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Noninvasive estimation of liver fibrosis and response to interferon therapy by a serum fibrogenesis marker, YKL-40, in patients with HCV-associated liver disease

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Abstract

AIM: To evaluate the clinical utility of serum fibrosis markers, including YKL-40, in patients with HCV-associated liver disease.

METHODS: A total of 109 patients with HCV-associated liver disease were enrolled. We measured serum type IV collagen, amino-terminal peptide of type III procollagen (PIIIP), hyaluronic acid (HA), YKL-40 levels and biochemical. Parameters by RIA or ELISA. Eighty-eight patients underwent liver biopsy, and 67 of 109 patients received interferon (IFN) therapy. We also investigated the relationship between the concentrations of serum fibrosis markers and histological fibrosis scores (METAVIR), and evaluated the changes of the levels of fibrosis markers before and after the IFN therapy.

RESULTS: The increase in serum levels of all markers, particularly HA, was correlated with the progression of liver fibrosis (for type IV collagen, $F = 9.076$, $P<0.0001$; for PIIIP, $F = 9.636$, $P<0.0001$; for HA, $F = 13.128$, $P<0.0001$; and for YKL-40, $F = 8.016$, $P<0.0001$). YKL-40 had strong correlation with HA ($r = 0.536$, $P<0.0001$). Based on the receiver operating curve (ROC), the ability of serum HA exceeded the abilities of other serum markers to determine fibrosis score 4 from fibrosis score 0-3 (AUC = 0.854). While YKL-40 was superior to other fibrosis markers for predicting severe fibrosis (F2-F4) from mild fibrosis (F0-F1) (YKL-40, AUC = 0.809; HA, AUC = 0.805). After IFN therapy, only YKL-40 values significantly decreased not only in the responder group, but also in the nonresponder group ($P = 0.03$).

CONCLUSION: YKL-40 may be a useful non-invasive serum marker to estimate the degree of liver fibrosis and to evaluate the efficacy of IFN therapies in patients with HCV-associated liver disease.

Key words: HCV; Liver fibrosis; YKL-40; Interferon

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INTRODUCTION

Hepatic fibrosis is the most important factor for estimating clinical outcome and determining therapeutic strategy, especially interferon therapy, in patients with hepatitis C virus (HCV)-associated liver diseases. Liver biopsy is currently the most reliable standard for assessing hepatic fibrosis, necrosis and inflammation[1,2]. However, liver biopsy has potential complications[3], and so is not repeatedly performed. Thus, there is a need to establish noninvasive monitoring methods for assessing the severity of hepatic fibrosis.

Several attempts have been made to find accurate noninvasive markers of disease activity and fibrosis. To date, several laboratory markers, such as platelet counts, ALT/AST ratio[4], or levels of hyaluronic acid (HA)[5], N-terminal propeptide of type III collagen (P III P)[6,7]or type IV collagen[8], have been proposed to represent hepatic fibrosis, focusing particularly on the diagnosis of advanced hepatic fibrosis. Some have combined several biochemical and clinical markers with scoring systems to predict the presence or absence of fibrosis[9]. However these scoring systems are somewhat complicated, since some analyses are not routinely available and the calculation system can be complex.

Among the single fibrosis markers, HA and PIIIP levels have been well studied in patients with chronic liver diseases[10]. HA is a glucosaminoglycan synthesized by the mesenchymal cells and degraded by hepatic sinusoidal cells by a specific receptor-mediated process. Serum levels of HA are highly correlated with advanced fibrosis and liver cirrhosis[5,11,12]. However, serum HA alone has limited value in predicting histological change over a treatment period. PIIIP, a product of collagen synthesis, correlates better with histological inflammation than fibrosis[10]. Hence, there is a need to develop a simple, accurate and reliable noninvasive marker for evaluating the severity of fibrosis.

YKL-40 (chondrex, human cartilage glycoprotein-39) is a recently described glycoprotein that belongs to the chitinase family[13]. YKL-40 mRNA was strongly expressed in human liver and arthritic articular cartilage[13,14] and was elevated in the synovial fluid[15] and serum with active rheumatoid arthritis[16], severe osteoarthritis[17] and alcoholic liver disease[6,18]. Although its physiological function is unknown in detail, YKL-40 is thought to contribute to tissue remodeling or degradation of the extracellular matrix[19]. Previous reports have indicated that the YKL-40 is a growth factor for fibroblasts and that YKL-40 acts synergistically with insulin-like growth factor 1 in stimulating the growth of fibroblasts[20], YKL-40 is also a growth factor for chondrocytes and synovial cells[15] and acts as a chemo-attractant for endothelial

cells and stimulates migration of these cells at a level comparable to that achieved by basic fibroblast growth factor[21]. Furthermore, YKL-40 modulates vascular endothelial cell morphology by promoting the formation of branching tubules, indicating that YKL-40 mayplay a role in angiogenesis by stimulating the migration and reorganization of vascular endothelial cells[21].

However, clinical use of serum YKL-40 level for the assessment of hepatic fibrosis in patients with HCV-associated liver diseases has not been elucidated. Furthermore, the response of fibrosis markers after interferon (IFN) therapy, a potential anti-fibrosis therapy, is not well understood. We questioned whether the fibrosis-related markers type IV collagen, PIIIP, HA and YKL-40 could discriminate between histological fibrosis stages, and reflect the histological response of post-interferon therapy in patients with HCV-associated liver diseases.

MATERIALS AND METHODS

Patients

One hundred and nine patients with chronic liver disease were studied and all patients had detectable serum HCV-RNA (Amplicor HCV 2.0; Roche Diagnostics, Branchburg, NJ, USA). Eighty-eight patients underwent liver biopsies for histological examination of the liver. Twenty-one patients, whose diagnosis was based on clinical, biochemical and imaging findings, were classified as having liver cirrhosis, because they had much risk for the biopsies. None of the patients had other causes of chronic liver injury, a history of habitual alcohol consumption nor hepatocellular carcinoma. All tissue and serum samples were obtained with informed consent.

Liver histological findings

Eighty-eight patients underwent liver biopsies as a part of clinical standard management. Tissue sections from the patients were stained with hematoxylin and eosin and evaluated for the stage of liver fibrosis and the grade of liver activity.

The patients were sub-classified into five groups based on the stage of liver fibrosis according to the METAVIR classification: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. The grade of liver activity was classified into four groups according to METAVIR classification: A0, no activity; A1, mild activity; A2, moderate activity; and A3, severe activity.

Serological and biochemical methods

We measured the levels of type IV collagen, PIIIP, HA and YKL-40 from patient serum samples that were stored at -20 ℃. The concentration of type IV collagen was determined with a commercial RIA kit (Panassay IV. C, Daiichi Chemical Co. Ltd. Tokyo, Japan). PIIIP concentration was measured by RIA-gnost PIIIP kit (Hoechst, Tokyo, Japan). ELISA kits were used for determination of HA concentrations (Chugai Pharmaceuticals, Tokyo, Japan) and YKL-40 concentrations (Quidel Corporation, San Diego, USA). We determined the concentrations of these markers before and after interferon (IFN) treatment. Serum albumin and alanine aminotransferase (ALT) were measured by routine methods. All biochemical data were expressed as median (range).

Receiver operating curve

To assess the ability of the four serum fibrosis markers for differentiating chronic hepatitis (fibrosis score F0-F3) from liver cirrhosis (F4), and for differentiating mild hepatitis (F0-1) from severe hepatitis (F2-4), we calculated the sensitivity and the specificity for each value of each fibrosis marker and then constructed receiver operating curves (ROC) by plotting the sensitivity against the reverse specificity (1 minus specificity) at each value. The diagnostic value of each serum marker was assessed by the area under the ROC. An area under the curve (AUC) of 1.0 is characteristics of an ideal test, whereas 0.5 indicates a test of no diagnostic value. The nearer a curve shifts to the top left-hand corner of the graph, the more useful marker is for the diagnosis. We determined the turning point of the curve to the best cut-off value for the diagnosis, and it was also a maximal value at the sum of the sensitivity and specificity. The diagnostic accuracy was calculated by sensitivity, specificity, positive and negative predictive values, considering significant fibrosis of the disease.

Assessment of response to IFN treatment

We also evaluated whether a marker was an independent predictor of the response to interferon (IFN) treatment in chronic hepatitis C. Sixty-seven patients with chronic hepatitis C received a 6-mo course of interferon-based therapy. We determined serum levels for each fibrosis marker and determined HCV-RNA for each patient at 6 mo after the therapy Patients whose HCV-RNA became positive after IFN treatment were classified into the non-virological responser (NVR) group, and those whose HCV-RNA remained negative after IFN treatment were classified as the sustained-virological responser (SVR) group. The NVR group was sub-divided into those patients whose ALT remained below 664 nkat/L l after IFN treatment as the biochemical responders (BR) group.

Statistical analysis

Results of serum fibrosis markers were expressed as box plots. The statistical significance was calculated by one-way ANOVA. The correlations between four serum fibrosis markers and biochemical data were evaluated by analysis of person's correlation coefficient. The levels of serum fibrosis markers in the patients with IFN treatment were analyzed by the paired *t* test. *P* values <0.05 were considered statistically significant.

RESULTS

Patient characteristics

Baseline demographic and laboratory values are summarized in Table 1 for the109 patients. The median age was 54 years, with a male predominance. The values of platelets and serum albumin decreased in the patients with HCV-associated liver disease according to the stage of liver fibrosis (F0-4) (Table 1).

Table 1 Characteristics of subjects

Fibrosis	*n*	M/F	Age (yr)	Platelet ($\times 10^4$/mm^3)	Albumin (g/L)	ALT (nkat/L)
F 0	5	3/2	35 (29-39)	19.9 (14.4-26.0)	42 (3.2-4.6)	182.6 (7-84)
F 1	27	17/10	51 (31-73)	15.8 (10.3-25.2)	41 (3.6-4.7)	1 477.4 (20-267)
F 2	13	8/5	57 (50-64)	13 (10.0-15.1)	39 (3.5-4.5)	1 543.8 (31-235)
F 3	34	22/12	55 (30-68)	12.2 (8.3-19.1)	39 (3.3-4.3)	1 062.4 (23-295)
F 4	30	12/18	59 (43-75)	9.5 (3.2-21.3)	34 (2.5-4.1)	996.0 (13-150)

M: male, F: female.

Correlation between serum markers and the stage of liver fibrosis

The relation of serum concentrations of type IV collagen, PIIIP, HA and YKL-40 to the stage of liver fibrosis is illustrated in Figure 1. Serum levels of type IV collagen, PIIIP, HA and YKL-40 increased with the progression of liver fibrosis in patients with HCV-associated liver disease. The overall significances by ANOVA of the difference of these five groups were as follow: for

type IV collagen, $F = 9.076$, $P<0.0001$; for PIIIP, $F = 9.636$, $P<0.0001$; for HA, $F = 13.128$, $P<0.0001$; and for YKL-40, $F = 8.016$, $P<0.0001$. Levels of each fibrosis marker had a strong correlation with the stage of liver fibrosis. HA values increased more significantly in F4 than other fibrosis markers. PIIIP and, particularly YKL-40, values increased according to the stage of liver fibrosis. The correlation between the grade of histological activity and serum fibrosis markers were evaluated with ANOVA. The overall significances in these four groups were as follows: for type IV collagen, $F = 3.385$, $P<0.0219$; for PIIIP, $F = 0.991$, $P = 0.4011$; for HA, $F = 0.277$, $P = 0.8417$; and for YKL-40, $F = 0.246$, $P = 0.8638$. Type IV collagen had a weak correlation with the grade of histological activity in liver histology, but other serum fibrosis markers did not show significant correlations.

Correlation between serum markers and biochemical parameters

The correlation between type IV collagen, PIIIP, HA, YKL-40 and biochemical liver function tests is shown in Table 2. Correlations were found between each fibrosis marker ($P<0.001$). There was a moderate correlation between type IV collagen and PIIIP ($r = 0.432$, $P<0.0001$). YKL-40 and HA showed the strongest correlation ($r = 0.536$, $P<0.0001$).

Table 2 Correlation between type IV collagen, PIIIP, HA, YKL-40 and parameters of liver function

	Type IV collagen	PIIIP	Hyaluronic acid	YKL-40
Type IV collagen		0.432[b]	0.250	0.258
PIIIP	0.432[b]		0.333	0.367
Hyaluronic acid	0.250	0.333		0.536[b,d]
YKL-40	0.258	0.258	0.536[b,d]	
Platelet	-0.453[b]	-0.105	-0.494[b]	-0.478[b]
Albumin	-0.091	0.127	-0.472[b]	-0.239
ALT	0.149	-0.046	-0.108	0.009

[b]$P<0.001$, [d]$P<0.0001$.

Laboratory liver test results revealed a moderate inverse correlation between serum albumin with the HA levels ($r = -0.472$, $P<0.0001$) and a moderate inverse correlation between platelets and type IV collagen ($r = -0.453$, $P = 0.0007$), HA ($r = -0.494$, $P = 0.0002$) or YKL-40 levels ($r = -0.478$, $P = 0.0003$).

Prediction of cirrhosis from chronic hepatitis

The ability of these serum markers to detect cirrhosis (F4) in patients with chronic hepatitis C (F0-3) was assessed using ROC. The best ROC derived from four serum fibrosis markers was applied by measuring the area under the curve (AUC), and the best ROC was that of HA (AUC = 0.854). Based on the ROC, the predictive ability of serum HA exceeded that of the other serum fibrosis markers (Figure 2). The selected cut-off values for diagnosing cirrhosis in patients with chronic hepatitis C was 183.5 ng/mL for serum HA with 80% sensitivity and 80% specificity (Table 3). The cut-off value for YKL-40 was 284.8 ng/mL with 80% sensitivity and 77% specificity, and that for PIIIP was 0.995 U/mL with 79% sensitivity and 66% specificity.

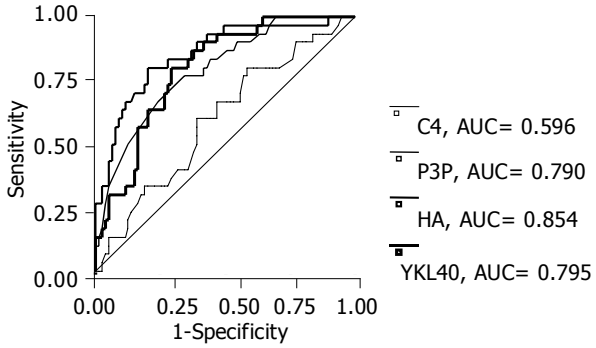

Figure 2 Receiver operating curves of type IV collagen, PIIIP, HA and YKL-40 for predicting stages greater than F3. The predictive ability of serum HA exceeded that of another serum markers (AUC = 0.854). The ability to predict cirrhosis (F4) from chronic hepatitis C (F0-3) was AUC = 0.795 for YKL-40 and AUC = 0.790 for PIIIP.

Table 3 Prediction of cirrhosis *vs* chronic hepatitis C by hyaluronic acid and YKL-40

	Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IV collagen (ng/mL)	6.55	60	61	61	60
PIIIP (U/mL)	0.995	77	66	69	67
HA (ng/mL)	183.5	80	80	80	80
YKL-40 (ng/mL)	284.8	80	71	73	78

Prediction of severe stage of fibrosis from mild stage of fibrosis

The utility of these markers to differentiate severe stage of fibrosis (F2-4) from mild stage of fibrosis (F0-1) was evaluated using ROC (Figure 3). The best ROC derived from four serum fibrosis markers was applied by measuring the area under the curve (AUC), and the best ROC was that of YKL-40 and HA (YKL-40, AUC = 0.809; HA, AUC = 0.805), exceeding those of PIIIP and

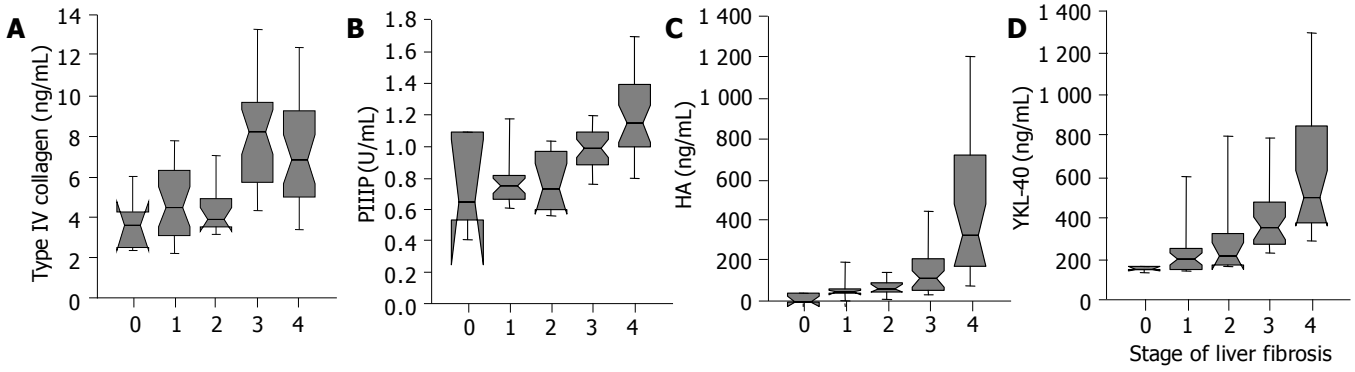

Figure 1 Serum levels of type IV collagen, PIIIP, HA and YKL-40 with respect to stage of liver fibrosis (F0-4). The box represents the interquartile range. The whiskers indicate the highest and lowest values, and the line across the box indicates the median value. Overall significance of differences among 5 groups was determined by ANOVA: for type IV collagen, $F = 9.076$, $P<0.0001$; for PIIIP, $F = 9.636$, $P<0.0001$; for HA, $F = 13.128$, $P<0.0001$; and for YKL-40, $F = 8.016$, $P<0.0001$.

type IV collagen. The cut-off values of YKL-40 and HA were 186.4 ng/mL and 75.7 ng/mL, respectively (Table 4). The sensitivity and specificity of these concentrations were 80% and 81% for YKL-40 and 75% and 81% for HA, respectively.

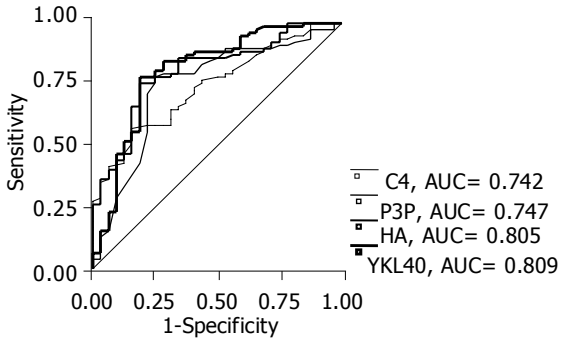

Figure 3 Receiver operating curves of type IV collagen, PIIIP, HA and YKL-40 for predicting stages greater than F1. YKL-40 and HA exceeded those of PIIIP and type IV collagen (YKL-40, AUC = 0.809; HA, AUC = 0.805).

Table 4 Prediction of severe hepatitis C versus mild hepatitis C by hyaluronic acid and YKL-40

	Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IV collagen (ng/mL)	5.75	65	69	67	66
PIIIP (U/mL)	0.835	78	75	76	77
HA (ng/mL)	75.7	75	81	79	76
YKL-40 (ng/mL)	186.4	78	81	80	79

Changes of serum markers after IFN therapy

The sixty-seven patients with HCV-associated liver disease received IFN therapy and were classified after therapy into NVR group (n = 44) and SVR group (n = 23). In SVR group, PIIIP, HA and YKL-40 levels significantly decreased after IFN treatment (for PIIIP, P<0.0001; for HA, P = 0.0077; for YKL-40, P = 0.0084). In contrast, in NVR group, PIIIP and HA levels did not change, whereas YKL-40 values significantly decreased (P = 0.03) (Figure 4). Furthermore, there were 9 patients in the BR group within the patients in NVR group (n = 44), and only one patient had normal ALT value before IFN therapy. Even in the BR group, only YKL-40 significantly decreased after IFN therapy (P = 0.0111). Moreover, there was a tendency for those in the SVR group to have lower YKL-40 values than those in the NVR group before IFN therapy (P = 0.084).

DISCUSSION

Hepatic fibrosis is the main determinant of clinical outcome and therapeutic efficacy in patients with HCV-associated liver disease. A single-pass liver biopsy is able to correctly diagnose the stage of fibrosis or presence of cirrhosis in 80% of patients[22]. However, liver biopsy is an invasive procedure with associated morbidity that carries a significant cost[3]. For these reasons, a reliable noninvasive fibrosis marker is required. In recent studies, simple noninvasive methods without biopsy to predict both significant fibrosis and cirrhosis have been investigated. Measurements of mixed parameters, such as the blood test[22], fibrotest[9] or aspartate aminotransferase (AST) to platelet ratio[4,23] indices, have been assessed as substitutes for liver biopsy, but these methods are difficult to calculate, do not reflect the mechanism of the liver fibrosis directly, and do not relate to the efficacy of IFN treatment in patients with HCV-associated liver disease.

In an attempt to search for more suitable markers for prediction of liver fibrosis severity, we investigated the novel marker, YKL-40, in addition to the well established fibrosis markers, type IV collagen, PIIIP and HA. In our study, YKL-40 and HA were found more useful than other markers for assessing the fibrosis stage. In particular, YKL-40 was most useful for monitoring the fibrosis of liver disease and for distinguishing extensive liver fibrosis from mild stage of liver fibrosis, enabling us to predict severe stage of fibrosis at 80% positive predictive value. HA appeared to be slightly better for prediction of cirrhosis (F4) from chronic hepatitis (F0-3) than YKL-40.

Type IV collagen is composed of a major triple-helix, an amino-terminal triple-helix (7S domain) and a carboxy-terminal globular domain[24]. We measured type IV collagen by RIA, which recognizes the 7S domain. The 7S domain is thought to be derived primarily from the degradation of already existing basement membrane, and it correlates better with liver fibrosis rather than intact type IV collagen[8]. PIIIP is a component of the extracellular matrix deposited in the space of Disse[25]. It is produced from type III procollagen in hepatic stellate cells and is released into the circulation in stoichiometric amounts[26]. PIIIP correlates better with inflammation and is thought to reflect primarily active hepatic fibrogenesis in chronic liver disease[27,28]. HA is a polysaccharide found in virtually all connective tissues, and in liver fibrosis[29], it is a component of the extracellular matrix[30]. In chronic hepatitis, HA is synthesized by the hepatic stellate cells and is metabolized in the liver endothelial cells[11]. With severe fibrosis in chronic hepatitis, increasing deposition of basement membrane components causes sinusoidal capillarization, diminishing HA clearance.

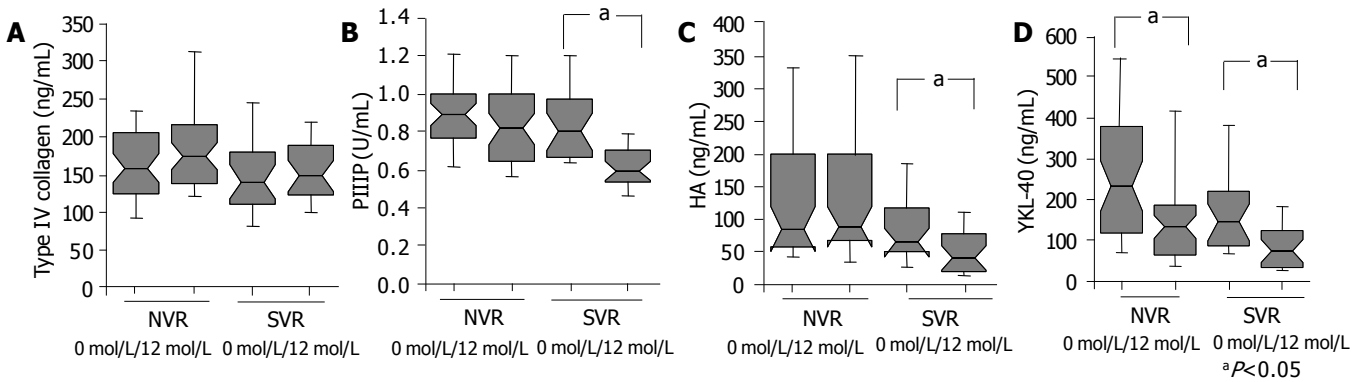

Figure 4 Serum levels of type IV collagen, PIIIP, HA and YKL-40 before and 6 months after IFN therapy in NVR and SVR patient groups. The box represents the interquartile range. The whiskers indicate the highest and lowest values, and the line across the box indicates the median value. The levels of all serum markers without type IV collagen 6 mo after IFN treatment were lower than those before IFN treatment in SVR group (^aP<0.05), while only YKL-40 levels decreased in NVR group (^aP<0.05).

HA levels increase, particularly in patients with cirrhosis[31]. Therefore, it is thought that type IV collagen and PIIIP levels reflect more fibrogenesis than fibrosis, whereas HA levels reflect fibrosis. YKL-40 is a mammalian 40 ku molecule[13] of the chitinase family. Though the biological function of YKL-40 is unknown, it is expressed in various diseases, such as liver disease with alcoholic cirrhosis[32], and recurrent breast cancer[33] or colorectal cancer[34]. YKL-40 is produced in a wide variety of cell types, including chondrocytes, synovial cells[15], activated macrophages[35], neutrophils[36], and in particular from cells located in tissues with increased remodeling/degradation or inflammation of the extracellular matrix, such as the hepatic stellate cells[37]. Based on our results and those from other groups[6], it appeared that serum levels of YKL-40 represented ongoing fibrosis like HA, in addition to fibrogenesis similar to type IV collagen and PIIIP of the liver disease. Serum YKL-40 levels were valuable for diagnosing mild stage of fibrosis (value<186.4), severe stage of fibrosis (186.4<value<284.8) and F4 (284.8<value) in our patients with HCV-associated liver disease. These results suggested that YKL-40 might be a new useful marker for monitoring liver fibrosis.

We also examined which fibrosis marker reflected the response to IFN therapy in patients HCV-associated liver disease. The natural course of fibrosis progression was studied based on a single biopsy and a suspected, rather than proven, duration of infection from the patient's history[38]. The natural course of fibrosis progression rate in patients with chronic hepatitis C was 0.133 units/year of the fibrosis score[39]. In contrast, persistently normal ALT levels correlated with slow progression of liver fibrosis (0.05 units/year)[40]. The short-term effects of IFN therapy on histological improvement are well documented and most studies have shown histological improvement at the end of treatment and/or within 1-2 years of follow-up[41], even though hepatitis C virus was not completely eradicated. Meta-analysis of the current data showed a correlation between biochemical response to IFN (*i.e.*, normalization of ALT) and histological improvement of inflammatory activity and showed a slower progression of the liver fibrosis in the NVR group than that in the untreated patients[42,43]. IFN treatment also reduced activity in the SVR group, and even in NVR patients at 6-12 mo after completion of IFN treatment.

We investigated the values of serum fibrosis markers before and 6 mo after IFN therapy. In the SVR group, the levels of PIIIP, HA and YKL-40 significantly decreased after IFN treatment. The YKL-40 levels lowered significantly not only in the BR group, but also in the NVR group after IFN therapy. These results in BR group, which were different from the previous report which demonstrated that plasma PIIINP was the only marker predicting treatment[7], suggested that the values of YKL-40 after IFN treatment might promptly reflect the improvement of liver inflammation, as well as fibrogenesis and tissue remodeling. We speculated that the serum YKL-40 changes might reflect the efficacy of the IFN treatment in the patients with HCV-associated liver disease more directly and dynamically than other fibrosis markers. In addition, YKL-40 might estimate the therapeutic effect before IFN treatment, since YKL-40 value was relatively lower in SVR group than that in NVR group. Additional studies will address these observations and the resulting YKL-40 characteristics may provide information about fibrosis status and fibrosis improvement.

In conclusion, our study demonstrates that serum YKL-40 reflects fibrosis and fibrogenesis in patients with HCV-associated liver disease. Furthermore, serum YKL-40 measurements may be a serological marker of liver fibrosis and may be used as a noninvasive marker for evaluating the efficacy of various therapies in these patients.

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