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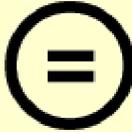
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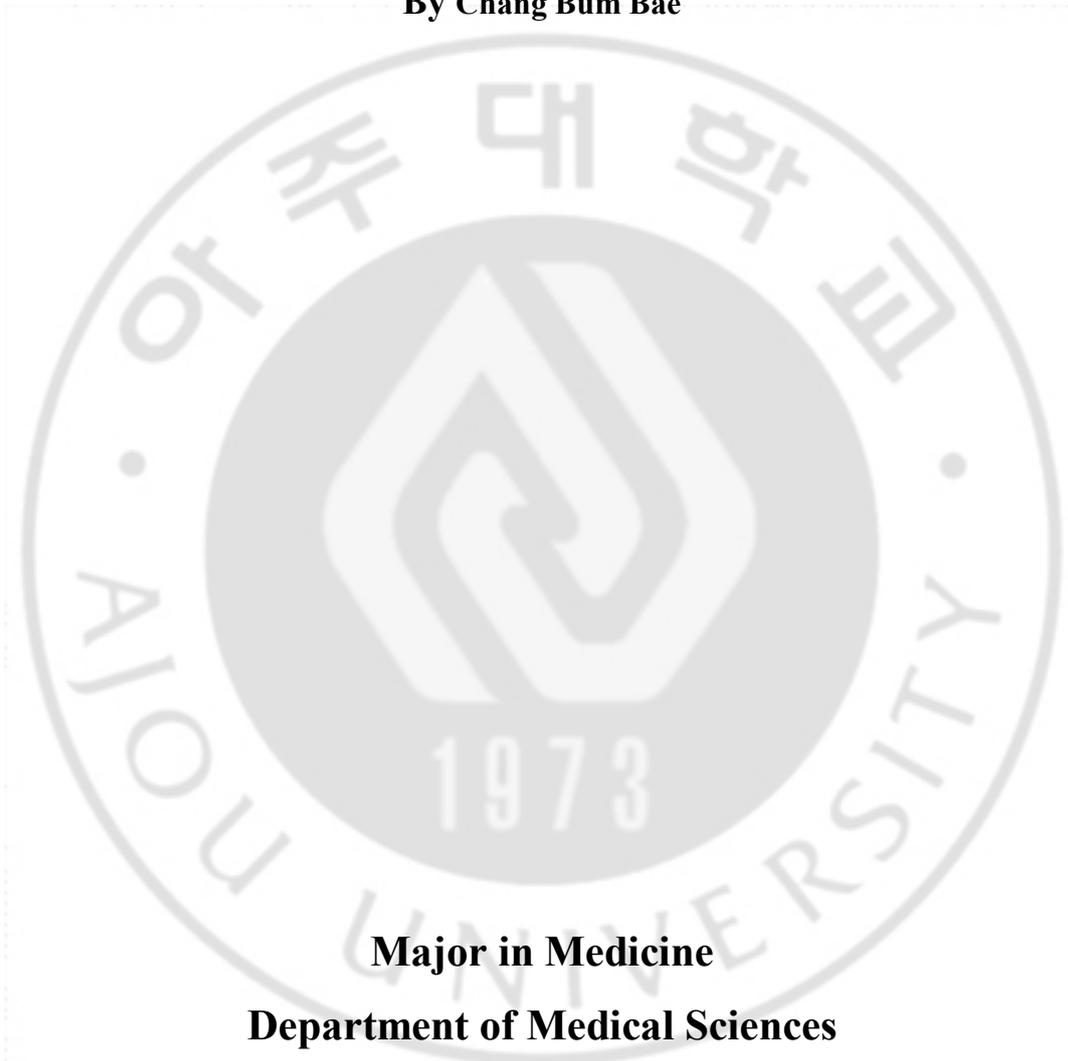
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**Caspase-cleaved fragments of cytokeratin-18 as a marker of
inflammatory activity in chronic hepatitis B virus infection**

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Ajou University in Partial Fulfillment of the Requirements for the Degree
of Master of Medicine**

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Caspase-cleaved fragments of cytokeratin-18 as a marker of inflammatory activity in chronic hepatitis B virus infection

Background: The differential diagnosis between inactive carrier and active hepatitis is important in patients with chronic hepatitis B (CHB) virus infection. Serum cytokeratin (CK)-18 fragments (M30 antigen) are proposed as biomarkers of apoptosis. We investigated whether serum M30 levels might help to characterize the various phases of CHB and predict the state of significant inflammation in patients with CHB.

Methods: A total of 339 CHB patients who underwent liver biopsy, were included. Serum M30 antigen levels were compared with inactive carriers (n=36), patients with HBeAg- negative hepatitis (n=80), HBeAg-positive hepatitis (n=141) and liver cirrhosis (n=82).

Results: Serum M30 antigen levels were correlated significantly not only with ALT ($r=0.315$, $p<0.001$) and AST ($r=0.544$, $p<0.001$), but also inflammatory grading score on liver biopsy ($r=0.240$, $p<0.001$). Multivariate analysis showed that AST ($P<0.001$), M30-antigen ($P=0.020$) and albumin ($P=0.009$) were the independent predictors of significant inflammation. The area under the curve of the receiver operating characteristic plot for the detection of significant inflammation of serum AST, M30-antigen and ALT levels were 0.872, 0.795 and 0.758 respectively. Combined serum M30 level (>344 U/L) and AST (>78 IU/L) measurements provided the most accurate identification of significant inflammation, showing 38.2% sensitivity, 96.1% specificity, 91.0% positive predictive value and 56.1% negative predictive value. We did not detect any significant differences in serum M30 levels between inactive HBV carrier and HBeAg-negative CHB.

Conclusions: CK-18 fragment levels are correlated with liver inflammation and serum M30 levels are associated with the presence of significant inflammation in patients with CHB.

Keyword: apoptosis, chronic hepatitis B, cytokeratin-18, significant inflammation

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I. INTRODUCTION

Chronic hepatitis B virus (HBV) infection remains a serious public health problem affecting more than 400 million people worldwide (Lavanchy D, 2004). According to the natural history, chronic HBV carriers can be presented with various clinical states as inactive HBV carriers, individuals with hepatitis B e antigen (HBeAg)-positive or HBeAg-negative chronic hepatitis B (CHB), or with liver cirrhosis (McMahon BJ, 2004). Inactive HBV carriers have low levels of HBV-DNA and normal serum aminotransferase and are at low risk to develop liver cirrhosis or hepatocellular carcinoma (HCC) (McMahon BJ, 2004). Since patients with HBeAg-negative CHB may often present with fluctuating serum ALT levels within the normal range and low viral replication, their differentiation from inactive HBV carriers may not be so evident (Hadziyannis SJ and Papatheodoridis GV, 2006; Manesis EK et al., 2003).

Caspase activation and apoptosis were found to be associated with the severity of inflammation in both experimental hepatitis and chronic hepatitis C virus (HCV) infection (Bantel H and Schulze-Osthoff K, 2003). There is increasing evidence that liver cell damage in chronic HBV infection is mediated by the induction of apoptosis (Malhi H et al., 2006). During apoptosis, caspases cleave cytokeratin (CK) which leads to the collapse of the cytoskeleton and the subsequent formation of apoptotic bodies (Galluzzi L et al., 2007). CK-18 is a major cytoplasmic intermediate filament protein in hepatocytes and epithelial cells (Omary MB et al., 2002). In vitro experiments have shown that the cellular release of CK-18 fragments into the extracellular space occurs during the intermediate stage of apoptosis as a consequence of caspase digestion (Oshima RG, 2002). The M30 apoptosis enzyme linked immunosorbent assay (ELISA) detects a neoepitope specific to apoptosis caused by caspase cleavage of CK-18 at aspartate 396 and may serve as a surrogate serum biomarker of hepatocyte apoptosis (Linder S, 2007).

Previous reports suggested that the serum levels of CK-18 are associated with the severity of liver disease in chronic HCV infection and non-alcoholic fatty liver disease (Bantel H et al., 2001; Wieckowska A et al., 2006). Serum M30 level was also reported as a marker to represent apoptotic activity in chronic HBV infection (Papatheodoridis GV et al., 2008).

Although histological examination of liver biopsies is the current gold standard for the detection of liver damage, laboratory tests are usually requested to assess hepatitis activity. The most popular test is serum alanine aminotransferase (ALT), followed by aspartate aminotransferase (AST). However, significant necroinflammation and fibrosis may be present despite persistently normal ALT levels in

chronic HBV and HCV carriers. For this reason, there is a strong need for better noninvasive markers to predict liver injury in patients with chronic viral hepatitis.

The aim of this study was to investigate whether serum levels of caspase-generated fragments of CK-18 may provide useful information on the extent of liver injury in various clinical conditions of HBV infection. Furthermore, we explored whether serum M30 measurement can be used to predict significant necroinflammation in patients with CHB.



II. MATERIAL AND METHODS

A. Patients

A total of 339 patients with chronic HBV infection were prospectively recruited from 6 medical centers (Ajou University Hospital, Hallym University Chuncheon, CHA University Hospital, Inje University Busan Paik Hospital, Pusan National University Hospital and Catholic University St. Vincent's Hospital) in South Korea from October 2005 to June 2009. All patients underwent liver biopsy and had not received antiviral treatment within 6 months prior to this study's enrollment. Patients were not included if they were detected with decompensated cirrhosis or other causes of liver disease, such as superinfection with other hepatitis viruses, autoimmune or metabolic liver disease and alcohol abuse, and hepatotoxic drug use.

The study population comprised of four subgroups at different stages of the natural course of chronic HBV infection according to the recent guideline by the American Association for the Study of Liver Diseases (Lok AS et al., 2009): 1) inactive HBV carrier (n=36), negative for HBeAg, serum HBV-DNA \leq 2,000 IU/mL, normal ALT ($<$ 40 U/L) for more than 12 months and inflammatory grade \leq 2 on liver biopsy 2) HBeAg-negative CHB (n=80), negative HBeAg and serum HBV-DNA $>$ 2,000 IU/mL and elevated ALT levels (above the upper limit of normal on $>$ 2 separate monthly determinations within the last 6 months) 3) HBeAg-positive CHB (n=141) was defined as patients with CHB who have positive HBeAg and 4) liver cirrhosis (n=82) was confirmed by liver biopsy. The study protocols were approved by the Institutional Review Board of Human Research of Ajou University Hospital (AJIRB-MED-KSP-11-372) and the local research ethics committees at all participating hospitals. Informed consent to participate in the study was obtained from all study subjects.

B. Serological test and measurement of cytokeratin 18 fragment (M30 antigen)

Serum samples for M30-antigen were obtained and routine blood tests were performed at the time of liver biopsy and were processed immediately. Routine blood tests were measured using standard methodologies. HBV markers included HBsAg, antibody to hepatitis B surface antigen, HBeAg, and antibody to hepatitis e-antigen (Abbot Laboratories). Serum HBV DNA was quantified using COBAS TaqMan polymerase chain reaction assay (Roche Diagnostics, Branchburg, NJ, USA; lower limit, 50 copies/mL).

Specific quantitative detection of caspase-cleaved and apoptosis-associated CK-18 (M30-antigen) in serum was carried out using sandwich enzyme immunoassay according to manufacturer's instructions

(M30-Apoptosense ELISA, Peviva AB, Sweden).

C. Histologic examination

Liver biopsy specimens were fixed with 10% formalin, routinely embedded in paraffin, and tissue sections were processed with hematoxylin and eosin, Masson's trichrome, and reticular fiber staining. Histologic examination was performed according to the Batts-Ludwig scoring system. Based on the Batts-Ludwig scoring system, grade 1 denotes necroinflammatory activities largely confined to the portal areas, grades 2-3 indicates an extension beyond the portal areas, and grade 4 signifies confluent necrosis in the form of bridging necrosis. All biopsy specimens were analyzed by two hepatopathologists (Y.B.Kim and Y.N. Park) who were blinded to patient information. At the end of a study, discrepancies between two pathologists were resolved by joint discussion at the microscope.

D. Statistical analysis

To compare M30-antigen levels among the four subgroups at different stages of the natural course of chronic HBV infection, statistical analysis was performed using the parametric independent t test and ANOVA test.

Univariate chi-square and *t*-test analyses were performed to identify variables that were significantly different between patients with (grade 3, 4) and without (grade 0, 1, 2) significant inflammation. To identify predictive factors for significant inflammation, we used multivariate logistic regression with forward selection for the significant variable on the univariate analysis ($P < 0.05$). We selected a cutoff value for each factor at the level greater than 90% specificity on the area under receiver operating characteristics (AUROC) curve. Diagnostic accuracies were evaluated by calculating sensitivities, specificities, positive predictive values (PPVs) and negative predictive values (NPVs). All statistical tests were two-sided and performed with SPSS software version 16.0 (SPSS Inc., Chicago, IL).

III. RESULTS

A. Patient characteristics

The baseline characteristics of the enrolled patients are outlined in Table 1. Three hundred and thirty nine patients (234 men and 105 women) between 18 and 70 years of age (mean, 42.98 ± 18.10 years) were included. According to the biochemical, virologic, serologic and histologic states, the patients were classified into four groups: inactive carriers, HBeAg-positive CHB patients, HBeAg-negative CHB patients and liver cirrhosis patients. The distribution of inflammatory activity grades was as follows: grade 1=43 (12.7%); grade 2=109 (32.2%); grade 3=116 (34.2%); and grade 4=71 (20.9%)(Table 1). The distribution of fibrosis stages of all patients was as follows. F0=12 (3.5%); F1=54 (15.9%); F2=100 (29.5%); F3=92 (27.1%); and F4=82 (24.2%).

As inactive carriers and HBeAg-negative CHB patients have similar serologic profiles but different prognoses and risks of disease progression, we compared biochemical, virologic and histologic parameters between the two groups. As expected, inactive carriers had significantly lower AST, ALT and serum HBV-DNA than those of HBeAg-negative CHB patients ($p < 0.001$)(Table 2). Histologic findings demonstrated that inactive carriers had a lower grade of inflammation ($p < 0.001$) and a lower stage of fibrosis ($p < 0.001$, $p = 0.009$) than those of HBeAg-negative CHB (Table 2).

Table 1. Baseline characteristics of the patients.

Characteristics	All patients (n=339)	Inactive carrier (n=36)	HBeAg(-) CHB (n=80)	HBeAg(+) CHB (n=141)	Liver cirrhosis (n=82)
Age (years)	42.98 ± 18.10	40.83 ± 10.41	43.42 ± 12.46	42.51 ± 9.53	46.48 ± 14.6
Gender (M/F)	234/105	30/6	54/26	96/45	54/28
AST (U/L)	87.24 ± 98.51	34.86 ± 17.46	94.45 ± 89.11	100.16 ± 92.2	80.89 ± 119.9
ALT (U/L)	122.3 ± 146.6	50.86 ± 44.75	135.6 ± 130.9	146.5 ± 151.4	98.54 ± 149.4
Platelet (x10 ³ /mm ³)	190.0 ± 64.04	211.0 ± 80.24	191.0 ± 60.33	204.0 ± 56.95	156.0 ± 58.70
GGT (U/L)	89.19 ± 177.3	100.1 ± 105.1	78.60 ± 94.89	78.60 ± 94.89	131.4 ± 484.9
HBV DNA (copies/ml)	6.9x10 ⁷ (60- 1.3x10 ⁹)	5.5x10 ² (60- 2.0x10 ³)	2.0x10 ⁷ (4.4x10 ² - 1.1x10 ⁸)	1.2x10 ⁸ (1.0x10 ³ - 1.3x10 ⁹)	1.9x10 ² (60- 2.5x10 ⁸)
Grade of Inflammation					
G1	43 (12.7%)	14 (38.9%)	7 (8.8%)	15 (10.6%)	7 (8.5%)
G2	109 (32.2%)	22 (61.1%)	22 (27.5%)	34 (24.1%)	31 (37.8%)
G3	116 (34.2%)	0	34 (42.5%)	54 (38.3%)	28 (34.1%)
G4	71 (20.9%)	0	17 (21.2%)	38 (27.0%)	16 (19.5%)
Stage of fibrosis					
F0	12 (3.5%)	3 (8.3%)	1 (1.2%)	8 (5.7%)	0
F1	54 (15.9%)	15 (41.7%)	15 (18.8%)	24 (17.0%)	0
F2	100 (29.5%)	7 (19.4%)	28 (35.0%)	65 (46.1%)	0
F3	91 (26.8%)	11 (30.6%)	36 (45.0%)	44 (31.2%)	0
F4	82 (24.2%)	0	0	0	82 (100%)
M30-antigen (U/L)	489.81	225.96	411.94	587.61	513.45

HBeAg, hepatitis B e antigen; CHB, chronic hepatitis B; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ - glutamyl transpeptidase.

Table 2. Comparison of biochemical, virologic and histologic profiles between inactive carriers and patients with HBeAg-negative chronic hepatitis B.

	Inactive carrier (n=36)	HBeAg(-) CHB (n=80)	p-value
AST (U/L)	34.86 ± 17.46	94.45 ± 89.11	< 0.001
ALT (U/L)	50.86 ± 44.75	135.6 ± 130.9	< 0.001
HBV DNA, (copies/ml)	5.5x10 ² (60-2.0x10 ³)	2.0x10 ⁷ (4.4x10 ² -1.1x10 ⁸)	< 0.001
Grade of inflammation	1.61 ± 0.49	2.76 ± 0.89	< 0.001
Stage of fibrosis	1.72 ± 1.00	2.24 ± 0.80	0.009

HBeAg, hepatitis B e antigen; CHB, chronic hepatitis B; AST, aspartate aminotransferase; ALT, alanine aminotransferase.



B. M30 antigen levels correlate with serum markers of liver inflammation and histologic inflammatory grades in chronic HBV infection

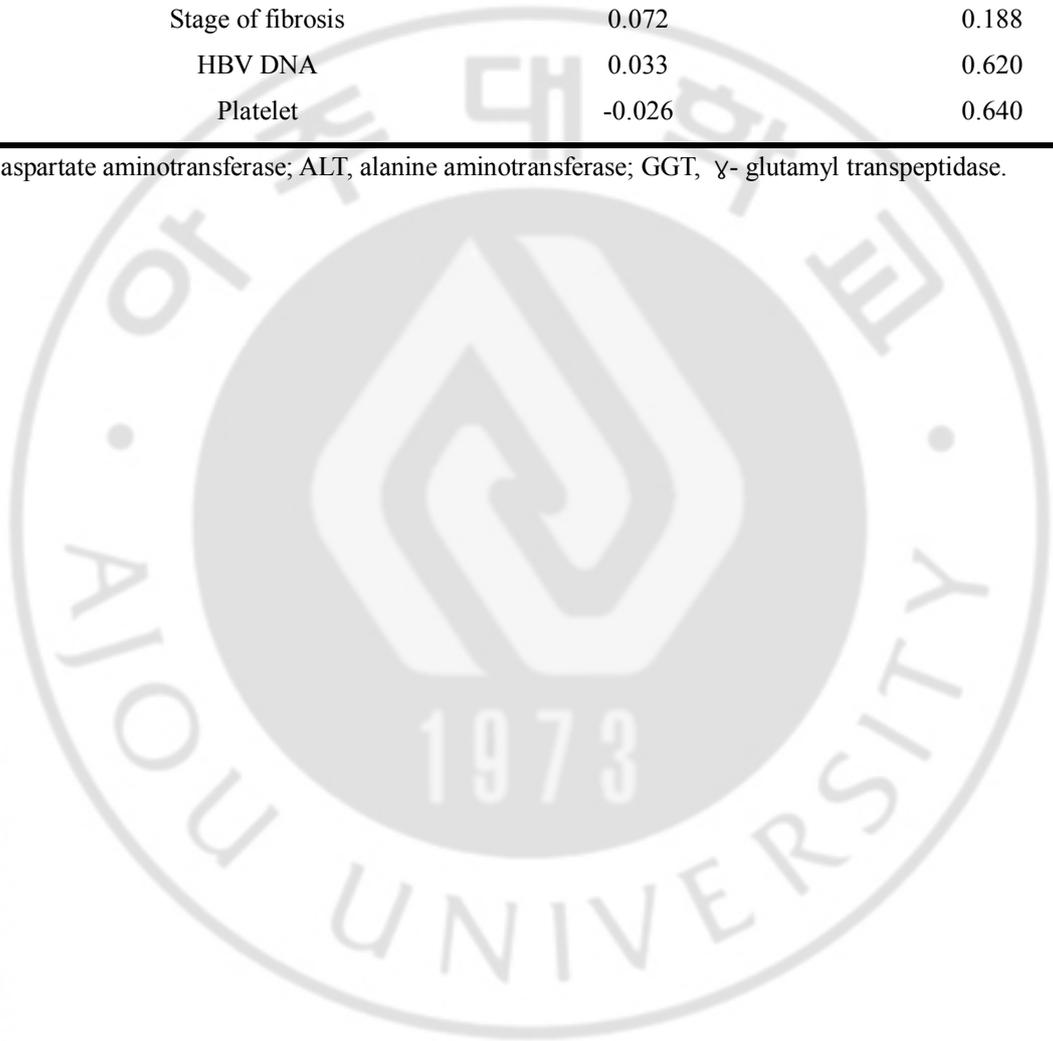
Serum M30 levels were correlated with other parameters indicating inflammatory liver injury in the serum of study subjects (Table 3). M30 antigen levels were found to significantly correlate not only with ALT ($r = 0.315$, $p < 0.001$) and AST ($r = 0.544$, $p < 0.001$), but also with total bilirubin ($r = 0.139$, $p = 0.011$), and albumin ($r = -0.189$, $p = 0.001$). Moreover, M30 levels were correlated with inflammatory grading score ($r = 0.240$, $p < 0.001$). In contrast, serum HBV-DNA levels ($r = -0.033$, $p = 0.620$), platelet counts ($r = -0.026$, $p = 0.640$), serum AFP levels ($r = 0.076$, $p = 0.308$) and liver fibrosis staging on liver biopsy ($r = 0.072$, $p = 0.188$) were not correlated with M30 serum levels.



Table 3. Relationship between M30-antigen and biochemical/histologic variables.

Rank	Variables	Correlation co-efficients (γ)	p-value
1	AST	0.544	0.000
2	ALT	0.315	0.000
3	Grade of inflammation	0.240	0.000
4	albumin	-0.189	0.001
5	bilirubin	0.139	0.011
6	GGT	0.091	0.134
7	Stage of fibrosis	0.072	0.188
8	HBV DNA	0.033	0.620
9	Platelet	-0.026	0.640

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ - glutamyl transpeptidase.

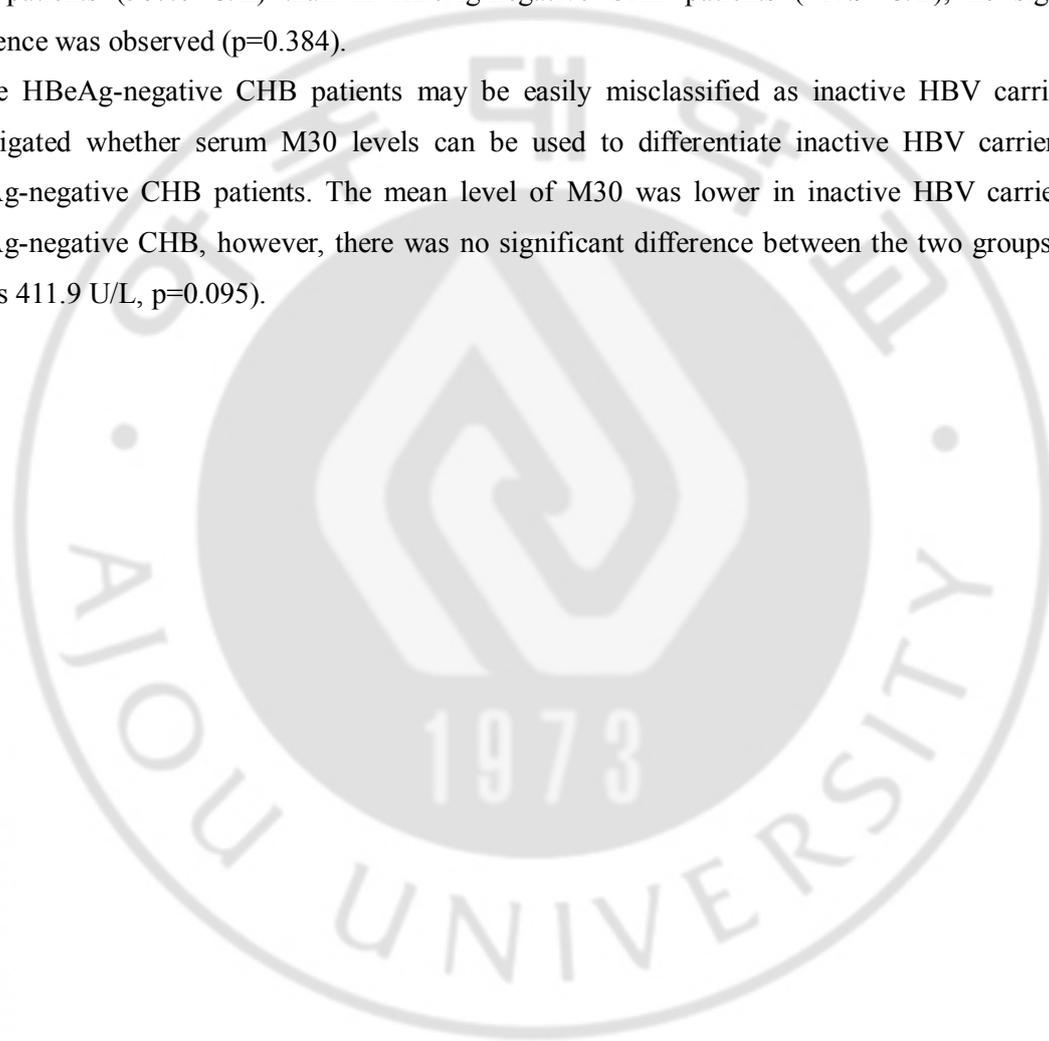


C. M30 antigen levels in different stages of chronic HBV infection

We assessed serum M30 levels of different groups of patients and compared them with each other. The mean levels of M30-antigen were lower in inactive carriers (225.9 U/L) than in CHB patients (524.0 U/L) and liver cirrhosis patients (513.4 U/L) (Table 1), but there was no statistical difference ($p=0.161$, $p=0.370$)(Fig. 1).

CHB patients ($n=221$) were divided into HBeAg-negative CHB patients ($n=80$) and HBeAg-positive CHB patients ($n=141$). Although the mean levels of M30-antigen were higher in HBeAg-positive CHB patients (587.6 U/L) than in HBeAg-negative CHB patients (411.9 U/L), no significant difference was observed ($p=0.384$).

Since HBeAg-negative CHB patients may be easily misclassified as inactive HBV carriers, we investigated whether serum M30 levels can be used to differentiate inactive HBV carriers from HBeAg-negative CHB patients. The mean level of M30 was lower in inactive HBV carriers than HBeAg-negative CHB, however, there was no significant difference between the two groups (225.9 U/L vs 411.9 U/L, $p=0.095$).



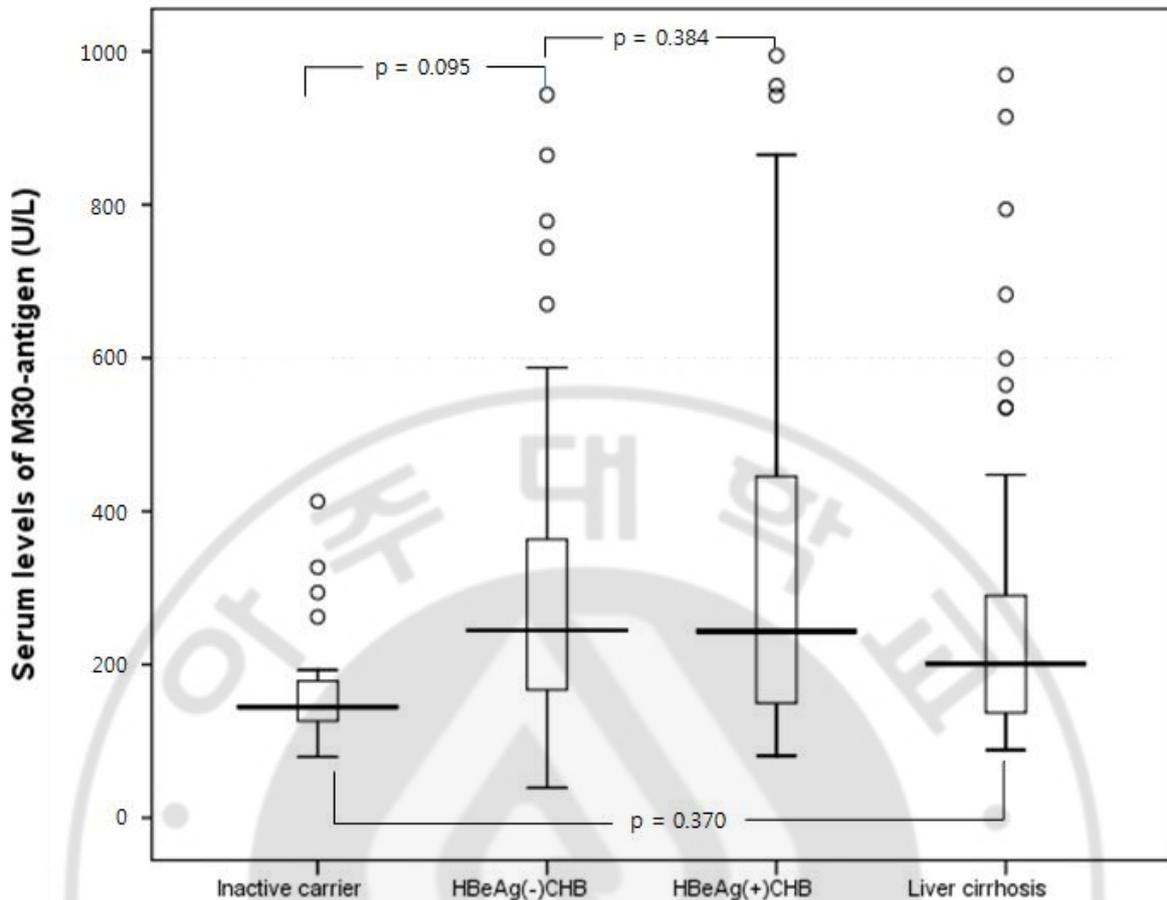


Figure 1. Serum levels of M30-antigen in various phases of chronic hepatitis B virus infection.

Box and whiskers plots express medians, and inter-quartile and overall ranges. The outlying values are plotted individually; 25 outlying values > 1000 U/I in all patients are not shown. There are no statistical differences in serum M30-antigen levels among the groups (Inactive carriers vs HBeAg-negative CHB, $p=0.095$; HBeAg-negative CHB vs HBeAg-positive CHB, $p=0.384$; Inactive carriers vs Liver cirrhosis, $p=0.370$). HBeAg, hepatitis B e antigen; CHB, chronic hepatitis B.

D. Serum M30 antigen and AST level as predictors of significant inflammation in chronic HBV infection

To investigate whether serum M30 levels might be associated with significant necroinflammatory activity in patients with CHB, the grade of inflammation was converted into a binomial variable of significant inflammation (Batts-Ludwig inflammatory activity grades 3 or 4) vs. no significant inflammation (grade 0, 1 or 2). These thresholds were selected because they are generally considered as indications for antiviral therapy in chronic viral hepatitis. Significant inflammation was present in 152 patients (44.9%).

To identify predictors of significant inflammation, we performed univariate and multivariate analyses. Univariate analysis of the variables revealed that AST, ALT, bilirubin, albumin, M30-antigen levels were significantly different between patients with and without significant inflammation (Table 4). Table 5 shows AUROCs of predictors for diagnosing significant inflammation. The ROC curve showed that serum AST, M30-antigen and ALT levels had good diagnostic accuracy for detecting significant inflammation in patients with CHB (AST: 0.872, M30-antigen: 0.795, ALT: 0.758) (Fig 2).

Next, multiple logistic regression analyses were performed, simultaneously controlling for all covariates with statistical significance ($P < 0.05$) from univariate analysis. Multiple logistic regression analysis by stepwise forward selection identified high AST ($P < 0.001$), high M30-antigen ($P = 0.020$) and low albumin ($P = 0.009$) as independent predictors of significant inflammation. Sensitivity, specificity, NPV and PPV for the diagnosis of significant inflammation using AST and M30-antigen levels are presented in Table 6. The cutoff values were chosen based on the ROC curve analysis to obtain a specificity of at least 90% in predicting significant inflammation. Although the sensitivity at a cutoff value of 344 U/L was not very high, M30-antigen level was capable of detecting significant inflammation with good specificity in patients with HBV infection (sensitivity; 45.7%, specificity; 89.5%). In addition, combined analysis of AST and M30-antigen was able to detect significant inflammation with 38.2% sensitivity, 96.1% specificity, 91.0% PPV and 56.1% NPV.

Table 4. Univariate and multivariate analyses for variables associated with significant inflammation.

	No significant inflammation Grade \leq 2 (n=152)	Significant inflammation Grade \geq 3 (n=187)	p-value	
			Univariate	Multivariate
Age (years)	42.21 \pm 14.32	49.38 \pm 14.93	0.004	
AST (U/L)	46.87 \pm 74.26	120.23 \pm 103.65	< 0.001	< 0.001
ALT (U/L)	67.21 \pm 91.65	107.23 \pm 166.71	< 0.001	
T. bilirubin (mg/dl)	0.78 \pm 0.39	0.97 \pm 0.58	< 0.001	
Albumin (g/dl)	4.31 \pm 0.41	4.05 \pm 0.53	< 0.001	0.009
M30-antigen (U/L)	318.95 \pm 1392.38	630.35 \pm 1366.13	< 0.001	0.020

AST, aspartate aminotransferase; ALT, alanine aminotransferase; T. bilirubin, total bilirubin.

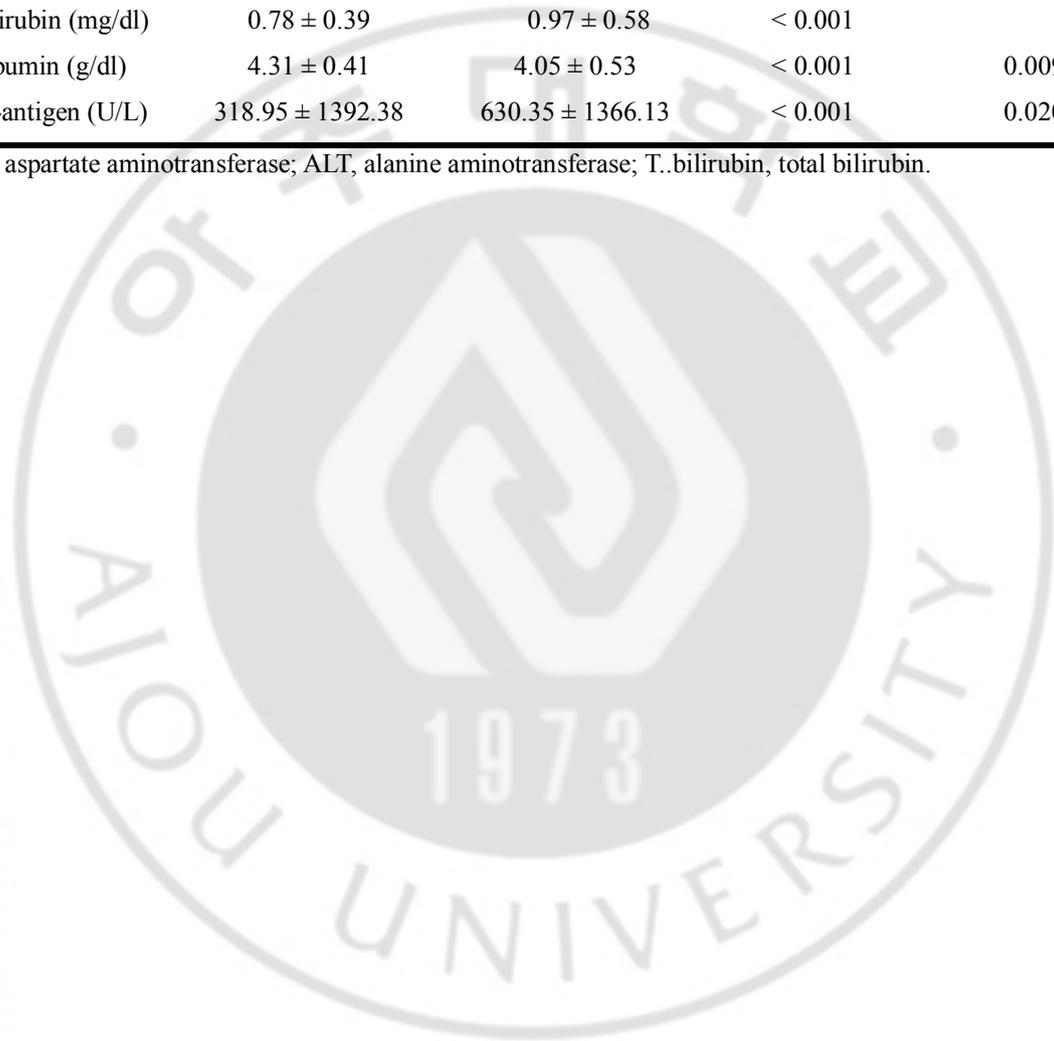


Table 5. Area under the receiver operating characteristics curves for predicting significant inflammation.

Rank	Variables	AUROC
1	AST	0.872
2	M30-antigen	0.795
3	ALT	0.758
4	Total bilirubin	0.668
5	Albumin	0.341

AST, aspartate aminotransferase; ALT, alanine aminotransferase; AUROC, area under the receiver operating characteristics.



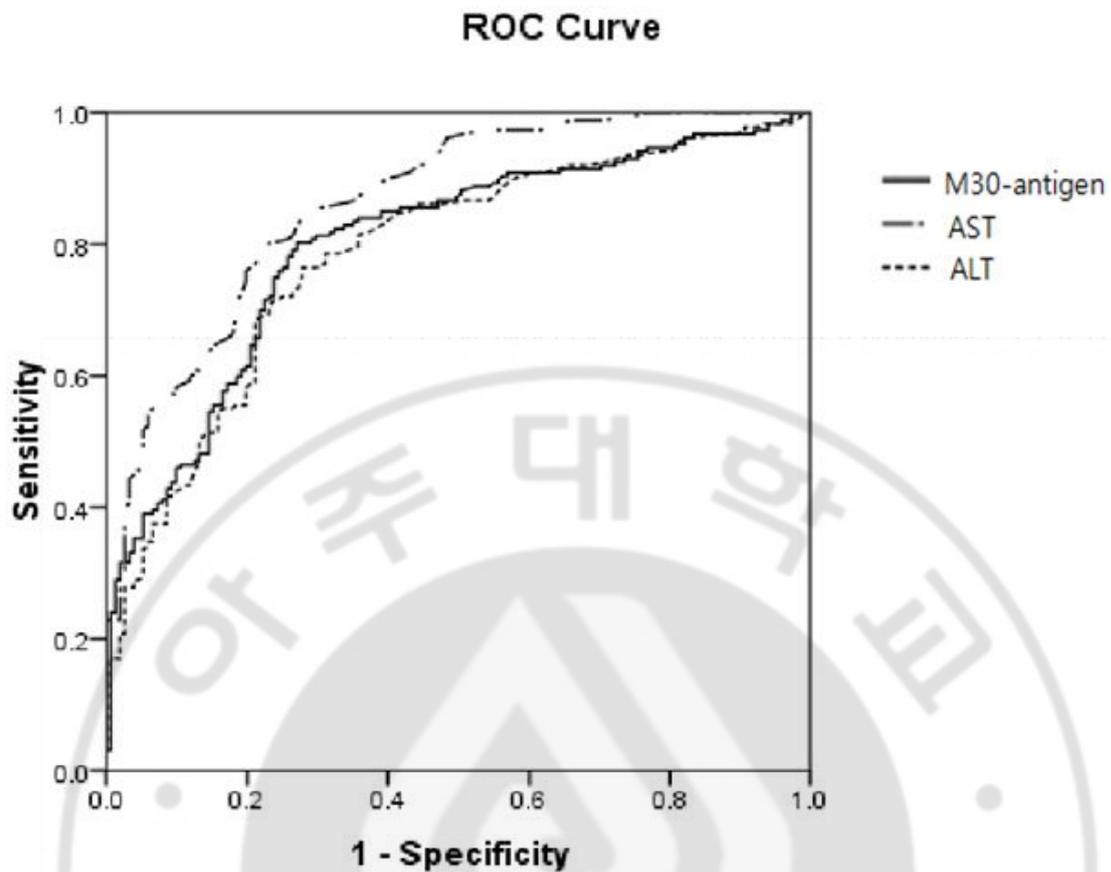


Figure 2. Receiver operating characteristics (ROC) curve of serum AST, ALT and M30-antigen for detecting significant inflammation in patients with chronic hepatitis B. The area under the curve of ROC plot for detecting significant inflammation of the serum AST, M30-antigen and ALT levels were 0.872, 0.795 and 0.758 respectively. AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table 6. Diagnostic values of AST and M30 in detecting patients with significant inflammation.

Total patients (n=339)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
AST \geq 78 IU/L	54.8	92.8	90.3	62.8
M30 \geq 344 U/L	45.7	89.5	84.2	57.6
AST \geq 78 IU/L or M30 \geq 344 U/L	62.4	86.3	84.7	65.3
AST \geq 78 IU/L and M30 \geq 344 U/L	38.2	96.1	91.0	56.1

AST, aspartate aminotransferase; PPV, positive predictive value; NPV, negative predictive value.



IV. DISCUSSION

In this study, we evaluated whether serum CK-18 fragment levels can serve as useful biomarkers of liver necroinflammation in the clinical spectrum of HBV infection. Serum M30 antigen levels were correlated with serum markers of liver inflammation and histologic inflammatory grades in patients with CHB. A combined analysis of AST and M30 antigen was able to detect significant inflammation with high specificity.

It is widely accepted that apoptosis of hepatocytes is a prominent feature of hepatitis B and C (Rust C and Gores GJ, 2000). Serum CK-18 fragment levels are considered as reliable markers of the severity of apoptosis of liver cells (Grassi A et al., 2004; Bantel H et al., 2001). Serum M30 antigen, a specific caspase-cleaved CK-18 fragment, detects only apoptotic cells, but not viable or necrotic cells (Linder S et al., 2004). Bantel et al have shown that serum levels of M30 antigen can be more sensitive markers in comparison with aminotransferase for the detection of early liver injury in HCV infection (Bantel H et al., 2004). The present study demonstrated that serum concentration of M30 was correlated with other laboratory markers of hepatic inflammation such as AST, ALT. Our finding of a close correlation between M30 levels and aminotransferase activities corresponds with other earlier studies on patients with CHC infection (Bantel H et al., 2004). Earlier studies demonstrated that serum caspase activity could not be used to distinguish different histological grades (Bantel H et al., 2004; Kronenberger B et al., 2005). In contrast to previous findings, we found a clear correlation between M30 levels and histological hepatic inflammation. HBV induced hepatocyte injury is a mixture of HBV-induced apoptotic and T cell-induced cell death (Baumert TF et al., 2007). In this regard, serum M30 levels appear to be an appropriate candidate for the diagnosis of liver necroinflammation.

Serum aminotransferase levels are biochemical markers reflecting liver damage and are important in the decision to initiate treatment for CHB. Many guidelines for management of CHB consider an elevated ALT level of more than 2 times the upper limit of normal as an indication for antiviral therapy (Lok AS, 2009; Liaw YF et al., 2008; EASL Clinical Practice Guidelines, 2009; Suk KT et al., 2012). However, there is increasing evidence that serum ALT level may not accurately reflect the histological status (Prati D et al., 2002). Previous reports have suggested that significant inflammation or fibrosis occurs in CHB patients with an ALT level of less than 2 times the upper limit of normal (Kumar M et al., 2008; Lai M et al., 2007). Therefore, finding non-invasive markers of liver inflammation in chronic HBV carriers is important.

Recent investigations have indicated that the best model for predicting significant inflammation in patients with CHB are age, HBV DNA levels, AST and albumin (Mohamadnejad M et al., 2006). We have recently shown that a simple scoring system including ALT, procollagen III N-terminal peptide and hyaluronic acid is an accurate non-invasive predictor of significant inflammatory activity in patients with CHB (Cho HJ et al., 2012). However, it is believed that serum hyaluronic acid and procollagen III N-terminal peptide are markers of liver fibrosis rather than inflammation in view of the biological functions of two markers, thus could not be generally accepted as predictors of liver inflammation. In the current study, the most accurate diagnosis of significant inflammation was achieved by combining M30 level (>344 U/L) and AST level (>78 IU/L), which showed 38.2% sensitivity, 96.1% specificity, 91.0% PPV and 56.1% NPV. Both serum markers appropriately reflected apoptosis and necrosis of hepatocytes. Ideally, the presence of significant inflammation might be predicted by a combination of two markers.

The differential diagnosis between active CHB and inactive carrier status is elusive due to fluctuations of HBV-DNA and frequent changes of serum ALT levels (Brunetto MR et al., 2001; Chen CJ et al., 2009; Feld JJ et al., 2007). Measurement of serum CK-18 fragment levels has been reported as a valuable tool for the correct diagnosis of a substantial proportion of hidden HBeAg-negative CHB cases. Papatheodoridis et al. have shown that serum CK-18 fragment levels were significantly higher in patients who were HBeAg-negative CHB than in inactive HBV carriers (Papatheodoridis GV et al., 2008). Similarly, Eren et al. have reported that serum levels of M30-antigen were significantly higher in both HBeAg-negative and HBeAg-positive CHB patients than those in inactive HBV carriers (Eren F et al., 2010).

Our results are different from other recent publications (Papatheodoridis GV et al., 2008; Eren F et al., 2010), and serum M30 level failed to discriminate inactive carrier and HBeAg-negative CHB. Even though serum M30 levels tended to be higher in patients with HBeAg-negative CHB than those who were inactive carriers, there was no significant difference between the two groups. The reason for the discrepancy between their data and ours is unclear. Strong points of our study are that liver histology data were available from all study subjects, we confirmed the classification of inactive carriers by liver biopsy and we were able to avoid misclassification caused by fluctuations of viral load in patients with CHB. In addition, we could also exclude other causes of liver injury such as steatohepatitis. Considering the small sample size of inactive HBV carriers in most investigations, prospective studies with larger cohorts are needed to determine their diagnostic value of serum caspase activity for differentiating inactive carriers from HBeAg-negative CHB patients.

V. CONCLUSION

In conclusion, the present study shows that serum CK-18 fragment levels correlate with liver necroinflammation, and the measurements of serum CK-18 fragments and AST levels could serve as valuable markers for the detection of significant inflammation in patients with CHB. More attention should be devoted to future research to search for more sensitive predictors of hepatic injury in patients with chronic hepatitis.



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만성 B형 간염 환자에서 염증반응 예측인자로서 Cytokeratin-18 분절의 유용성

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목적: 만성 B형 간염 환자에서 비활동성 보균자와 활동성 간염환자를 구분하는 것은 치료 방향을 결정할 때 중요하다. 본 연구는 세포 자멸의 생물학적 표지자로 알려진 cytoke-
ratin-18분절이 만성 B형 간염의 염증 반응 정도를 예측할 수 있는 지에 대해 조사하였다.

방법: 간 조직 검사를 시행한 만성 B형 간염 환자 총 339명 중에서 비활동성 보균자 36명, HBe항원 음성 간염 환자 80명, HBe항원 양성 간염 환자 141명, 간경화증 환자 82명을 대상으로 하였다.

결과: 혈청 M30 항원은 ALT, AST, 간 조직검사 결과와 통계학적으로 유의한 관계 ($p=0.000$)를 나타냈고 간의 중요한 염증 반응을 나타내는 독립인자로 AST ($p<0.001$), M30 항원 ($p=0.020$), ALT ($p=0.009$)가 확인되었다. M30 항원과 AST를 함께 측정할 경우 특이도 96.1%, 양성 예측도 91.0%로 의미 있는 결과를 확인하였지만 비활동성 보균자와 HBe항원 음성 간염환자에서 M30 항원의 유의한 차이는 나타나지 않았다.

결론: Cytokeratin-18 분절 수치는 만성 B형 간염환자에서 간의 중요한 염증 반응과 관련이 있음을 확인하였다.

핵심어: 세포 자멸, 만성 B형 간염, cytoke-
ratin-18 분절, 중요한 염증 반응