Original Article

THAI J GASTROENTEROL 2006
Vol. 7 No. 2
May - Aug. 2006
83

Efficacy of Serum Hyaluronic Acid Level for Predicting Liver Fibrosis in Patients with Nonalcoholic Fatty Liver Disease

Pawitpok C, M.D.*
Kongtawelert P, M.D.[†]
Ua-arayaporn S, M.D.**
Punyarit P, M.D.[#]
Piyanirun W, M.D.*
Chutaputti A, M.D.*

ABSTRACT

Background: Non alcoholic fatty liver disease (NAFLD) is a common liver disease that comprises a wide spectrum, ranging from fatty liver alone to steatohepatitis and progress to the end stage of liver disease. Serum hyaluronic acid is a glycosaminoglycan produce from hepatic stellate cells which is responsible for liver fibrogenesis.

Objective: To evaluate clinical significant of serum hyaluronic acid as a non invasive marker for prediction of liver fibrosis in NAFLD.

Methods: Twenty-seven patients with biopsy proven NAFLD were studied. Histopathology finding were graded and staged. The concentration of serum hyaluronic acid was measured by radioimmunoassay method. BMI, fasting blood sugar, liver function test and lipid profiles were also measured.

Results: In twenty-seven patients with NAFLD, the mean age was 47.9 years. Diabetes mellitus was found in 7 patients (45.4%). Only 2 patients had normal BMI (<23 kg/m^2), with a mean of 27.4 kg/m^2. Significant liver fibrosis defined as fibrosis score F2, F3 or F4 were present in 9 patients (33.3%). Factors correlated with significant fibrosis were age, platelet count and serum hyaluronate but factor that not correlated with fibrosis was hypercholesterolnemia. Serum hyaluronic acid levels were significantly higher in fibrosis group (mean 373 ± 184.7 ng/dl) than non significant fibrosis group (mean 156.7 ± 64.6 ng/dl, p = 0.008). When a receiver operating characteristic analysis was performed, the cut off point that achieved the highest accuracy for predicting fibrosis was 218.5 ng/dl. With this concentration the sensitivity, specificity, accuracy, positive predictive value and negative predictive value were 77.78%, 88.89%, 85.18%, 77.78%, 88.89%, respectively.

Conclusions: The serum levels of hyaluronic acid were correlated well with the degree of liver fibrosis in NAFLD. Thus, serum hyaluronic acid can be a useful marker for predicting liver fibrosis in NAFLD.

Key words : Fatty liver, serum Hyaluronic acid, Fibrosis

[Thai J Gastroenterol 2006; 7(2): 83-87]

* *Division of Digestive and Liver Disease, Department of Medicine, Phramongkutklao Hospital, Bangkok, Thailand*
† *Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand*
** *Department of Pathology, Phramongkutklao College,* # *Army Institute of Pathology, Phramongkutklao Medical Center, Bangkok, Thailand.*

Background

Nonalcoholic fatty liver disease (NAFLD) represent a large spectrum of liver diseases ranging from fatty liver alone to steatohepatitis and may progress to cirrhosis and liver failure that increase liver related morbidity and mortality[1,2].

Prognosis of NAFLD is depended on degree of liver dysfunction and stage of the disease. Degree of liver dysfunction is generally acquired from albumin, bilirubin and prothrombin time but they usually are unchanged until late stage. Stage of liver disease reflects by degree of liver fibrosis. Liver biopsy remains the reference method to access severity of fibrosis; however, because it has some limitation such as interobsever variation, sampling error, cost and complication such as hemorrhage or death[3]. Therefore, several biochemistry markers have been proposed as non invasive marker of liver fibrosis.

Ideally, the marker would be based on accurate and reproducible test that could be performed repeatedly which minimal disruption to patients. The extracellular matrix (ECM) refers to a group of macromolecules that deposit and altered composition in fibrotic liver[4,5]. Potential of fibrosis marker includes products of collagen synthesis or degradation (eg. Procollagen IV C Peptide, Procollagen IV N Peptide7-S collagen)[6], enzyme in matrix biosynthesis or degradation (eg. Tissue inhibitor of metalloproteinase; TIMP)[7], extracellular matrix glycoprotein and proteoglycan or glycosaminoglycan (eg. Hyaluronic acid)

Hyaluronic acid (HA) is the best single marker assay that reflects ECM concentration[8]. Several studied had demonstrated that HA level correlate with degree of hepatic fibrosis in many chronic liver disease such as chronic viral hepatitis, hemochromatosis, alcoholic liver disease and NAFLD[9-12].

The aim of this study was to assess discriminative ability of HA level to predict the presence of fibrosis in NAFLD.

Patients and Methods

Patients

Twenty-seven patients with a diagnosis of NAFLD (16 men, 11 women) were enrolled in this study. The diagnosis of NAFLD was a persistent increase of aminotransferase level at least 1.5 times of the upper normal limit for more than 3 months before enrolled in this study and histological of liver biopsy was proven as NAFLD.

The exclusions criteria include: consumption of alcohol more than 20 gm/day for female and 30 gm/day for male, pregnancy, malignancy, previous gastrointestinal bypass surgery, received drug induce steatosis (corticosteroid, tamoxifen, amiodarone, metrotrexate, estrogen, chloroquin or penhexilene maleate) and presence other form of liver disease (primary biliary cirrhosis, autoimmune hepatitis, Wilson's disease, chronic viral hepatitis B or C and hereditary hemochromatosis).

Methods

Biochemical Data

Blood sample collected on the day of liver biopsy after an overnight fasting. Laboratory test includes complete blood count, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, alkaline phosphatase (ALP), prothrombin time (PT), INR, fasting glucose, total cholesterol, triglyceride, low density lipoprotein (LDL), high density lipoprotein (HDL) and hyaluronic acid (HA). Hyaluronic acid levels was determined by using competitive inhibition base ELISA as previously described[11].

Histological Data

All liver biopsy samples were examined by two pathologists (US, PP) who were blind for the clinical and biochemical conditions of each patient. Biopsy specimens were fix in 10% neutralized formaldehyde, embedded in paraffin and section were stained with hematoxylin-eosin and Masson's trichrome. Histological assessment of the liver fibrosis was done according to Brunt, *et al*.[13] Scoring system as follow: F0, no fibrosis; F1, perisinusoidal fibrosis without portal fibrosis; F2, perisinusoidal fibrosis with focal or extensive periportal fibrosis; F3, presence of focal or extensive bridging fibrosis and F4, cirrhosis. Significant liver fibrosis was defined as a score of F2 or more. Then, patients were divided into two groups: group 1 had no significant fibrosis (F0 and F1 scores) and group 2 had significant fibrosis (F2, F3 and F4 scores).

Statistical analysis

Results were expressed as mean ± standard variation. The continuous variables were compared using the 2-tailed Student's t test. Categorical variables were comparing with Fisher's exact test. The diagnostic values of the clinical variables were assessed by cal-

culating the area under the receiver operating characteristic (ROC) curves, which also used to assess the best cut off points to identify the presence of significant fibrosis. The diagnostic accuracy was calculated by sensitivity, specificity and positive and negative predictive value (PPV and NPV). One way ANOVA was used to compare data between fibrosis groups (F0-F4). The SPSS statistical software (Version 11.5) was used for statistic analysis. A P value less than 0.05 was considered statistically significant.

RESULTS

Twenty-seven patients were enrolled in this study. The mean age was 47.9 years. Diabetes mellitus was found in 7 patients (45.4%). Only 2 patients had normal BMI (<23 kg/m^2), with a mean of 27.4 kg/m^2 but not significantly different in the two groups. The mean ALT level was higher than the mean AST level and the mean AST/ALT ratio was 0.55. Nine patients (33.3%) had significant fibrosis on liver biopsy; the rest of the patients had non significant fibrosis. Main characteristics and mean HA concentration were listed in Table 1. On univariated analysis, the factor correlated with significant fibrosis were age, platelet count and serum hyaluronate. Factor associated with non significant fibrosis on histopathology was cholesterol level. Dyslipidemia occurred in 15 patients (4 in significant fibrosis group, 11 in non significant fibrosis group, P = 0.448). No serious complication related to liver biopsy was found in our study. Only minor complication such as pain that relieved by medication was found in 7 patients.

Mean level of HA in each fibrosis group are shown

Table 1 Clinical and biochemical data of the 27 patients with univariate comparison among patients according to the presence or absence of significant fibrosis.

Variable	All patients (N = 27)	Significant fibrosis (Stages F2-4; n = 9)	No significant fibrosis (Stages F0, F1; n = 18)	P value
Demographics				
Age (yrs)	47.9 (10.7)	54.9 (12.0)	44.4 (7.9)	0.044
Sex				
Male (%)	16 (59.3)	6 (66.7)	10 (55.6)	0.692
Female(%)	11 (40.7)	3 (33.3)	8 (44.4)	
BMI (kg/m^2)	27.4 (3.8)	25.7 (4.4)	28.3 (3.2)	0.091
Diabetes mellitus (%)	7 (25.9)	4 (44.4)	3 (16.7)	0.175
Fasting blood sugar (mmol/L)	101.2 (23.6)	109 (26.4)	97.1 (21.7)	0.207
Liver test results				
AST (IU/L)	79.6 (36.1)	88.6 (38.7)	5.2 (35.1)	0.375
ALT (IU/L)	144.0 (54.5)	146.4 (66.1)	142.8 (49.8)	0.875
AST/ALT ratio	0.5 (0.2)	0.6 (0.2)	0.5 (0.1)	0.124
Total bilirubin (mmol/L)	0.9 (0.4)	1.0 (0.3)	0.9 (0.4)	0.611
Albumin (mmol/L)	4.5 (0.4)	4.4 (0.6)	4.6 (0.3)	0.440
Hematologic test results				
Platelets ($\times 10^3$/µl)	267.1 (78.1)	221.3 (61.2)	290.0 (76.8)	0.028
Leukocytes ($\times 10^3$/µl)	7.2 (1.4)	7.1 (1.4)	7.2 (1.4)	0.881
Prothrombin time	11.7 (1.5)	12.5 (2.0)	11.3 (1.1)	0.131
INR	1.0 (0.1)	1.0 (0.2)	0.9 (0.1)	0.276
Lipid profiles				
HDL (mmol/L)	49.1 (16.8)	49.7 (25.8)	48.9 (10.9)	0.933
LDL (mmol/L)	147.4 (53.2)	119.3 (55.8)	161.4 (47.4)	0.051
Cholesterol (mmol/L)	217.0 (50.8)	187.3 (38.5)	231.9 (50.5)	0.029
Triglycerides (mmol/L)	184.3 (126.4)	205.2 (101.6)	173.9 (138.7)	0.554
Hyaluronic acid (ng/ml)	228.8 (155.0)	373.1 (184.8)	156.7 (64.6)	0.008

Abbreviation: BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL, high density lipoprotein; LDL, low density lipoprotein.

in Table 2. Mean HA level in patient group F0, F1, F2 and F3 were 170.4 ng/ml, 135.2 ng/ml, 383.2 ng/ml and 370.2 ng/ml, respectively. When mean HA level of each fibrosis grade were compared, we found the mean level of HA in F0 group was not significantly different from F1, but the mean level of HA in F0, F1 were significantly lower than that of F2 and F3 level. The mean levels of HA in F2 and F3 were not significantly different.

When we used the cut off level of serum HA level at 218.5 ng/ml, sensitivity, specificity, positive predictive value, negative predictive value and accuracy to discriminate between significant fibrosis and non significant fibrosis patients were 77.78%, 88.89%, 77.78%, 88.89% and 85.18, respectively, A receiver operating characteristic (ROC) curve was generated and the area under curve was 0.846 (Figure1).

Table 2 Mean level of hyaluronic acid according to fibrosis stages

Fibrosis score	N	Serum HA (ng/ml)
F0	11	170.4 ± 88.9*
F1	7	135.2 ± 59.0#
F2	2	383.2 ± 353.9**
F3	7	370.2 ± 156.8

* p <0.05 to compare with F2, F3, p >0.05 to compare with F1
# p <0.05 to compare with F2, F3
** p >0.05 to compare with F3

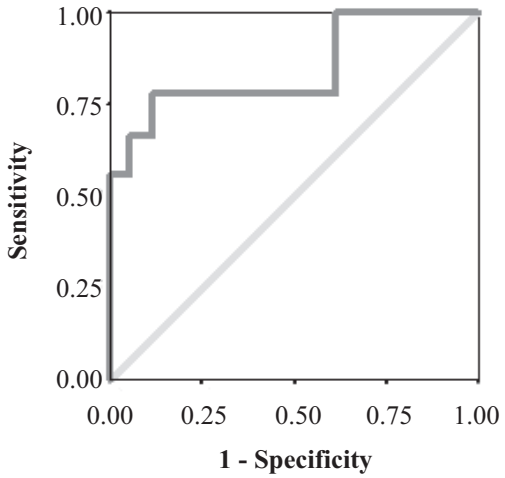

Figure 1 ROC curve of serum HA distinguishing patients with and without significant fibrosis. The area under curve was 0.846

DISCUSSION

In this study, the marker of liver fibrosis; Hyaluronic acid correlated well with the degree of liver fibrosis. The validly of HA in prediction of significant fibrosis was calculated by the receiver operating characteristic curve. By using cut off level at 218.5 ng/ml, the sensitivity was 77.78% and specificity was 88.89%. Our findings were consistent with previous report suggesting high serum HA correlates with a significant degree of fibrosis in NAFLD. Although the cut off level was not well described, Fabrice, *et al*.[12] found that none of patients with HA level <35 μg/L had significant fibrosis. Sakukawa, *et al*.[14] reported that the cut off level of HA to predict fibrosis was >50 ng/ml, with sensitivity and specificity were 68.8% and 82.8%, respectively. Combination of HA and type 4 collagen 7S domain had a higher specificity (92%).

The other factors that correlated with fibrosis in this study were age and platelets count. Platelets count was correlated with liver dysfunction and might be more useful to predict the presence of severe fibrosis compare with other markers of liver fibrosis[15]. Several studies have shown that age, diabetes mellitus, serum ALT and AST/ALT ratio were predictors of advance liver disease and fibrosis stage[16-18]. In our study overweight was found in 25 patients and diabetes mellitus was fond in 7 patients. However, both factors were not correlated with the degree of fibrosis. The AST/ALT ratio was less than 1 in all patients but not useful to discriminate between significant and non significant fibrosis. Surprisingly, the level of cholesterol was significant lower in fibrosis group but the explanation of these finding is unclear.

A limitation of the present study was that the small number of patients. Thus, further studies are needed to determine the best cut off value of HA level to predict liver fibrosis in NAFLD.

In conclusion, the biochemical markers of liver fibrosis, hyaluronic acid can be used a marker to predict the degree of hepatic fibrosis in patients with NAFLD.

REFERENCES

1. Metteloni CA, Younossi ZM, Gramlich T, *et al*. Non alcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999; 116: 1413-9.

2. Falch-Yttler Y, McCoollough AJ, Younossi ZM, *et al*. Clinical features and natural history of nonalcoholic steatosis syndromes. Semin Liver Dis 2001; 21: 17-26.
3. Picani F, Sagnelli E, Pasguale G, *et al*. Complication following percutaneous liver biopsy: a multicenter retrospective study on 68,276 biopsies. J Hepatol 1986; 2: 167-73.
4. Burt AD.C.L Oakley lecture. Cellular and molecular aspects of hepatic fibrosis. J Pathol 1993; 170: 105-14.
5. Franch P, Hahn EG. Significance of serum matrix proteins in the diagnosis of cirrhosis. In: Clement B, Guillouzo A editors. Cellular and molecular aspects of cirrhosis. Montrouge, France: John Libbey Eurotext; 1992. p. 333-43.
6. George DK, Ramm GA, Walker NI, *et al*. Elevated serum type IV collagen: a sensitive indicator of the presence of cirrhosis in hemochromatosis. J Hepatol 1999; 31: 47-52.
7. Walsh KM, Timms P, Campbell S, *et al*. Plasma levels of matrix metalloproteinase-1 and -2 (TIMP1 and TIMP2) as non invasive markers of liver disease in chronic hepatitis C: comparison using ROC analysis. Dig Dis Sci 1999; 44: 624-30.
8. Barry TK, Nezam A. Non invasive ascessment of liver fibrosis. Clin Liver Dis 2005; 9: 667-83.
9. McHutchison JG, Blatt LM, De Medina M, *et al*. Measurement of serum hyaluronic acid in patients with chronic hepatitis C and its relationship to liver histology. Consensus interferon Study group. J Gastroenterol Hepatol 2000; 15: 945-51.
10. Frebourg J, Delpech B, Bercoff E, *et al*. Serum hyaluronate in liver disease: study by enzymimmunological assay. Hepatology 1986; 6: 392-5.
11. Tangkitvanich P, Kongtawelert P, Pothacharoen P, *et al*. Serum hyaluronan; a marker of liver fibrosis in patient with chronic liver disease. Asian Pacific J Allergy Immunol 2003; 21: 115-20.
12. Fabrice L, Claude B, Romaine M, *et al*. Predictor of liver fibrosis in patient with features of the metabolic syndrome reguardless of alcohol consumption. Hepatology 2004; 39: 1639-46.
13 Brunt EM, Janney CG, Di Bisceglie AM, *et al*. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999; 94: 2467-74.
14. Shimadal M, Hashimoto E, Kaneda H, *et al*. Non alcoholic steatohepatitis: risk factor for liver fibrosis. Hepatol Res 2002; 24: 429-38.
15. Angulo P, Keach JC, Batts KP, *et al*. Independent predictors of liver fibrosis patients with nonalcoholic steatohepatitis. Hepatology 1999; 30: 1356-62.
16. Teli MR, James OFW, Burt AP, *et al*. The natural history of Non alcoholic fatty liver: a follow up study. Gastroenterology 1995; 22: 1714-9.
17. Ratziu V, Giral P, Charlotte F, *et al*. Liver fibrosis in overweight patients. Gastroenterology 2000; 118: 1117-23.
18. Powell EE, Cooksley WGE, Hanson R, *et al*. The natural history of non alcoholic steatohepatitis: a follow up study of forty two patients for up to 21 years. Hepatology 1990; 11: 74-80.