[原著］Comparison of clinical utility between type IV collagen 7S domain and hyaluronate in patients with chronic viral liver disease

Comparison of clinical utility between type Ⅳ collagen 7S domain and hyaluronate in patients with chronic viral liver disease

Hiroshi Sakugawa, Hiroki Nakasone, Yuko Kawakami, Tomofumi Nakayoshi, Fukunori Kinjo, Atsushi Saito, Minoru Ikema*, Akihiro Yamashiro** and Tomokuni Nakayoshi***

First Department of Internal Medicine, Faculty of Medicine, School of Medicine, University of the Ryukyus, Okinawa, Japan
**Ohama Daiichi Hospital, **Hokubu-chiku Ishikai Hospital,*
****Ginowan Kinen Hospital, Okinawa, Japan*

(Received on August 18, 1995, accepted on September 5, 1995)

ABSTRACT

To compare the clinical utility of type Ⅳ collagen 7S domain and hyaluronate, as markers in the evaluation of liver fibrosis, 184 patients with biopsy-proven chronic viral liver disease (32; type B, 152; type C) were involved in this study. The two markers correlated well with the grade of liver fibrosis. The correlation coefficients of 7S-collagen and hyaluronate with the extent of fibrosis were 0.747 and 0.813, respectively. Very high values were seen more frequently in hyaluronate than in 7S-collagen, particularly in patients with moderate and severe fibrosis. Both markers also correlated strongly with conventional liver function tests except for alanine aminotransferase. In conclusion, serum hyaluronate was a more sensitive marker and seemed to more strongly correlate with the degree of hepatic fibrosis than type Ⅳ collagen 7S domain. *Ryukyu Med., 15(3)133~137, 1995*

Key words: biochemical marker, liver fibrosis, chronic hepatitis, liver cirrhosis

INTRODUCTION

Chronic viral hepatitis is a progressive liver disease caused by chronic infection with hepatotrophic virus type B or C and is characterized by mononuclear inflammatory cell infiltration in mainly the portal tracts. Continuing damage to hepatocytes results in increasing connective tissue intension in the portal tracts as well as periportal areas. As the disease progresses, there is increasing fibrotic disruption of the liver architecture and nodular regeneration, leading to liver cirrhosis. Thus, evaluation of the degree of liver fibrosis is important for the assessment of the stage of chronic viral liver disease[1].

Liver biopsy has been the only reliable method for the evaluation of the extent of liver fibrosis. However, this technique is invasive, and considerable sampling error has been seen.

Several serum biochemical markers of liver fibrosis have recently become available in clinical practice[2]. There have been a few reports assessing the clinical utility of these biochemical markers[3-7]. Of these biochemical markers, both type Ⅳ collagen 7S domain and hyaluronate are promising in the evaluation of liver fibrosis, particularly in chronic viral liver disease[7,8]. The literature concerning the clinical utility of both biochemical markers in patients with chronic viral liver disease published in English and Japanese from 1975 to 1994 was reviewed and analyzed. We recognized that there is no report comparing the clinical utility of the two markers.

The aim of this study was to compare the usefulness of these two markers in predicting the degree of histological liver fibrosis and assess the correlation between these markers and conventional liver function biochemical tests.

SUBJECTS AND METHODS

The subjects of this study were 184 patients with biopsy-proven chronic viral liver disease who were admitted to our institutes between April, 1989 and December, 1994. There were 116 males and 68 females aged 18-77 years (Mean±S.D.=49.7±14.0). The subjects included 32 with type B chronic liver disease positive for hepatitis B surface antigen (HBsAg), determined by enzyme immunoassay (Enzygnost, Behring, Germany) and 152 with type C chronic liver disease positive for antibody to hepatitis C virus (anti-HCV), measured by second generation ELISA (UBI-HCV-EIA, United Biochemical Inc., NY). The histological diagnosis according to the standard histological criteria[9] of the 184 subjects were as follows; chronic persistent hepatitis, 49; chronic active hepatitis, 81;

liver cirrhosis, 54.

As control, 40 healthy persons, consisting of 22 males and 18 females aged 27-75 years (49.7±12.2), were selected at their health check-up. They met the following criteria; normal alanine aminotransferase (ALT) level, negative for both HBsAg and anti-HCV, and normal ultrasonographic findings. Informed consent for this study was obtained from each person.

Blood samples from most of the patients were taken after an overnight fast in the morning on the day of liver biopsy, and in the remaining patients, samples were taken within one month of liver biopsy.

Type Ⅳ collagen 7S domain was measured with a type Ⅳ collagen 7S domain RIA kit (Nippon CPC Co., Tokyo, Japan) and serum hyaluronate levels were determined by sandwich binding protein assay using a commercially available kit (Chugai Co., Tokyo, Japan). Serum ALT activity, plasma albumin concentration, zinc turbidity test (ZTT), fasting total serum bile acid (TBA), indocyanine green retention rate at 15 minutes (ICGR15), and peripheral blood platelet count were all examined using conventional methods at the central laboratory in the Ryukyu University Hospital, Okinawa, Japan.

Liver biopsy specimens were obtained by the True-Cut needle, fixed in 10% formalin, and stained with hematoxylin-eosin and with the Mallory-Azan method. The degree of histological liver fibrosis was evaluated according to the classification of the histological staging proposed by Yano et al., that is, grade 1: fibrous portal expansion limited to the portal zone, grade 2: portal elongation but no bridging fibrosis, grade 3: partial bridging fibrosis, grade 4: whole bridging fibrosis, grade 5: cirrhosis[10].

Statistics

The non-parametric Mann and Whitney U-test was used to compare serum 7S-collagen and hyaluronate concentration between controls and each grade group of fibrosis as well as between the neighboring groups (e.g. between grade 1 and 2, or grade 2 and 3). Spearman rank correlation coefficients were calculated to evaluate the relationship between the histological degree of liver fibrosis and serum fibrotic markers as well as other laboratory tests.

RESULTS

Relation between serum 7S-collagen, hyaluronate and the degree of liver fibrosis

Type Ⅳ collagen 7S domain: Serum level of type Ⅳ collagen 7S domain correlated well with histological grade of liver fibrosis ($\rho=0.747$). Even in patients with grade 1 and 2 liver fibrosis, significant difference was found in the 7S-collagen levels when compared with the normal control ($p<0.05$ and $p<0.05$, respectively). However, most of the patients with grade 1 and 2 showed normal levels of 7S-collagen. When 7S-collagen levels were compared between the neighboring groups of fibrotic

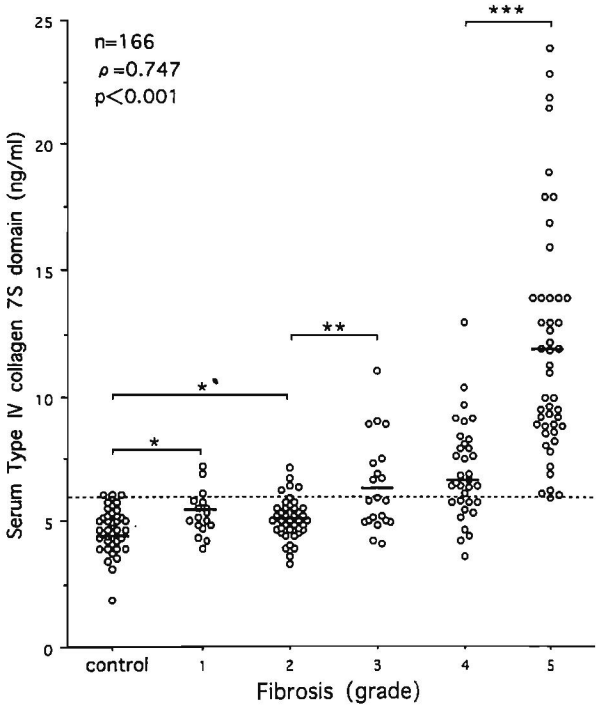

Fig.1 Correlation between serum type Ⅳ collagen 7S domain concentration and the degree of liver fibrosis in patients with chronic viral liver disease. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.0001$ The interrupted line indicates the upper limit of the reference range

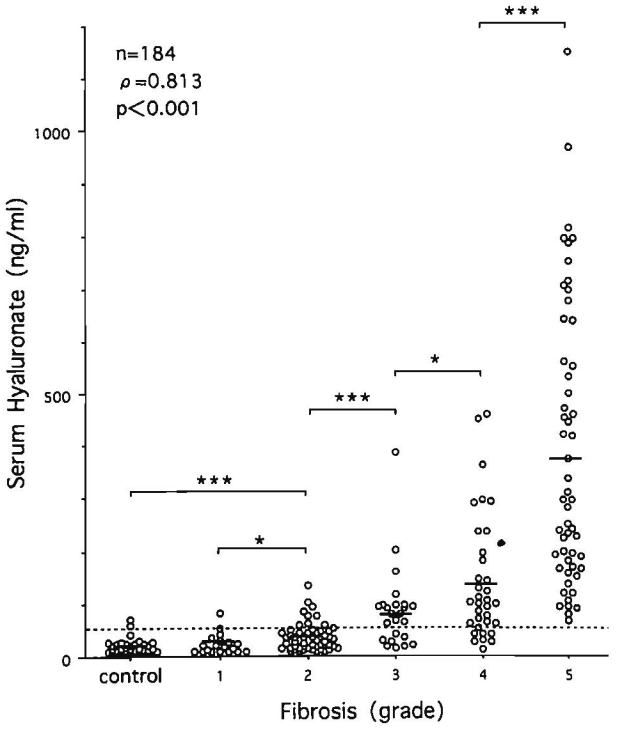

Fig.2 Correlation between serum hyaluronate concentration and the degree of liver fibrosis in patients with chronic viral liver disease. $^{*}p<0.05$, $^{***}p<0.0001$ The interrupted line indicates the upper limit of the reference range

Table 1 Correlation between various laboratory tests and the grade of liver fibrosis in patients with chronic viral liver disease (by the Spearman's rank correlation coefficient)

	n	Correlation coefficient	
7S-collagen	166	0.747	$p<0.0001$
Hyaluronate	184	0.813	$p<0.0001$
ALT	179	0.116	$p=0.059$
ZTT	178	0.544	$p<0.0001$
Albumin	157	−0.515	$p<0.0001$
TBA	133	0.614	$p<0.0001$
ICGR15	121	0.628	$p<0.0001$
Platlet	177	−0.746	$p<0.0001$

ALT: alanine aminotransferase, ZTT: zinc turbidity test TBA: total bile acid, ICGR15: indocyanine green retention rate at 15 minutes

Table 2 Correlation between serum biochemical markers of liver fibrosis and conventional laboratory tests in patients with chronic viral liver disease

	7S-collagen (r)	Hyaluronate (r)
ALT	0.010	0.073
ZTT	0.534	0.410
Albumin	−0.688	−0.581
TBA	0.635	0.480
ICGR15	0.646	0.549
Platlet	−0.579	−0.586

ALT: alanine aminotransferase, ZTT: zinc turbidity test TBA: total bile acid, ICGR15: indocyanine green retention rate at 15 minutes

change, significant difference was seen between grade 2 and 3 ($p<0.01$) and between grade 4 and5 ($p<0.0001$), Most patients with both grade 4 and 5 had abnormal high value of 7S-collagen with the frequency of 67.6% and 95.7%, respectively (Fig.1).

Hyaluronate: Serum hyaluronate level strongly correlated with the degree of liver fibrosis ($\rho=0.813$). There was no difference in hyaluronate level between patients with grade 1 and the normal control. On the contrary, patients with grade 2 or with more severe fibrosis showed significantly higher serum hyaluronate concentration than the control. Moreover, each comparison between the neighboring group of the grade showed statistically significant difference. Very high values were seen more frequently in hyaluronate than in 7S-collagen, particularly in patients with moderate and severe fibrosis (Fig.2).

Relation between other laboratory tests and the degree of liver fibrosis

In conventional liver function biochemical tests, ALT did not correlate with the grade of liver fibrosis, but other tests evaluated in this study showed strong correlation. However, these correlation were weaker than those seen between the biochemical markers of fibrosis and the grade of liver fibrosis. There was a strongly negative correlation between peripheral platelet count and the grade of liver fibrosis. The correlation coefficient of platelet count with the grade of fibrosis was higher than those with the liver biochemical tests as well as that with 7S-collagen (Table 1).

Relation between serum markers of liver fibrosis and other laboratory tests

Both type Ⅳ collagen 7S domain and hyaluronate levels correlated well with the conventional liver function tests except for ALT, and also strongly correlated with the platelet count. Type Ⅳ collagen 7S tended to be more closely correlated with these laboratory tests than hyaluronate (Table 2).

DISCUSSION

Chronic viral hepatitis is a slowly progressive liver disease with fluctuating liver cell damage. The progress (stage) of the disease implies various factors such as, fibrosis, circulation disorder, impairment of liver function and others. These factors are also mutually related.

In conventional liver function tests, ALT and aspartate aminotransferase represent inflammatory activity, whereas

ZTT, albumin, TBA and ICGR15 can assess the progress of the disease. It is described that ZTT correlates strongly with gamma-globulin as well as IgG concentration. Hypergamma-globulinemia is usually seen in patients with cirrhosis, and ZTT increases with progression of chronic liver disease. Plasma albumin concentration represents synthetic activity of the liver and negatively correlates with progression of liver disease. Since bile acid and ICG are mainly taken up and metabolized in liver cells, those serum levels are affected by hepatic circulation disorder. Using these conventional liver biochemical tests, we can assess the state of the liver (circulation disorder, impairment of liver function) in patients with chronic liver disease. However until recently, there had been no test by which we could evaluate the extent of liver fibrotic change.

Type Ⅳ collagen is the component of the basement membrane of the small vessels[11] and increased in the liver tissue and blood stream with the progression of liver fibrotic change. On the other hand, hyaluronate is the component of extra-cellular matrix synthesized largely by mesenchymal cells[12], probably by fat-storing cells and is taken up and degraded by liver endothelial cells[13]. Both 7S-collagen and hyaluronate are thought to be associated with capillarization of hepatic sinusoid[11,14].

In this study, the two biochemical markers strongly correlated with the extent of liver fibrosis. When the performance of the two markers in patients with chronic hepatitis are compared, hyaluronate tended to rise even in patients with chronic hepatitis showing mild to moderate fibrosis, whereas, 7S-collagen began to rise in more advanced cases, that is, chronic hepatitis of grade 4 or cirrhosis.

All patients with cirrhosis (except for two patients who showed normal 7S-collagen level and high value of hyaluronate) showed increased levels of both hyaluronate and 7 S-collagen. The increasing levels of the two biochemical markers in cirrhosis patients were usually more than 7 ng/ml and more than 100 ng/ml, respectively.

Type Ⅳ collagen 7S as well as hyaluronate did not correlate with ALT, which reflects liver cell necrosis. It is well known that the degree of liver cell necrosis is not associated with the stage of chronic hepatitis. On the other hand, there was significant correlation between the biochemical markers of liver fibrosis and other conventional liver function tests (albumin, ZTT, TBA, ICGR15).

All liver biochemical tests evaluated in this study except for ALT strongly correlated with the grade of liver fibrosis. However, the correlation coefficients of these tests with the extent of liver fibrosis were lower than those of the two fibrotic markers, and clinical utility of these tests seemed to be limited. For example, serum albumin concentration was usually normal even in patients with liver cirrhosis, particularly in those with liver cirrhosis at the compensated stage. TBA and ICGR15 tended to vary within the same stage, so that tests seemed difficult to assess clinically.

Decreased peripheral platelet count is usually seen in advanced liver disease and is mostly caused by secondary hypersplenism. Blood platelet count correlates with the stage of chronic liver disease, and might be attributed to the strong association between liver fibrotic change and hypersplenism which may result from hepatic circulation disorder.

In conclusion, both type Ⅳ collagen 7S domain and hyaluronate correlated well with the degree of hepatic fibrosis, the degree of which represents the stage of chronic liver disease. The correlations were stronger than those seen in conventional liver function tests. However, correlation coefficient of platelet count with liver fibrosis was between that of hyaluronate and of 7S-collagen. Serum hyaluronate was the more sensitive marker and seemed to have stronger correlation with the degree of liver fibrosis than type Ⅳ collagen 7S domain.

REFERENCES

1) Desmet, V.J., Gerber, M., Hoofnagle, J.H., Manns, M., and Scheuer, P.J.: Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 19: 1513-1520, 1994.
2) Plebani, M., and Burlina, A.: Biochemical markers of hepatic fibrosis. Clin. Biochem. 24: 219-239, 1991.
3) Nyberg, A., EngstromLaurent, A., and Loof, L.: Serum hyaluronate in primary biliary cirrhosis-A biochemical marker for progressive liver damage. Hepatology 8: 142-146, 1988.
4) Hasegawa, A., Schuppan, D., Ohnishi, K., Okuda, K., and Hahn, E.G.: Serum concentration of the carboxy-terminal cross-linking domain of procollagen type Ⅳ (NC1) and the animoterminal propeptide of procollagen type Ⅲ (PⅢP) in chronic liver disease. J. Hepatol. 10: 17-22, 1990.
5) Ramadori, G., Zohrens, G., Manna, M., Rieder, H., Dienes, H.P., Hess G., and Meyer Zum Buschenfelde, K.-H.: Serum hyaluronate and type Ⅲ collagen aminoterminal propeptide concentration in chronic liver disease. Relationship to cirrhosis and disease activity. Eurp. J. Clin. Invest. 21: 323-330, 1991.
6) Yamada, S., Suou, T., Kawasaki, K., and Yoshikawa, N.: Clinical significance of serum 7S collagen in various liver diseases. Clin. Biochem. 25: 467-470, 1992.
7) Guechot, J., Poupon, R.E., Giral, P., Balkau, B., Giboudeau, J., and Poupon, R.: Relationship between procollagen Ⅲ aminoterminal propeptide and hyaluronate serum levels and histological fibrosis in primary biliary cirrhosis and chronic viral hepatitis C. J. Hepatol. 20: 388-393, 1994.
8) Murawaki, Y., Ikuta, Y., Koda, M., and Kawasaki, H.: Serum type Ⅲ procollagen peptide, type Ⅳ collagen 7S domain, central triple-helix of type Ⅳ collagen and

tissue inhibitor of metalloproteinases in patients with chronic viral liver disease: Relationship to liver histology. Hepatology 20: 780-787, 1994.
9) International group.: Acute and chronic hepatitis revisited. Lancet 2: 914-919, 1977.
10) Yano, M., Yatsuhashi, H., Inoue, O., Inokuchi, K., and Koga, M.: Epidemiology and long term prognosis of hepatitis C virus infection in Japan. Gut 34: S13-16, 1993.
11) Timpl, R., Wiedermann, H., and Van Delden, V.: A network model for the organization of type Ⅳ collagen in basement membranes. Eur. J. Biochem. 120: 203-211, 1981.
12) Gressner, A.M., and Haarmann, R.: Hyaluronic acid synthesis and secretion by rat liver fat storing cells. Biochem. Biophys. Res. Commun. 151: 222-229, 1988.
13) Erikson, S., Fraser, J.R.E., Laulent, T.C., Pertoft, K., and Smedsrod, D.: Endothelial cells are a site of uptake and degradation of hyaluronic acid in the liver. Exp. Cell Res. 144: 223-228, 1983.
14) Ueno, T., Inuzuka, S., Torimura, T., Shakado, S., Sakata, R., Sakamoto, M., Sata, M., Yoshida, H., and Tanikawa, K.: Serum hyaluronate in liver disease (in Japanese). Acta Hepatologica Japonica 32: 767-774, 1991.