significantly decreased in liver cirrhosis patients, but not in acute hepatitis or chronic hepatitis patients. Decreased plasma fibronectin was correlated poorly with 18 laboratory tests, including liver function tests, and inflammatory marker determinations performed prior to peritoneoscopy. A correlation was found between the decreased plasma fibronectin and the severity of fibrotic, inflammatory and necrotic changes of the liver. These results suggested that the level of plasma fibronectin may reflect the severity of tissue injury resulting from chronic liver diseases.

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Fibronectin is a high molecular weight glycoprotein which can crosslink to collagen, fibrin, heparin, hyaluronic acid, gelatin and factor XIII (1, 2). Fibronectin is considered to have at least two molecular forms, cellular fibronectin and plasma fibronectin (3). Plasma fibronectin has been reported to act in blood coagulation, scar formation and opsonization before phagocytosis (4). A remarkable decrease in plasma fibronectin has been observed in patients with fulminant hepatitis (5). It has been reported that fibronectin might play an important role in the process of liver fibrosis, since the deposition of fibronectin in large amount was often found in fibrotic lesions (6). Although the production site of plasma fibronectin has not been fully understood, the hepatocyte is one of the candidates, since hepatocytes in culture is proved to synthesize fibronectin (7). From these evidences, it is considered that the liver might be an important organ involved in the metabolism of plasma fibronectin. Therefore, it would be important to investigate the level of plasma fibronectin in association with various liver diseases. Previously it was reported that plasma fibronectin was increased in some types of liver diseases such as recurrent cholestasis of pregnancy and liver cirrhosis (8). Recently many other researchers have studied the level of plasma fibronectin in patients with various liver diseases, but it is still an open question whether it is associated with histological changes of the liver, or not.

In this paper, we applied a modified enzyme-linked immunosorbent assay for mea-
suring plasma fibronectin and investigated
the level of plasma fibronectin in various
liver diseases, which were diagnosed histo-
logically.

Materials and Methods

Subjects. Ninety patients with various liver
diseases and 10 normal subjects were included
in the study. All the patients were admitted to the
Okayama University Medical School Hospital
during the period from May 1982 to March 1983.
Diseases were diagnosed histologically with a liver
specimen obtained by needle biopsy under perito-
neoscopy. Diseases included in this study were
acute hepatitis in the convalescent stage (13
cases), chronic hepatitis (40 cases), liver cirrho-
sis (17 cases) and other liver diseases including
alcoholic liver injury, fatty liver and chronic per-
sistent hepatitis (20 cases). The patients were all
free of bacterial infectious diseases while fibro-
nectin was being determined. The laboratory data
used in statistical analysis were obtained during a
week prior to the peritoneoscopic examination.
The laboratory tests included erythrocyte sedi-
mentation rate, C-reactive protein, total biliru-
bin, direct bilirubin, glutamate oxaloacetate trans-
aminase, glutamic pyruvic transaminase, alkaline
phosphatase, cholinesterase, γ-glutamyl transpept-
dase, thymol turbidity test, zinc sulfate turbidity
test, total protein, albumin, γ-globulin, A/G, total
cholesterol, fractional disappearance rate of indo-
cyanine green, prothrombin time.

Plasma sample. Three milliliters of blood
were collected by venipuncture and immediate-
ly transferred in a test tube containing 1.0 mg
EDTA. The plasma was separated by centrifu-
gation at 3000 rpm for 15 min and stored in a plastic
tube at −20°C until used.

Assay for plasma fibronectin. Plasma fibro-
nectin was determined by a direct enzyme-linked
immunosorbent assay (ELISA) modified from the
method reported by Rennard et al. (9). A micro-
titer plate (Nunc Immuno Plate II, Nunc Lab.)
was coated with anti-human fibronectin rabbit se-
rum (Cappel Lab.) diluted 1000 fold with 20 mM
carbonate buffer, pH 9.6, containing 0.02% so-
dium azide. The remaining active sites of the
plate were blocked with 1% ovalbumin in 20 mM
sodium phosphate containing 0.15 M sodium chlo-
ride (PBS). The plate thus prepared was used
as the immunosorbent. Plasma samples and stan-
dard fibronectin (Bethesda Research Lab,) serially
diluted with PBS were applied to the plate. Anti-
human fibronectin goat immunoglobulin-horseradish
peroxidase conjugate (Cappel Lab.) diluted 200
times with PBS were next applied as a probe for
detecting fibronectin bound to the plate. Color
development of the enzyme reaction was carried
out in a dark box using a substrate solution of
40 mg/ml of o-phenylenediamine and 0.01% 
H₂O₂ in 0.1 M phosphate buffer, pH 5.0, for
1 h. The reaction was stopped by adding 4N
H₂SO₄, and color development was read at 492 nm
with a spectrophotometer (Microelisa Mini Reader
MR590, Dynatech).

Peritoneoscopic study. Peritoneoscopy was
performed under local anesthesia. The distortion
from the normal structure to nodules of the liver
lobes was graded into 5 degrees according to
Shimada's code number system (10).

Histological study of liver-biopsy specimens.
Liver tissue was obtained by needle biopsy under
peritoneoscopy. The specimens were immediate-
ly fixed in Bouin’s solution, dehydrated by pass-
aging through an ethanol series, and embedded in
paraffin. The specimens were sectioned and rou-
tinely stained with hematoxylin-eosin and azan.
Fibrosis, cellular infiltration and necrosis were
graded into 4 degrees according to the degree of
severity. Grades 1, 2, 3 and 4 represented
“minimum”, “mild”, “moderate” and “severe”
changes, respectively. The severity of fibrosis
was determined by the width of the azan-stained
fibrous area in the interstitial space and the pre-
sence of portal-portal veins and portal-central vein
connections. Liver cirrhosis which showed all of
these findings was graded “severe”. The severity of
cellular infiltration was determined by the num-
ber of inflammatory cells in Glisson’s area and
the degree of destructive changes of the limiting
plate due to the infiltration of the inflammatory
cells. The severity of necrosis was determined
by the width and number of necrotic areas.

Results

An enzyme immunosorbent assay was de-
Alteration of Plasma Fibronectin in Liver Diseases

Fig. 1 Calibration curve for standard plasma fibronectin.

Fig. 2 Plasma fibronectin in patients with various liver diseases. AH, Acute hepatitis; CH, Chronic hepatitis; LC, Liver cirrhosis.

Fig. 3 Relationship between plasma fibronectin and lobular distortion. The lobular distortion in the peritoneoscopic pictures of the liver surface was classified according to Shimada’s code number system: 100, normal lobular structure with no fibrosis in Glisson’s capsule; 200, normal lobular structure with moderate fibrosis of Glisson’s capsule; 300, presence of a network formed by portal-portal and portal-central vein connections; 400, mounds or paving stone-shaped nodules; 500, spherical nodules. No cases with code number 500 were seen in this study.
acute hepatitis patients, $278.0 \pm 125.7 \mu g/ml$ for chronic hepatitis patients, $246.7 \pm 90.0 \mu g/ml$ for liver cirrhosis patients, and $275.7 \pm 94.8 \mu g/ml$ for patients with other liver diseases (Fig. 2). The liver cirrhosis group showed a significantly lower mean value than the normal subjects. The fibronectin of the acute hepatitis group was not significantly different from that of normal subjects. It was further investigated whether or not the decreased plasma fibronectin in patients with the liver diseases studied was related to any other clinical data. However, the plasma fibronectin was not significantly correlated to any laboratory data examined. Among the peritoneoscopic findings of the liver surface, including lobular distortion, size of the nodule and other markers of pathological changes of the liver, only the degree of the distortion of the liver lobes had a relationship to the level of plasma fibronectin. Fibronectin was lower in patients with more severely distorted liver as shown in Fig. 3. A greater degree of fibrosis, cellular infiltration and necrosis in the biopsy specimen were also found in patients with lower levels of fibronectin (Fig. 4a, b and c). These findings indicate that a significant decrease in plasma fibronectin in liver cirrhosis patients is correlated not only with fibrotic changes but also with inflammatory and necrotic changes of the liver tissue.

Discussion

Many types of immunoassay, such as electroimmunoassay (11), immunoturbidimetric assay (12), single radial immunodiffusion (13-15), radioimmunoassay (16) and enzyme immunoassay (17, 18), have been used for determining the level of plasma fibronectin. We have developed an enzyme-linked immunosorbent assay and determined the level of plasma fibronectin in patients with various liver diseases diagnosed histologically. The assay method was sensitive enough to follow the concentration of plasma fibronectin throughout the clinical course with
a small plasma sample.

We found that plasma fibronectin was significantly decreased in liver cirrhosis patients, but not in patients with acute hepatitis, chronic hepatitis or other liver diseases including alcoholic liver injury, fatty liver and persistent hepatitis when compared with that of normal subjects. Matsuda et al. reported that plasma fibronectin was high in patients with acute hepatitis, chronic hepatitis and liver cirrhosis, except decompensated cirrhosis (13). On the contrary, Kojima reported decreased plasma fibronectin in acute hepatitis, chronic hepatitis and liver cirrhosis patients (14). Our results were not consistent with either report. The acute hepatitis patients in our study were all in the convalescent stage and showed normal plasma levels, but several cases of acute hepatitis with severe jaundice and a prolonged prothrombin time showed a low plasma fibronectin level (data not shown).

We investigated the reason for decreased plasma fibronectin in peritoneoscopic and histological studies. Our results showed that plasma fibronectin was decreased in relation to the severity of the distortion of the liver lobes determined by peritoneoscopy, and also to fibrosis, infiltration of inflammatory cells and necrosis found in biopsied specimens. These results suggested that the level of plasma fibronectin might reflect the severity of tissue injury due to chronic liver diseases. The deposition of fibronectin in fibrotic and necrotic areas and increased catabolism of fibronectin are possible explanations of the decreased plasma fibronectin. Enhanced consumption of fibronectin as an opsonin due to overactivation of the reticuloendothelial system might be another reason for the decreased plasma fibronectin in liver disease patients. The fact that albumin and the prothrombin time had no correlation to the decreased plasma fibronectin suggests that the production of fibronectin in liver parenchymal cells has little effect on the concentration of fibronectin in the plasma.

Other factors involved in changes in the plasma level of fibronectin in liver disease patients must be investigated if the determination of fibronectin is to be used as a laboratory test to estimate the states of the severity of liver diseases.

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References


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