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HBsAg Levels Didn't Predict Significant Liver Histology in Patients with HBeAg Negative Chronic Hepatitis B

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ABSTRACT

Background: Serum hepatitis B surface antigen (HBsAg) level is a serum marker that correlates with intrahepatic covalently closed circular DNA (cccDNA) and could also predict liver-related diseases but the role in predicted significant liver histology in treatment naive HBeAg-negative CHB patients that had indication for liver biopsy as AASLD and APASL guidelines is still unknown.

Aims: To evaluate the association of serum HBsAg level, intrahepatic HBsAg and other clinical and laboratory parameters with significant liver necroinflammation as defined by histologic activity index (HAI) necroinflammatory activity score ≥ 4 or Metavir necroinflammatory activity score ≥ 2 and significant liver fibrosis as defined by Metavir fibrosis score ≥ 2 in treatment naïve HBeAg-negative CHB patients which had HBV DNA level ≥ 2,000 IU/mL and age ≥ 40 years or ALT 40-79 U/L.

Methods: Twenty-two patients were prospectively included in the study. Serum HBsAg level that was performed by enzyme immunoassay and other parameters were collected within 2 weeks of liver biopsy. Immunohistochemical (IHC) staining for HBsAg in liver tissue was examined in terms of location, area of involvement and intensity.

Results: Mean age of patients was 48±9 years. Male was equal to female. There were 5 patients (23%) with significant liver inflammation and 7 patients (32%) with significant liver fibrosis. Serum HBsAg level did not associated with both significant liver inflammation and fibrosis [median (IQR) 3.45 (0.45) vs. 3.50 (0.34), p = 0.225 and 3.35 (0.45) vs 3.50 (0.49), p = 0.698]. Intra-hepatic HBsAg also did not have association in all terms of evaluation. Factors that associated with significant liver inflammation were lower BMI [21.55 (2.59) kg/m^2 vs. 24.69 (5.21) kg/m^2, p = 0.046] and higher alkaline phosphatase (85.0±16.7 vs. 59.4±11.7 U/L, p = 0.001). While factor that associated significant liver fibrosis were lower age (42.7±8.4 vs. 50.7±8.2 years, p = 0.047). On multivariate analysis, only HBV DNA level > 5.5 log IU/mL could predict significant liver fibrosis (OR 28.012, 95% CI, 1.631-481.240 p = 0.022) and its sensitivity, specificity, positive predictive value and negative predictive value were 71.4%, 93.3%, 83.3% and 87.5% respectively. There were no factors which predict significant liver inflammation.

Conclusions: Serum HBsAg level and intrahepatic HBsAg didn't associate with significant liver histology in HBeAg-negative CHB patients that had indication for liver biopsy as recommendation by AASLD and APASL guidelines. HBV DNA level > 5.5 log IU/mL was the only factor that could predict significant liver fibrosis in this group of patients.

Key words : HBsAg, chronic hepatits B, HBeAg negative, liver histology, liver fibrosis

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INTRODUCTION

Liver inflammation and fibrosis evaluations are important part for severity assessment and treatment decision in management of chronic hepatitis B (CHB) patients. Gold standard for evaluation in grading of liver inflammation and staging of liver fibrosis is a liver biopsy[1-3]. However, it is an invasive procedure with minimal but significant risk of morbidity and mortality[4].

Nowadays, transient elastography (TE) is an alternative non-invasive method to liver fibrosis assessment in CHB patients[5]. Although, the cut-off of liver stiffness (LS) values in determining degree of fibrosis were still controversy and LS values had good accuracy in only advance fibrosis or cirrhosis[6-8]. Otherwise, it couldn't evaluate grading of liver inflammation. The Aspartate Platelet Ratio Index (APRI) is another option that has been validated by investigators; somehow it also has only fair accuracy in significant fibrosis prediction[9-10].

Serum hepatitis B surface antigen (HBsAg) level is a serum marker that correlated with covalently closed circular DNA (cccDNA) intra-hepatic markers of hepatitis B virus (HBV)[11-14] and could also predict phases and some clinical endpoints in CHB patients. Serum HBsAg level more than 1,000 IU/mL could predict HBV-DNA more than 2,000 IU/mL[15] and increase risks of hepatitis flare, cirrhosis and hepatocellular carcinoma (HCC) in hepatitis B e antigen (HBeAg)-negative CHB patients[16-17]. In Guner, et al. study which included HBeAg-negative genotype D CHB patients with normal alanine aminotransferase (ALT) to as high as 399 U/L, found to have significant higher serum HBsAg level in patients with significant liver fibrosis[18]. Otherwise, serum HBsAg level more than 25,000 IU/mL could predict insignificant liver fibrosis in HBeAg-positive CHB patients[19]. Nevertheless, there is no data about association of serum HBsAg level and liver histology in HBeAg-negative CHB patients in our region that is mainly genotype C[20] and need to evaluate liver inflammation and fibrosis by liver biopsy as The American Association for the Study of Liver Diseases (AASLD) and The Asian Pacific Association for the Study of the Liver (APASL) guidelines including HBV DNA level ≥ 2,000 IU/mL and ages more than 40 years or elevated ALT levels between 1-2 of upper limit of normal (ULN)[1,3].

The aim of this study was to evaluate association of serum HBsAg level and other clinical and laboratory parameters including TE and APRI to liver histology in HBeAg-negative CHB patients that needed to be evaluated liver inflammation and fibrosis by liver biopsy as AASLD and APASL guidelines which were the most common used guidelines in our regions with a prospective cross-sectional study. The authors also demonstrated intra-hepatic HBsAg to further understanding its association. If associations were found, serum HBsAg level or other clinical and laboratory parameters might become an alternative method in liver inflammation and fibrosis assessment.

PATIENTS AND METHODS

Patients

All CHB patients visited at outpatient clinic of Division of Gastroenterology or the NKC institute of Gastroenterology and Hepatology, Prince of Songkla University, Songkla, Thailand between November 2012 and February 2014 were screened to include in this study.

Inclusion criteria were age more than 20 years, positive HBsAg more than 6 months, negative HBeAg, HBV DNA more than 2,000 IU/mL, naïve HBV treatment and positive indication for liver biopsy according to AASLD and APASL guidelines including ALT 40-79 U/L (1-2 of ULN) or age ≥ 40 years[1,3]. We also added positive family history of cirrhosis or HCC into another indication for liver biopsy due to Thai national guideline[21] (only 1 patient in our study was biopsy by this indication).

Exclusion criteria were co-infection with hepatitis C virus (HCV) or human immunodeficiency virus (HIV) infection, alcohol intake more than 20 g/day, cirrhosis as documented by either physical examination, laboratory or radiology, HCC, pregnancy, contraindication to liver biopsy including the presence of ascites, international normalized ratio (INR) more than 1.5, activated partial thromboplastin time (aPTT) more than > 1.5 times or platelet < 100,000/mm^3[4] and refuse to participate in the study. Written informed consents were obtained from all patients before enrollment. The study was approved by the Ethical Committee of Prince of Songkla University and in accordance with Helsinki Declaration of 1975.

The primary end point was association of HBsAg levels with liver histology in HBeAg-negative CHB

patients that needed liver biopsy as AASLD and APASL guidelines. The secondary end points were the association of clinical and laboratory parameters including transient elastography (TE) and the Aspartate Platelet Ratio Index (APRI) and intrahepatic HBsAg with liver histology.

All of the eligible patients were collected basic clinical and laboratory data, HBsAg levels, and underwent LS measurement by TE within 2 weeks of the day of liver biopsy. The basic clinical and laboratory data were age, gender, underlying diseases, family history of cirrhosis or HCC, body mass index (BMI), HBV DNA levels, liver function tests, complete blood counts (CBC).

Quantification of HBsAg and HBV DNA Levels

Serum HBsAg levels were quantified using the Architect HBsAg QT (Abbott Laboratories) with a detection range of 0.05 to 250 IU/mL according to manufacturer's instructions[22,23]. If the HBsAg level was more than 250 IU/mL, samples were diluted to 1:100 to 1:1000 to obtain a reading within the calibration curve range. Serum HBV-DNA levels were quantified by COBAS AmpliPrep/COBAS TaqMan assay (Roche Diagnostic Systems Inc, Mannheim, Germany) with a detection range of 20-1.7x10^8 IU/mL.

LS measurements by TE

LS was determined by using Fibroscan®) (Echosens, Paris, France) as described by Sandrin[24]. TE operator was a physician who had previously performed LS by Fibroscan® at least 30 patients (N. J.). The operators performed at least 10 valid measurements at each time. An LS value is expressed in median (kilopascal; kPa). LS values included in this study needed to have inter-quartile range (IQR) over median ratio less than 30% and success rate more than 80%.

Liver histology

Liver histology was evaluated by a pathologist (S.K.) who was expert in the gastroenterological pathology, blinded to any other clinical and laboratory data and tested for intra-observer variation. The degrees of liver necroinflammation were assessed by the histologic activity index (HAI) necroinflammatory activity score (from 0-18)[25] and the Metavir necroinflammatory activity score. The degrees of liver fibrosis were assessed by the Metavir fibrosis score (from 0-4)[26].

Immunohistochemistry (IHC) for HBsAg in liver tissue

HBsAg immunostaining was performed on liver tissue in paraffin block as previously described. Mouse monoclonal against HBsAg (M3506, Dako, Carpinteria, CA) was primary antibody and then staining by BenchMark XT automated staining system (Ventana, Tucson, AZ)[27]. One pathologist blinded to clinical data examined liver tissue immunohistochemistry by location, area of involvement and intensity. Locations were reported as cytoplasm or cytoplasmic membrane[28-29]. Areas of involvements were grouped into more or less than 50% involvement. Finally, intensities were semi-quantitatively divided into mild, moderate or mark intensity[30].

Definition

"Significant liver inflammation" was defined by HAI necroinflammatory activity score ≥ 4 or Metavir necroinflammatory activity score ≥ 2, while "significant liver fibrosis" was defined by Metavir fibrosis score ≥ 2. If one of the above definitions were found, they could also be called as "significant liver histology". These levels represented at least moderate inflammation or significant fibrosis and also changed management of the patients due to treatment indication.

Statistical analysis

The baseline characteristics were presented as means with standard deviation or medians with interquartile range (IQR) according to type of data distribution. Comparison of means used Independent-samples *t*-test, while medians used Mann-Whitney U test. Factors that had p-value < 0.1 were further univariate analysis by Fisher exact test and multivariate analysis by multiple logistic regression. Statistical significance was set at $p < 0.05$.

RESULTS

Characteristics of Patients

Twenty-two eligible patients were included in the study. Baseline characteristic of all patients divided according to liver pathology were shown in Table 1. Male and female genders were equal, as well as, posi-

Table 1. Baseline characteristics of 22 HBeAg-negative CHB patients included in the study.

Parameters	All patients (n = 22)	Liver inflammation			Liver fibrosis			Liver inflammation or fibrosis		
		Significant[a] (n = 5)	Insignificant (n = 17)	*p*- value	Significant[b] (n = 7)	Insignificant (n = 15)	*p*- value	Significant[c] (n = 8)	Insignificant (n = 14)	*p*- value
Male/female, n (%)	11/11 (50/50)	1/4 (5/18)	10/7 (45/32)	0.127	4/3 (18/14)	7/8 (32/36)	0.647	4/7 (18/32)	4/7 (18/32)	1.000
Age, mean ± SD, years	48 ± 9	41.8 ± 8.3	50.1 ± 8.4	0.068	42.7 ± 8.4	50.7 ± 8.2	0.047	44.4 ± 9.1	50.4 ± 8.4	0.134
BMI, median (IQR), kg/m^2	23.87 (4.14)	21.55 (2.59)	24.69 (5.21)	0.046	22.64 (2.86)	24.96 (6.55)	0.217	22.48 (2.93)	25.21 (6.61)	0.088
Positive family history of cirrhosis or HCC, n (%)	11/11 (50)	3/2 (14/9)	8/9 (36/41)	0.611	3/4 (14/18)	8/7 (36/32)	0.647	4/7 (18/32)	4/7 (18/32)	1.000
HBsAg levels, median (IQR), log IU/mL	3.45 (0.45)	3.20 (1.19)	3.50 (0.34)	0.225	3.35 (0.45)	3.50 (0.49)	0.698	3.28 (0.42)	3.53 (0.33)	0.275
HBV DNA levels, median (IQR), log IU/mL	4.75 (1.96)	5.77 (3.07)	4.49 (1.45)	0.327	5.81 (2.89)	4.35 (3.97)	0.053	5.79 (2.90)	4.42 (1.40)	0.172
TB, mean ± SD, mg/dL	0.55 ± 0.22	0.40 ± 0.11	0.60 ± 0.23	0.08	0.44 ± 0.20	0.61 ± 0.22	0.109	0.46 ± 0.19	0.61 ± 0.23	0.120
DB, mean ± SD, mg/dL	0.15 ± 0.07	0.13 ± 0.05	0.16 ± 0.07	0.288	0.14 ± 0.08	0.16 ± 0.06	0.458	0.14 ± 0.07	0.16 ± 0.07	0.580
AST, median(IQR), U/L	28 (16)	44 (30)	27 (11)	0.071	32 (22)	28 (17)	0.572	33 (23)	27.5 (11)	0.231
ALT, mean ± SD, U/L	35.4 ± 17.3	47.4 ± 21.8	31.9 ± 1.47	0.077	36.1 ± 20.1	35.1 ± 1.64	0.896	38.8 ± 20.4	33.5 ± 15.8	0.507
ALP, mean ± SD, U/L	65.2 ± 1..67	85.0 ± 16.7	59.4 ± 11.7	0.001	69.3 ± 16.7	63.3 ± 16.9	0.443	74.3 ± 20.9	60.0 ± 11.6	0.051
GGT, median (IQR), mg/dL	19 (13)	24 (16)	17 (14)	0.347	24 (21)	17 (13)	0.459	24 (17)	17 (14)	0.274
Albumin, mean ± SD, g/dL	4.47 ± 0.13	4.40 ± 0.16	4.49 ± 0.11	0.153	4.44 ± 0.17	4.49 ± 0.11	0.468	4.46 ± 0.17	4.48 ± 0.11	0.784
Globulin, mean ± SD, g/dL	2.96 ± 0.34	3.16 ± 0.27	2.91 ± 0.35	0.151	3.03 ± 0.36	2.93 ± 0.35	0.559	3.00 ± 0.34	2.94 ± 0.36	0.718
INR, mean ± SD,	0.98 ± 0.07	1.00 ± 0.11	0.97 ± 0.05	0.527	0.99 ± 0.07	0.97 ± 0.07	0.554	1.01 ± 0.88	0.96 ± 0.05	0.103
White blood cells, mean ± SD, /mm^3	6,650 ± 1,329	7,168 ± 1,267	6,497 ± 1,345	0.333	6,803 ± 1,209	6,578 ± 1,416	0.721	6,773 ± 1,123	6,579 ± 1,470	0.752
Absolute neutrophil count, mean ± SD, /mm^3	3,456 ± 1,132	4,302 ± 1,691	3,207 ± 823	0.055	3,762 ± 1,608	3,313 ± 863	0.399	3,748 ± 1,489	3,289 ± 890	0.372
Absolute lymphocyte count, mean ± SD, /mm^3	2,405 ± 565	2,222 ± 527	2,459 ± 579	0.423	2,339 ± 531	2,435 ± 595	0.719	2,345 ± 492	2,439 ± 618	0.718
Hemoglobin, mean ± SD, g/dL	13.5 ± 1.3	13.7 ± 1.1	13.5 ± 1.4	0.781	13.6 ± 1.0	13.5 ± 1.5	0.793	13.8 ± 1.0	13.4 ± 1.5	0.503
Platelet, mean ± SD, x10^3/mm^3	234.5 ± 50.1	223.4 ±70.2	237.8 ± 44.8	0.586	215.3 _ 55.7	243.5 ± 46.5	0.228	222.6 ± 55.6	241.2 ± 47.5	0.414
AFP, median (IQR), ng/mL	2.23 (1.68)	1.95 (1.06)	2.35 (2.12)	0.256	1.95 (1.27)	2.24 (2.16)	0.217	2.10 (1.14)	2.29 (2.53)	0.246
LS values, mean ± SD, kPa	7.12 ± 2.92	9.16 ± 3.00	6.59 ± 2.72	0.084	8.06 ± 2.56	6.77 ± 3.06	0.347	8.59 ± 2.83	6.37 ± 2.75	0.087
APRI, mean ± SD	0.35 ± 0.21	0.54 ± 0.37	0.30 ± 0.09	0.210	0.45 ± 0.33	0.30 ± 0.10	0.283	0.45 ± 0.31	0.30 ± 0.10	0.206

Abbreviations: CHB, chronic hepatitis B; BMI, body mass index; TB, total bilirubin; DB, direct bilirubin; AST, aspartate aminotransferace; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyltransferase; INR, international normalized ratio; CRP, c-reactive protein; ESR, erythrocyte sedimentation rate; AFP, α-fetoprotein; LDH, lactate dehydrogenase; LS, liver stiffness; APRI, AST to platelet ratio index.

[a]Significant liver inflammation (HAI necroinflammatory activity score ≥4 or Metavir necroinflammatory activity score ≥2)

[b]Significant liver fibrosis (Metavir fibrosis score ≥2)

[c]Significant liver inflammation or fibrosis

Table 2. Univariate analysis in predictor of significant liver histology.

Parameters	Significant Liver inflammation			Significant liver fibrosis			Significant liver inflammation or fibrosis		
	n/N	%	*p*- value	n/N	%	*p*- value	n/N	%	*p*- value
Age ≤ 40 years	3/7	42.9	0.160	4/7	57.1	0.107	-	-	-
Age ≤ 42 years	4/8	50	0.039	5/8	62.5	0.032	-	-	-
Age ≤ 50 years	4/11	36.4	0.155	5/11	45.5	0.181	-	-	-
BMI < 23 kg/m^2	4/9	44.4	0.067	-	-	-	5/9	55.6	0.135
BMI < 24.5 kg/m^2	5/13	38.5	0.049	-	-	-	7/13	53.8	0.052
BMI < 25 kg/m^2	5/15	33.3	0.114	-	-	-	8/15	53.3	0.020
TB < 0.45 mg/dL	4/8	50	0.039	-	-	-	-	-	-
AST > 19 U/L	4/19	21.1	0.558	-	-	-	-	-	-
AST > 30 U/L	4/8	50	0.021	-	-	-	-	-	-
AST > 40 U/L	3/5	60	0.055	-	-	-	-	-	-
AST > 50 U/L	1/1	100	0.227	-	-	-	-	-	-
ALT > 30 U/L	4/12	33.3	0.218	-	-	-	-	-	-
ALT > 40 U/L	3/8	37.5	0.233	-	-	-	-	-	-
ALP > 78 U/L	4/5	80	0.003	-	-	-	4/5	80	0.039
Absolute neutrophil count > 4,300/mm^3	3/4	75	0.024	-	-	-	-	-	-
LS values ≥ 7.0 kPa	3/10	30	0.406	-	-	-	5/10	50	0.221
LS values ≥ 8.5 kPa	3/5	60	0.233	-	-	-	5/8	62.5	0.072
LS values ≥ 9.5 kPa	3/5	60	0.055	-	-	-	3/5	60	0.233
LS values ≥ 11.5 kPa	2/2	100	0.043	-	-	-	2/2	100	0.121
HBV DNA levels > 4 log IU/mL	-	-	-	5/14	35.7	0.490	-	-	-
HBV DNA levels > 5 log IU/mL	-	-	-	5/11	45.5	0.181	-	-	-
HBV DNA levels > 5.5 log IU/mL	-	-	-	5/6	83.3	0.004	-	-	-
HBV DNA levels > 6 log IU/mL	-	-	-	3/4	75	0.077	-	-	-

tive and negative family history of cirrhosis or HCC.

The mean age was 48 years. There were 4 (18%) patients with both significant liver inflammation and fibrosis, 1 (5%) patients with only significant inflammation and 3 (14%) patients with only significant liver fibrosis. The indication for liver biopsy in 4 patients with less than 40 years were elevated ALT in 3 patients and positive family of cirrhosis or HCC in 1 patient. The pathological finding in this group resulted in significant liver fibrosis in 3 patients and both significant liver inflammation and fibrosis in 1 patient.

The patients with significant liver inflammation were significant lower BMI [21.55 (2.59) kg/m^2 vs. 24.69 (5.21) kg/m^2, $p = 0.046$] and higher alkaline phosphatase (85.0±16.7 vs. 59.4±11.7 U/L, $p = 0.001$) than the patients without significant liver inflammation. While patients with significant liver fibrosis were significantly younger (42.7±8.4 years vs. 50.7±8.2, $p = 0.047$) than the patients without significant liver fibrosis. When combined patients with significant liver inflammation and significant liver fibrosis into the same group, they didn't show significant different in the baseline characteristics compared with patients without significant liver histology.

Serum HBsAg level and liver histology in HBeAg-negative CHB patients

HBsAg levels didn't associated with significant liver inflammation [3.20 (1.19) log IU/mL vs. 3.50 (0.34), $p = 0.225$], significant liver fibrosis [3.20 (1.19) log IU/mL vs. 3.50 (0.34), $p = 0.698$] or significant liver histology [3.28 (0.42) log IU/mL vs. 3.53 (0.33), $p = 0.275$] (Table 1).

Table 3. Multivariate analysis in predictor of significant liver fibrosis.

Parameters	Odd ratio	*p*- value	95% confidence interval
Age ≤ 42 years	7.403	0.139	0.523-104.716
HBV DNA levels > 5.5 log IU/mL	28.012	0.022	1.631-481.240

Table 4. Immunohistochemistry (IHC) for HBsAg in HBeAg-negative CHB patients.

Parameters of HBsAg	Liver inflammation			Liver fibrosis			Liver inflammation or fibrosis		
	Significant[a] (n = 5)	Insignificant (n = 17)	*p*-value	Significant[b] (n = 7)	Insignificant (n = 15)	*p*-value	Significant[c] (n = 8)	Insignificant (n = 14)	*p*-value
Location									
- C and M, n/n (%)	3/5 (60)	15/17 (88.12)	0.210	6/7 (85.7)	12/15 (80)	1.000	6/8 (75)	12/14 (85.7)	0.602
- C or M only, n/n (%)	2/5 (40)	2/17 (11.8)		1/7 (14.3)	3/15 (20)		2/8 (25)	2/14 (14.3)	
Area of involvement									
- ≥ 50%, n/n (%)	3/5 (60)	13/17 (76.5)	0.585	5/7 (71.4)	11/15 (73.3)	1.000	5/8 (62.5)	11/14 (78.6)	0.624
- < 50%, n/n (%)	2/5 (40)	4/17 (23.5)		2/7 (28.6)	4/15 (26.7)		3/8 (37.5)	3/14 (21.4)	
Intensity									
- Mark intensity, n/n (%)	4/5 (80)	11/17 (64.7)	1.000	5/7 (71.4)	10/15 (66.7)	0.613	6/8 (75)	9/14 (64.3)	0.490
- Mild to moderate intensity, n/n (%)	1/5 (20)	6/17 (35.3)		2/7 (28.6)	5/15 (33.3)		2/8 (25)	5/14 (35.7)	

Abbreviations: C, cytoplasm; M, cytoplasmic membrane.

Predictors of significant liver histology in HBeAg-negative CHB patients

Many factors were found to predict significant liver inflammation in univariate analysis, including age ≤ 42 years, BMI < 24.5 kg/m^2, total bilirubin < 0.45 mg/dL, aspartate aminotransferace > 30 U/L, alkaline phosphatase > 78 U/L, absolute neutrophil count > 4,300/mm^3, ceruloplasmin > 20 mg/dL and LS values ≥ 11.5 kPa (Table 2), nevertheless, they didn't reach statistical significance in multivariate analysis. The same results were also discovered by significant liver histology group. BMI < 25 kg/m^2 and ALP > 78 U/L associated with significant liver histology but they also weren't significance in multivariate analysis.

The liver fibrosis group had age ≤ 42 years and HBV DNA levels > 5.5 log IU/mL as a predictor of significant liver fibrosis in univariate analysis. HBV DNA levels > 5.5 log IU/mL were the only factor that also significant predictor on multivariate analysis (OR 28.012, 95% CI, 1.631-481.240 $p = 0.022$) (Table 3). Sensitivity, specificity, positive predictive value and negative predictive value were 71.4%, 93.3%, 83.3% and 87.5% respectively.

IHC for HBsAg and liver histology in HBeAg-negative CHB patients

Our study also demonstrated HBsAg activity in the liver histology (Table 4) by IHC. As serum HBsAg levels, HBsAg in the liver didn't associated with significant liver histology by various terms of evaluation including HBsAg location, area of HBsAg involvement and HBsAg intensity.

Discussion

In management of CHB patients, liver histology evaluation is an important part for severity assessment and treatment decision. Although liver biopsy is a gold standard to determine the stage of fibrosis and degree of necroinflammation, it is an invasive procedure with minimal but significant risk of morbidity and mortality[4]. Though many non-invasive methods were applied to replace this invasive procedure.

Nowadays, serum HBsAg level is becoming to be a useful test that can utilize in many conditions in CHB patients. It could predict HBV levels and liver-related diseases, and also significant liver fibrosis in

HBeAg-positive CHB patients[15-18]. However, it didn't associate with significant histology in HBeAg-negative CHB patients with normal or minimally elevated ALT in our study. Martinot-Peignoux M, *et al.* also found the same results with HBeAg-negative CHB patients with varying degree of ALT[31]. We also demonstrated HBsAg in liver tissue which was already proved to be associated with the viral replication template known as cccDNA[14] and also found that it did not associate with significant histology. These findings suggested that serum HBsAg level wasn't potential tool in liver histology assessment in HBeAg-negative CHB patients.

Patients with significant liver inflammation had significant lower BMI and higher alkaline phosphatase than another groups. However, these findings were not found in other studies in HBeAg-negative CHB patients with varying degree of ALT[32-34]. Larger trials in the same fashion as our study were need to clarify influence of these factors.

In the part of liver fibrosis, HBV DNA level was the only statistically significant predictive factor on our study with OR of 28.012 at level more than 5.5 log IU/mL. This was supported by other studies. Croagh CM *et al.* study HBV DNA level was found to be predictors of significant fibrosis in HBeAg-negative CHB patients with varying ALT with OR of 1.3 for every 1 log increment[33]. Association was also found in Mohamadnejad M *et al.* study with the sensitivity of 74% and specificity of 80% at cutoff level to 4.21 log IU/mL[32]. HBV DNA levels also correlate with advance fibrosis in HBeAg-negative CHB patients with normal ALT in Xiao L *et al* study[34]. In contrast, this finding was not found in HBeAg-positive CHB patients[19,32-34]. Further studies are needed to explain variable outcomes between different phase of CHB patients. Though, HBV DNA level with cut-off value at 5.5 log IU/mL might be a promising serum marker in predicting liver fibrosis in HBeAg-negative CHB patients with positive indication for liver biopsy as recommendation by AASLD and APASL guidelines.

Younger age was another factor that associated with liver fibrosis. This was against the basic knowledge that fibrosis would progress and eventually develop cirrhosis with more advance age[1-3]. This finding might be explained by selection bias. The authors excluded cirrhosis which could be a final outcome in younger patients with significant histology, though, patients with advance age but still did not progress to cirrhosis might represent a group of patients which actually had an insignificant histology. If cirrhosis was counted into this study, the result should be an opposite way.

Although TE and APRI correlated with degree of liver fibrosis, it was not associated with significant liver fibrosis from our study. TE seemed to accurately predict advance fibrosis especially fibrosis stage 4 or cirrhosis from meta-analysis studies, but unfortunately, its accuracy in predict significant liver fibrosis (fibrosis stage ≥ 2) was just fair. These studies demonstrated that LS values between fibrosis stage 1 and 2 were nearly similar[6-8]. TE might be an appliance in predict advance but not significant fibrosis, thus it should not be use to guide treatment initiation except LS values were high enough to reflect advance fibrosis or cirrhosis. Not surprisingly, APRI did not associate with significant liver histology. This was correlated with Degos F *et al.* study. APRI also had better accuracy in diagnosis of cirrhosis more than significant fibrosis[10].

There is no predictor for significant liver histology in this study. Even if HBV DNA level could significantly predict significant liver fibrosis, it did not associated with significant liver histology. Insignificant association with significant liver inflammation and small numbers of patients with significant liver histology might explain this finding.

The strength of our trial was the patients that included in the study. The authors collected patients in the prospective manner and strictly selected the patient that really needed liver biopsy as guideline recommendation. Though the result could be apply in the clinical practice. Small number of total patients was limitation in this study.

In conclusion, serum HBsAg level didn't associate with significant liver histology in HBeAg-negative CHB patients that had indication for liver biopsy as recommendation by AASLD and APASL guidelines. HBV DNA level > 5.5 log IU/mL was the only factor that could predict significant liver fibrosis in this group of patients.

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