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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.

Key words : plasma fibronectin, liver diseases, liver biopsy

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 histologically.

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 peritoneoscopy. Diseases included in this study were acute hepatitis in the convalescent stage (13 cases), chronic hepatitis (40 cases), liver cirrhosis (17 cases) and other liver diseases including alcoholic liver injury, fatty liver and chronic persistent hepatitis (20 cases). The patients were all free of bacterial infectious diseases while fibronectin 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 sedimentation rate, C-reactive protein, total bilirubin, direct bilirubin, glutamate oxaloacetate transaminase, glutamic pyruvic transaminase, alkaline phosphatase, cholinesterase, γ-glutamyl transpeptidase, thymol turbidity test, zinc sulfate turbidity test, total protein, albumin, γ-globulin, A/G, total cholesterol, fractional disappearance rate of indocyanine green, prothrombin time.

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

Assay for plasma fibronectin. Plasma fibronectin was determined by a direct enzyme-linked immunosorbent assay (ELISA) modified from the method reported by Rennard *et al.* (9). A microtiter plate (Nunc Immuno Plate II, Nunc Lab.) was coated with anti-human fibronectin rabbit serum (Cappel Lab.) diluted 1000 fold with 20 mM carbonate buffer, pH 9.6, containing 0.02% sodium azide. The remaining active sites of the plate were blocked with 1% ovalbumin in 20 mM sodium phosphate containing 0.15 M sodium chloride (PBS). The plate thus prepared was used as the immunosorbent. Plasma samples and standard 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% H_2O_2 in 0.1 M phosphate buffer, pH 5.0, for 1 h. The reaction was stopped by adding 4N H_2SO_4, 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 immediately fixed in Bouin's solution, dehydrated by passing through an ethanol series, and embedded in paraffin. The specimens were sectioned and routinely 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 presence 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 number 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-

veloped for determining plasma fibronectin using anti-human fibronectin rabbit immunoglobulin and anti-human fibronectin goat immunoglobulin-horseradish peroxidase conjugate. A calibration curve using standard fibronectin was dose responsively linear between the range of 80-200 ng/ml as shown in Fig. 1. Thus, fibronectin in the patients' plasma was determined with plasma appropriately diluted within above range.

The mean values ±2SD of plasma fibronectin were 343.2±120.0 μg/ml for normal subjects, 363.9±126.4 μg/ml for

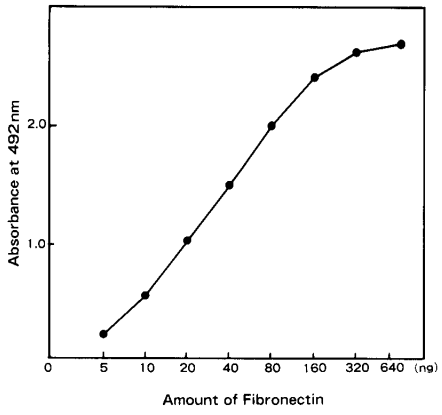


Fig. 1 Calibration curve for standard plasma fibronectin.

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

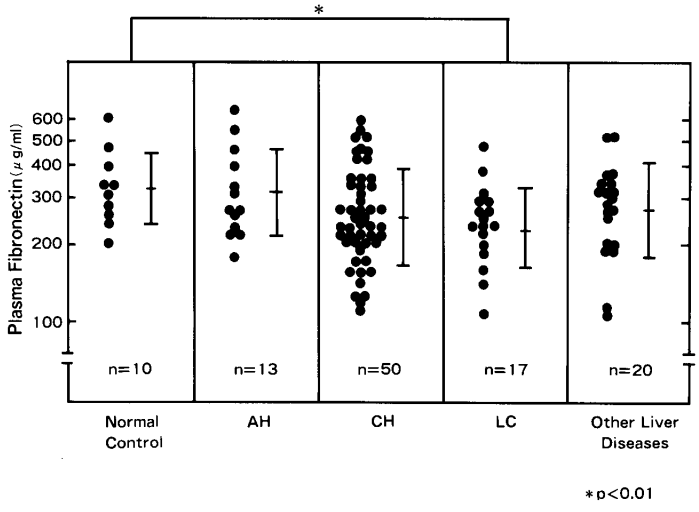
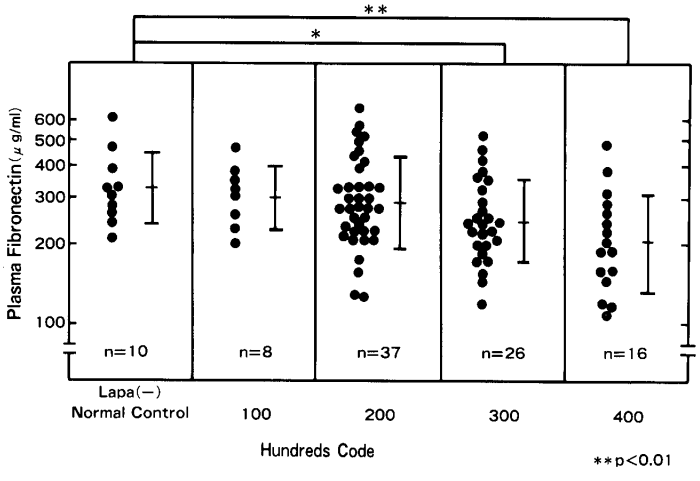


Fig. 3 Relationship between plasma fibronectin and lobular distortion. The lobular distortion in the peritoneoscopic pictures of the liver surface was classified according to Shimada's code number system: 100, normal lobular structure with no fibrosis in Glisson's capsule; 200, normal lobular structure with moderate fibrosis of Glisson's capsule; 300, presence of a network formed by portal-portal and portal-central vein connections; 400, mound or paving stone-shaped nodules; 500, spherical nodules. No cases with code number 500 were seen in this study.



acute hepatitis patients, 278.0±125.7 μg/ml for chronic hepatitis patients, 246.7±90.0 μg/ml for liver cirrhosis patients, and 275.7±94.8 μg/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.

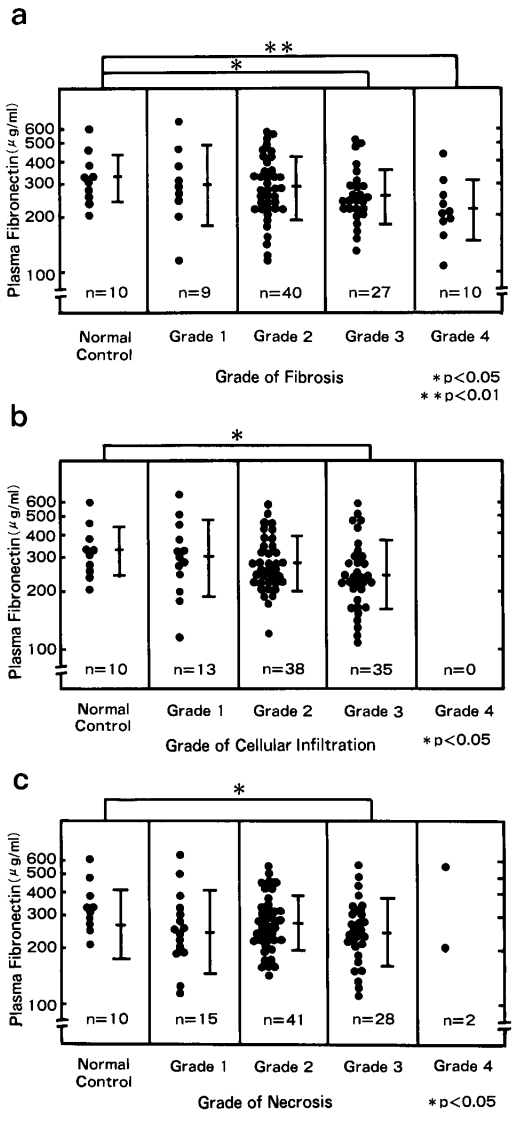


Fig. 4 Relationship between plasma fibronectin and fibrosis (a), cellular infiltration (b) and necrosis (c) in the liver. Fibrosis, cellular infiltration and necrosis seen in biopsy specimens were classified into 4 degrees of severity : Grade 1, minimum change ; Grade 2, mild change ; Grade 3, moderate change ; Grade 4, severe change.

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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