Korean J Gastroenterol Vol. 74 No. 6, 341-348
https://doi.org/10.4166/kjg.2019.74.6.341
pISSN 1598-9992 eISSN 2233-6869

ORIGINAL ARTICLE

KJG

만성 B형간염, 비알코올성 지방간 질환 환자에서 혈청 Asialo-α1-acid Glycoprotein의 진행성 간섬유화, 간경변증 예측력 연구

김승업[1,2,3], 전미영[1,3], 임태섭[1,3]

연세대학교 의과대학 내과학교실[1], 소화기병 연구소[2], 세브란스병원 연세간센터[3]

Diagnostic Performance of Serum Asialo-α1-acid Glycoprotein for Advanced Liver Fibrosis or Cirrhosis in Patients with Chronic Hepatitis B or Nonalcoholic Fatty Liver Disease

Seung Up Kim[1,2,3], Mi Young Jeon[1,3] and Tae Seop Lim[1,3]

Department of Internal Medicine[1], Institute of Gastroenterology[2], Yonsei University College of Medicine; Yonsei Liver Center, Severance Hospital[3], Seoul, Korea

Background/Aims: The utility of asialo-α1-acid glycoprotein (AsAGP) for assessing the fibrotic burden is unknown. This study examined the diagnostic performance of the AsAGP level for advanced liver fibrosis or cirrhosis in patients with chronic hepatitis B (CHB) or nonalcoholic fatty liver disease (NAFLD).
Methods: From July to December 2018, 48 patients with CHB and 75 with NAFLD were recruited prospectively. Transient elastography was used as the reference standard for liver fibrosis, and the cutoff liver stiffness values were defined as 10.0 kilopascal (kPa) for ≥F3 and 12.0 kPa for F4 in CHB patients, and 9.0 kPa for ≥F3 and 11.8 kPa for F4 in NAFLD patients.
Results: To predict stage ≥F3 and F4 fibrosis, the areas under the receiver operating characteristic curves of the AsAGP level in patients with CHB were 0.788 (95% CI 0.647-0.930; p=0.005) and 0.825 (95% CI 0.674-0.976; p=0.004), respectively. The cutoff AsAGP levels in patients with CHB that maximized the sum of the sensitivity and specificity values were 1.31 (sensitivity 100.0%, specificity 52.6%) and 1.55 (sensitivity 75.0%, specificity 80.0%), respectively. In contrast, the AsAGP level was similar regardless of the fibrosis stage in patients with NAFLD (all p>0.05 between the stages).
Conclusions: The AsAGP level showed acceptable diagnostic accuracy in predicting advanced liver fibrosis and cirrhosis in patients with CHB but not in those with NAFLD. Further studies will be needed to validate the diagnostic performance of the AsAGP level in patients with NALFD. **(Korean J Gastroenterol 2019;74:341-348)**

Key Words: Fibrosis; Hepatitis B; Non-alcoholic fatty liver disease; Liver cirrhosis

INTRODUCTION

An accurate assessment of the severity of liver fibrosis in patients with chronic hepatitis B (CHB) is essential not only for predicting the long-term clinical course but also for determining if and when to begin antiviral therapy. The most recent guidelines on the management of CHB propose that the presence of significant fibrosis and detectable HBV DNA

Received April 26, 2019. **Revised** July 9, 2019. **Accepted** August 13, 2019.

교신저자: 김승업, 03722, 서울시 서대문구 연세로 50-1, 연세대학교 의과대학 내과학교실
Correspondence to: Seung Up Kim, Department of Internal Medicine, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea. Tel: +82-2-2228-1944, Fax: +82-2-393-6884, E-mail: ksukorea@yuhs.ac, **ORCID:** https://orcid.org/0000-0002-9658-8050

Financial support: This study was supported by Diagen Inc. The funders had no role in study design, data collection and analysis, decision to publish, or manuscript preparation.

Conflict of interest: None.

indicate the need for antiviral therapy because the maintenance of viral suppression can reduce the incidence of liver-related complications, including hepatocellular carcinoma (HCC), in patients with CHB who have significant fibrosis or cirrhosis.[1-3]

Among the categories of nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH) is the most progressive and severe condition.[4] NASH is defined as steatosis in the presence of hepatocyte damage, inflammation, and/or subsequent scarring and a replacement of tissue with type I collagen. In contrast to the benign prognosis of simple steatosis, approximately 10-29% of patients with NASH develop cirrhosis within 10 years, which is often followed by liver-related complications, including HCC.[5] Therefore, similar to CHB, the assessment of fibrosis progression in patients with NAFLD is important for identifying those at high risk and determining the optimal time to commence medical interventions.

Thus far, liver biopsy is the gold standard for assessing liver fibrosis, but it has disadvantages, such as invasiveness, cost, risk of complications, lack of available expert practitioners, and intra/interobserver variability.[6] A recent study reported that the serum asialo-α1-acid glycoprotein (desialylated α1-acid glycoprotein; AsAGP) level is elevated in patients with liver diseases compared to healthy controls.[7] In addition, the AsAGP level showed acceptable diagnostic accuracy for liver cirrhosis and HCC.[8] On the other hand, its ability to predict the fibrotic burden in patients with chronic liver diseases is not known. Therefore, this study examined the diagnostic performance of the AsAGP level for the fibrotic burden, as assessed by transient elastography (TE, FibroScan®; EchoSens, Paris, France), in patients with CHB or NAFLD.

SUBJECTS AND METHODS

1. Patients

Patients with CHB and NALFD, who underwent TE (FibroScan®; EchoSens) at Yonsei Liver Center, Severance Hospital, Yonsei University College of Medicine, Seoul, South Korea, from July 2018 to December 2018 were recruited into this prospective study. CHB was defined as the presence of the serum HBV surface antigen for >6 months.[9] NAFLD was defined as a controlled attenuation parameter (CAP) value >250 dB/m, as assessed by TE (FibroScan®; EchoSens), which indicates the presence of fat in the liver after excluding the secondary causes of fat accumulation in the liver, such as significant alcohol consumption.[10]

The exclusion criteria were as follows: 1) age <19 years, 2) alcohol ingestion in excess of 40 g/day for >5 years, 3) malignancy, 4) an ALT level >5×the upper limit of normal, 5) total bilirubin level >2.0 mg/dL, 6) HCV infection, 7) TE (FibroScan®; EchoSens) failure or invalid liver stiffness (LS) values, 8) heart failure, 9) ascites, 10) pregnancy, 11) decompensated liver diseases, 12) refusal to provide informed consent, and 13) deviations from the protocol.

The study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. Written informed consent was obtained from each participant or responsible family member. The Institutional Review Board of Severance Hospital approved the study (2018-0483-001).

2. Recruitment information

Initially, this prospective clinical trial assessed the diagnostic accuracy of AsAGP in patients with HBV and NAFLD using the LS values determined using TE (FibroScan®; EchoSens) as the gold standard. The calculated sample size of each fibrosis stage from F0 to F4 was 21 (105 each for HBV and NAFLD; 210 in total). On the other hand, this clinical trial was terminated earlier than expected because of the delayed enrollment beyond the planned study period, which led to a final sample size of 123 (48 with HBV and 75 with NAFLD).

3. Assessment of LS by TE (FibroScan®; EchoSens)

LS was assessed by TE (FibroScan®; EchoSens), which relies on a calculation of the liver elasticity from the velocity of a low-frequency elastic wave transmitted through the liver, has good diagnostic accuracy for advanced fibrosis or cirrhosis in patients with various chronic liver diseases, including CHB and NAFLD.[11,12] Therefore, the LS values determined by TE (FibroScan®; EchoSens) were used as a reference.

A well-trained technician, who was blinded to the patients' clinical and laboratory data, performed TE (FibroScan®; EchoSens). Details of the technique and examination procedure are reported elsewhere.[13-20] The results are expressed in kilopascal (kPa). The interquartile range is an index of the intrinsic variability among the LS values and corresponds to the range of LS values encompassing 50% of the valid measurements (i.e., between the 25th and 75th percentiles). The

median value was considered representative of the elastic modulus of the liver. Only procedures with at least 10 valid measurements, a success rate of at least 60%, and a ratio of the interquartile range to median <30% were considered reliable.

4. Grading of fibrosis stage according to LS and fibrosis-4 index

The fibrosis stage was determined based on the established cutoff LS values (10.0 kPa for ≥F3 and 12.0 kPa for F4 in patients with CHB; 9.0 kPa for ≥F3 and 11.8 kPa for F4 in patients with NAFLD).[21,22] To validate the results based on the LS value, the fibrosis-4 index was also calculated using the following formula: age (years)×AST (U/L)/[platelets (10^9/L)×ALT (U/L)]$^{1/2}$. The fibrosis-4 index ≥2.67 indicated a high probability of advanced fibrosis.[23]

5. Measurement of the serum AsAGP level

The serum AsAGP level was measured by an antibody-lectin sandwich immunoassay using an AceGP® ELISA kit (Diagen Inc., Seoul, Korea), as described previously.[8]

6. Statistical analyses

The data are expressed as the mean±standard deviation, median (range), or number (%), where appropriate. When comparing the baseline characteristics between the two groups, a chi-squared test and a Fisher's exact test were used for the categorical variables, and a Student's t-test and a Mann-Whitney U-test was used for the continuous variables. The correlation between AsAGP and other continuous variables was assessed using Pearson's correlation. The diagnostic performance of the noninvasive indices was assessed using the receiver operating characteristic (ROC) curves and the areas under the ROC curves (AUCs). The cutoff values that maximized the sum of the sensitivity and specificity were calculated from the ROC curves. Multivariable binary logistic regression was used to identify the independent predictors of advanced fibrosis or cirrhosis. Statistical analyses were performed using SAS software version 9.1.3 (SAS institute, Cary, NC, USA). A p-value <0.05 was considered significant.

RESULTS

1. Baseline characteristics of the study population

This study screened 131 patients (49 with CHB and 82 with NAFLD) for inclusion. After exclusion of eight patients, the remaining 123 (48 with CHB and 75 with NAFLD) were analyzed. Table 1 lists the baseline characteristics of the study population. The mean age of the study population (74

Table 1. Baseline Characteristics of the Study Population

Variable	All (n=123)	HBV (n=48)	NAFLD (n=75)
Demographic variables			
Age (years)	47.3±13.7	47.6±11.4	47.1±15.1
Male	74 (60.2)	27 (56.3)	47 (62.7)
Diabetes	23 (18.7)	4 (8.3)	19 (25.3)
Hypertension	33 (26.8)	11 (22.9)	22 (29.3)
Antiviral therapy	-	11 (22.9)	-
Laboratory variables			
Aspartate aminotransferase (U/L)	37.2±23.0	32.2±14.8	40.0±26.4
Alanine aminotransferase (U/L)	37.7±27.1	33.5±21.5	40.2±29.9
Total bilirubin (mg/dL)	0.8±0.4	0.9±0.4	0.8±0.4
Serum albumin (g/dL)	4.4±0.4	4.3±0.3	4.5±0.4
Transient elastography			
Liver stiffness (kPa)	9.0±9.4	10.4±12.5	8.1±6.7
Controlled attenuation parameter (dB/m)	288.3±48.5	254.4±47.3	310.0±35.2
AsAGP (μg/mL)	1.37±0.26	1.44±0.30	1.32±0.21

Values are presented as mean±standard deviation or n (%).
HBV, hepatitis B virus; NAFLD, nonalcoholic fatty liver disease; kPa, kilopascal; AsAGP, asialo-α1-acid glycoprotein.

males and 49 females) was 47.3 years. Diabetes and hypertension were identified in 23 (18.7%) and 33 (26.8%) patients, respectively. The mean AST and ALT levels were 37.2 and 37.7 U/L, respectively. The mean LS and CAP values were 9.0 kPa and 288.3 dB/m, respectively. The mean AsAGP level was 1.37 μg/mL. The mean AsAGP levels of the patients with HBV and those with NAFLD were 1.44 and 1.32 μg/mL, respectively. Among the patients with HBV, 11 (22.9%) were receiving antiviral therapy.

2. Correlations among the AsAGP level, LS, and other variables

Table 2 lists the correlations among the AsAGP level, LS, and other variables. The AsAGP level showed a significant negative correlation with the serum albumin level (r=-0.207, p=0.024) and CAP value (r=-0.291, p<0.001). LS showed a significant positive correlation with the AST level (r=0.419), ALT level (r=0.305), and total bilirubin level (r=0.180) (all p<0.05). On the other hand, the LS did not correlate significantly with the AsAGP level (p=0.170).

3. Distributions of the LS-based fibrosis stage and corresponding AsAGP level

Table 3 lists the distributions of the LS-based fibrosis stage

Table 2. Correlation between the AsAGP, Liver Stiffness, and Other Variables

Variable	AsAGP		Liver stiffness	
	Correlation coefficient	p-value	Correlation coefficient	p-value
Age (years)	0.176	0.052	0.130	0.150
Aspartate aminotransferase (U/L)	-0.006	0.946	0.419	<0.001
Alanine aminotransferase (U/L)	-0.124	0.179	0.305	0.001
Total bilirubin (mg/dL)	0.146	0.111	0.180	0.049
Serum albumin (g/dL)	-0.207	0.024	-0.114	0.216
Liver stiffness (kPa)	0.124	0.170	-	-
Controlled attenuation parameter (dB/m)	-0.291	<0.001	0.075	0.412
AsAGP (μg/mL)	-	-	0.124	0.170

kPa, kilopascal; AsAGP, asialo-α1-acid glycoprotein.

Table 3. Distribution of Liver Stiffness-based Fibrosis Stage and Corresponding AsAGP Level

Fibrosis stage	HBV (n=48)		p-value	NAFLD (n=75)		p-value
	Patients	AsAGP		Patients	AsAGP	
Each fibrosis stage						
F0	20 (41.7)	1.36±0.25		21 (28.0)	1.39±0.25	
F1	16 (33.3)	1.40±0.28		20 (26.7)	1.27±0.19	
F2	2 (4.2)	1.29±0.22	0.014[a]	21 (28.0)	1.32±0.22	0.349[a]
F3	2 (4.2)	1.40±0.04		8 (10.7)	1.26±0.15	
F4	8 (16.7)	1.77±0.34		5 (6.7)	1.26±0.13	
Advanced fibrosis						
F0-2	38 (79.2)	1.37±0.26	0.002	62 (82.7)	1.33±0.23	0.851
F3-4	10 (20.8)	1.69±0.34		13 (17.3)	1.26±0.14	
Liver cirrhosis						
F0-3	40 (83.3)	1.38±0.25	<0.001	70 (93.3)	1.32±0.22	0.760
F4	8 (16.7)	1.77±0.34		5 (6.1)	1.26±0.13	

Values are presented as mean±standard deviation or n (%).
AsAGP, asialo-α1-acid glycoprotein; HBV, hepatitis B virus; NAFLD, nonalcoholic fatty liver disease.
[a]One-way analysis of variance test.

and corresponding AsAGP level. Among the 48 patients with CHB, the mean AsAGP level increased with increasing fibrosis stage (1.36 μg/mL for F0 [n=20, 41.7%], 1.40 μg/mL for F1 [n=16, 33.3%], 1.29 μg/mL for F2 [n=2, 4.2%], 1.40 μg/mL for F3 [n=2, 4.2%], and 1.77 μg/mL for F4 [n=8, 16.7%]; p=0.014 by one-way ANOVA). The mean AsAGP level of the patients with stage F0-2 (n=38, 79.2%) or F0-3 (n=40, 83.3%) fibrosis was significantly lower than that of the patients with F3-4 (n=10, 20.8%) (1.37 vs. 1.69 μg/mL, p=0.002) or F4 (n=8, 16.7%) fibrosis (1.38 vs. 1.77 μg/mL; p<0.001).

Among the 75 patients with NAFLD, 21 (28.0%), 20 (26.7%), 21 (28.0%), 8 (10.7%), and 5 (6.7%) had F0, F1, F2, F3, and F4 fibrosis, respectively. The AsAGP level did not differ significantly according to the fibrosis stage (mean AsAGP 1.26-1.39) (p=0.349 by one-way ANOVA). No significant difference in the AsAGP level was observed between the patients with F0-2 (n=62, 82.7%) or F3-4 (n=13, 17.3%) fibrosis and those with F0-3 (n=70, 93.3%) or F4 (n=5, 6.1%) fibrosis (all p>0.05).

4. Independent predictors of advanced fibrosis and cirrhosis

Table 4 lists the results of univariable and subsequent multivariable analysis. Older age (OR=1.143; 95% CI 1.014-1.289; p=0.028), higher AST level (OR=1.082; 95% CI 1.008-1.161; p=0.030), and higher AsAGP level (OR=18.818; 95% CI 1.104-320.675; p=0.043) were selected as independent predictors of advanced fibrosis (F3-4), whereas a higher AST level (OR=1.126; 95% CI 1.014-1.251; p=0.007) and higher AsAGP level (OR=78.400; 95% CI 1.848-3,326.437; p=0.023) were selected as independent predictors of cirrhosis (F4).

5. Diagnostic performance of AsAGP for LS-based advanced fibrosis and cirrhosis

The ability of the AsAGP level to predict LS-based advanced fibrosis and cirrhosis in patients with CHB was next evaluated (Table 5). The AUCs for the AsAGP level for predicting LS-based advanced fibrosis and cirrhosis was 0.788 (95% CI 0.647-0.930, p=0.005) and 0.825 (95% CI 0.674-0.976, p=0.004), respectively. The cutoff values that maximized the

Table 4. Predictors of Advanced Fibrosis Stage (F3-4) and Cirrhosis (F4) in Patients with HBV

Variable	Advanced fibrosis			Cirrhosis		
	Univariable	Multivariable		Univariable	Multivariable	
	p-value	OR (95% CI)	p-value	p-value	OR (95% CI)	p-value
Age (years)	0.004	1.143 (1.014-1.289)	0.028	0.001	1.172 (0.990-1.387)	0.065
Male	0.331			0.081		
Diabetes	0.831			0.644		
Hypertension	0.159			0.292		
Aspartate aminotransferase (U/L)	0.008	1.082 (1.008-1.161)	0.030	0.007	1.126 (1.014-1.251)	0.026
Alanine aminotransferase (U/L)	0.344			0.259		
Controlled attenuation parameter (dB/m)	0.739			0.541		
AsAGP	0.007	18.818 (1.104-320.675)	0.043	0.004	78.400 (1.848-3,326.437)	0.023

HBV, hepatitis B virus; OR, odds ratio; CI, confidence interval; AsAGP, asialo-α1-acid glycoprotein.

Table 5. Diagnostic Accuracy of AsAGP to Predict Liver Stiffness-based Advanced Fibrosis Stage (F3-4) and Cirrhosis (F4) in Patients with HBV

Diagnostic index	F3-4	F4
AUC	0.788	0.825
95% CI	0.647-0.930	0.674-0.976
p-value	0.005	0.004
Cutoff value	1.31	1.55
Sensitivity	100.0%	75.0%
Specificity	52.6%	80.0%

AsAGP, asialo-α1-acid glycoprotein; HBV, hepatitis B virus; AUC, area under curve; CI, confidence interval.

sum of the sensitivity and specificity were 1.31 for advanced fibrosis (sensitivity 100% and specificity 52.6%) and 1.55 for cirrhosis (sensitivity 75.0% and specificity 80.0%). When the fibrosis-4 index was used to define the fibrotic burden in the subgroup with the availability of the fibrosis-4 index value (n=21 with HBV), the AUC value to predict the advanced fibrosis stage was 0.947 (95% CI 0.847-1.000; p=0.042).

DISCUSSION

Several noninvasive surrogates, such as TE (FibroScan®; EchoSens), FibroTest (Biopredictive, Paris, France), and enhanced liver fibrosis, have been proposed to assess the degree of liver fibrosis. On the other hand, the patency and high cost of the device have limited their widespread use in clinical practice. Recently, altered glycosylation of plasma glycoproteins has been reported in various liver pathologies.[24] For example, increased AGP fucosylation in the ascitic fluid of patients with cirrhosis,[24,25] haptoglobin fucosylation in patients with alcoholic liver disease,[26,27] serum cholinesterase fucosylation in patients with cirrhosis,[28,29] fucosylation of α-fetoprotein and other serum glycoproteins in patients with HCC,[30,31] and serum AsAGP in patients with cirrhosis and HCC[7,8] have been reported.

In this study, the AsAGP level was not associated with the AST or ALT level. Furthermore, the AsAGP level increased significantly with the fibrosis stage in patients with CHB but not in those with NAFLD. In addition, the AsAGP level showed acceptable diagnostic performance for advanced fibrosis and cirrhosis. To the best of the authors' knowledge, this is the first study to assess the diagnostic performance of AsAGP and define the optimal cutoff values for advanced fibrosis and cirrhosis in a homogenous population of Asian patients with CHB.

These findings have several clinical implications. First, patients who underwent AsAGP testing and an LS-based fibrosis assessment, which shows excellent diagnostic performance in Asian patients with CHB, were recruited prospectively as a reference.[11] In addition, the optimal cutoff AsAGP levels for advanced fibrosis and cirrhosis for patients with CHB were proposed. Although validation studies will be needed, the proposed cutoff values can be used as a reference in future studies involving Asian patients with CHB. Second, the diagnostic performance of AsAGP was acceptable (AUCs of 0.788 for advanced fibrosis and 0.825 for cirrhosis), despite the relatively small population. Future studies involving more patients will be needed to validate these results. Third, the mean AsAGP level increased with increasing liver fibrosis stage. Although longitudinal validation will be required, these results suggest that the AsAGP level can be used to trace the changes in the fibrotic burden in patients with CHB. Fourth, in contrast to LS, which can be overestimated in the presence of hepatic necroinflammation or congestion,[32] the AsAGP level was not correlated significantly with the AST or ALT level (p>0.05).

On the other hand, patients with a higher ALT level (>5×upper limit of normal) were excluded to maintain the diagnostic accuracy of TE (FibroScan®; EchoSens) in assessing the fibrotic burden as the gold standard, but further validation will be needed to confirm the lack of a correlation between AsAGP and high ALT levels. Fourth, although the exact reason is unclear, several issues, such as an unbalanced distribution of fibrosis stage and early termination of trial leading to a relatively small sample size, might potentially be associated with the negative results in patients with NAFLD. Well-designed studies will be needed to solve this issue.

This study had several limitations. First, although the participants were recruited prospectively, the study was limited by its cross-sectional design. Therefore, it is unclear if repeated measurements of the AsAGP level would enable tracking of the progression of fibrosis and related clinical outcomes, such as the occurrence of hepatic decompensation and HCC. Therefore, the prognostic value of AsAGP should be evaluated. Second, the distribution of the fibrosis stage was skewed because of the relatively small sample size and recruitment from a tertiary hospital, possibly resulting in spectrum bias. Third, the AsAGP level was not correlated significantly with the fibrotic burden in patients with NAFLD. Because the diagnostic performance of a given noninvasive test tends to increase with the prevalence of higher-stage fibrosis, a study involving more patients with advanced fibrosis or cirrhosis will be needed. Fourth, the use of antivirals and hepatotonics was permitted. On the other hand, this probably did not influence the results because the LS measured by TE (FibroScan®; EchoSens) represents the remaining fibrotic burden in the liver at a particular point in time. Finally, the LS value assessed using TE (FibroScan®; EchoSens), not liver biopsy, was used as the gold standard for assessing the fibrotic burden in this study. Thus, these results should be interpreted cautiously.

In conclusion, the AsAGP level predicted advanced fibrosis or cirrhosis acceptably in Asian patients with CHB but not in those with NAFLD. The proposed optimal cutoff AsAGP level for patients with CHB may have utility as a reference in future research. Further largescale studies will be needed to validate the results.

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