Int J Clin Exp Med 2019;12(6):7569-7576
www.ijcem.com /ISSN:1940-5901/IJCEM0089713

Original Article

Clinical prognosis of chronic hepatitis B patients with concurrent HBeAg/Anti-HBe detection and possible mechanism

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Received November 19, 2018; Accepted April 9, 2019; Epub June 15, 2019; Published June 30, 2019

Abstract: Objective: The aims of this study were to analyze the clinical characteristics and treatment outcomes of such special group, and to explore possible mechanisms. Methods: A retrospective cohort including 326 antiviral treatment-naive CHB patients with concurrent HBeAg/Anti-HBe were studied. Clinical and laboratory data were collected. An improved 5 mol/L urea elution method was used for in vitro HBeAg and anti-HBe binding experiments. Results: Of the 326 patients, mean titers of HBeAg, anti-HBe were 1.48 ± 2.85 PEIU/mL and 0.53 ± 0.29 S/CO, respectively. The mean level of ALT was 213.91 ± 398.38 U/L. At week 48, the treated group achieved higher ALT normalization rate, undetectable HBV DNA rate, HBeAg seroconversion rate, and HBsAg seroclearance rate (84.8% vs 23.6%; 46.1% vs 8.7%; 6.1% vs 0.6%; and 72.1% vs 19.3%, all $P < 0.05$). Sub-group analysis was carried out in the treated group: 64.3% and 14.3% patients achieved HBeAg seroconversion and HBsAg seroclearance in PEG-IFN α-2a group, both significantly higher than NAs group ($P = 0.006$ & $P = 0.010$). The mean affinity index of the concurrent patients was significantly lower than the HBeAg(-)/Anti-HBe(+) patients (95.42 ± 36.26 vs 122.10 ± 46.10, $P < 0.001$). Conclusions: The prevalence of concurrent HBeAg/anti-HBe detection is often accompanied by a high level of ALT, and to effectively suppress viral replication and achieve HBeAg seroconversion, Peg-IFN is preferred. In addition, decreased affinity between HBeAg and anti-HBe is a probably explanation.

Keywords: Concurrent, hepatitis B e antigen, anti-HBe, seroconversion, mechanism

Introduction

During the natural history of chronic hepatitis B (CHB), the loss of hepatitis B e antigen (HBeAg) and the production of antibody to HBeAg (Anti-HBe) (HBeAg seroconversion) is generally associated with undetectable serum HBV DNA, normalization in alanine aminotransferase (ALT) levels with improvement in liver histology, which is viewed as a significant milestone and endpoint for treatment of HBeAg-positive CHB patients [1-3]. Furthermore, patients who undergo HBeAg seroconversion, spontaneous or treatment-induced, usually have a lower incidence of cirrhosis and hepatocellular carcinoma (HCC) [4, 5]. As highly sensitive techniques for antigen/antibody detection have been developed, a special serological pattern in CHB patients whereby concurrent HBeAg and anti-HBe is sometimes possible seen in clinical practice, even in antiviral treatment-naive patients [6-8].

The prevalence of HBeAg in HBsAg carriers is 80% before 20 years old and decreases to less than 10% after 40 years old [9], suggesting that HBeAg/anti-HBe seroconversion generally occurs during the third or fourth decade of life. Several studies have already reported a few patients with concurrent detection of both markers [10, 11]. However, little is known about the clinical outcomes and the possible mechanisms leading to this unique serological pattern of the e-system, especially in such group. The aims of this cohort were to: 1. Analyze the clinical characteristics of antiviral treatment-naive patients with concurrent HBeAg/Anti-HBe detection; 2. Study the clinical outcomes of these patients according to different treatment strategies; 3. Explore the possible mechanisms

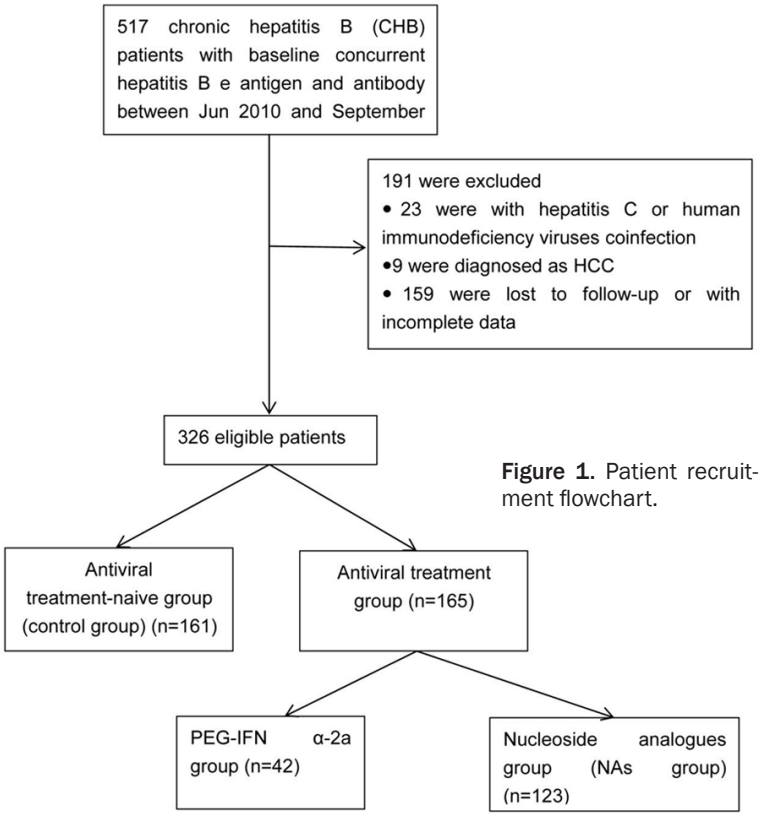

Figure 1. Patient recruitment flowchart.

leading to this unique serological pattern of the e-system.

Patients and methods

Patients and study design

This single-center, retrospective investigator-initiated cohort studied 617 chronic hepatitis B (CHB) patients with concurrent hepatitis B e antigen and antibody detected by Abbott i2000 instrument from Nanfang Hospital, Southern Medical University between Jun 2010 and Jan 2018. Of these, 326 patients with complete data were included in final analysis (**Figure 1**). All patients fulfilled the following criteria: (i) HBsAg positive for more than 6 months; (ii) Persistent or intermittent elevation of ALT serum levels within 12 months before entry; (iii) Aged not less than 14 years old. (iv) Antiviral treatment-naive prior to Admission. Patients with decompensated liver disease (ascites, variceal bleeding or encephalopathy) or renal impairment (serum creatinine ≥ 1.5 mg/dl) were excluded. Other exclusion criteria were as follows: coinfection with human immunodeficiency virus, hepatitis C virus, or hepatitis D virus; a history of alcohol or drug abuse within the preceding 2 years; documented or suspected hepatocellular carcinoma (HCC); prior organ transplantation, or prnancy/lactation. The study was approved by the Ethics Committee of Nanfang Hospital, Southern Medical University. The experiments were carried out in accodance with the approved guidelines and the "informed" consent was obtained from all subjects.

Patients were divided into antiviral treatment-naive group (control group) and treated group according to with/without antiviral treatment after enrollment. In designing more suitable therapies, the treated group was further divided into two groups: PEG-IFN α-2a group and nucleoside analogues group (NAs group). All patients accepted no less than 24 weeks of treatment.

Serum assays

The serum HBV DNA level was measured with the Cobas Taqman HBV Kit (Roche Diagnostics; lower limit of detection, 20 IU/mL). HBeAg, Anti-HBe, anti-HBc were detected using an Architect assay (Abbott Laboratories, Abbott Park, IL). The HBeAg value is presented as PEIU/mL and its titre > 0.1 PEIU/mL was regarded as positive. While anti-HBe value is presented as sample to cut-off ratios (S/CO) and its titre < 1.0 S/CO was taken as positive. Liver function tests, including ALT and total bilirubin, were detected by Olympus AU5421 automatic biochemical detector.

Efficacy endpoints and definitions

The primary endpoint of this study was that the serum HBV DNA (< 20 IU/ml) became PCR undetectable, and/or serum alanine aminotransferase (ALT) normalization (≤ 40 IU/l). The secondary endpoints included HBeAg loss or

Table 1. Baseline characteristics among PEG-IFN α-2a group, NAs group and control group

	PEG-IFN α-2a group (n = 42)	NAs group (n = 123)	Control group (n = 161)	*P* value	*P*1	*P*2
Males (%)	30 (71.4)	98 (79.7)	120 (74.5)	0.453	0.269	0.520
Age (year)	24 ± 11	37 ± 11	34 ± 13	< 0.001	< 0.001	0.816
Undetectable HBV DNA rate, n (%)	5 (11.9)	14 (11.4)	20 (12.5)	0.965	0.927	0.331
HBsAg (IU/mL)	1714.32 ± 1988.62	3562.83 ± 13939.96	3144.64 ± 11647.77	0.684	0.451	0.968
HBeAg (PEIU/mL)	1.67 ± 1.25	1.16 ± 1.67	1.67 ± 3.73	0.296	0.071	0.191
Anti-HBe (S/CO)	0.54 ± 0.31	0.54 ± 0.28	0.52 ± 0.29	0.823	0.929	0.533
ALT (U/L)	185.03 ± 177.18	206.42 ± 319.36	227.17 ± 485.02	0.802	0.743	0.553

ALT, alanine aminotransferase. *P* value for the overall groups; *P*1 value for PEG-IFN α-2a group vs NAs group; *P*2 value for the treated group vs the control group.

seroconversion at week 48, and/or HBsAg loss or seroconversion at week 48.

Detection of Anti-HBe relative affinity index

To further explore the possible mechanism for the concurrence of HBeAg and anti-HBe, an improved 5 mol/L urea elution method was used to measure the relative affinity of anti-HBe with 68 HBeAg(-)/anti-HBe(+) and 82 HBeAg(+)/anti-HBe(+) serum samples. The first step was to set Standard and testing sample wells. Then 50 μl standard and testing sample were added to relevant well, while nothing was added to the blank well. The enzyme marker was added at 50 μl and the neutralizing antigen was also added to each well except the blank well and covered with an adhesive strip and incubated for 30 minutes at 37°C. The second step was to wash each well for once, then add 200 μl 5 M urea or PBST solution respectively and let stand for 10 minutes at room temperature later. Aspirate each well and washed 3 times. The third step was to add 100 μl of HRP-conjugate reagent to each well, covered with an adhesive strip and incubated for 30 minutes at 37°C. Each well as aspirated and washed, repeating the process four times. After the last wash, any remaining Wash Solution was removed by aspirating or decanting and invert the plate and blot it against clean paper towels. Chromogen solution A was added at 50 μl and chromogen solution B 50 μl was added to each well. The solution was gently mixed and incubated for 15 minutes at 37°C (Protect from light). Then 50 μl of the Stop Solution was added to each well. The last step was to read the Optical Density (O.D.) at 450/630 nm using a microtiter plate reader within 30 minutes.

The Relative affinity index was defined as the PBST contrast OD value/urea after elution OD value * 100 in our research. Additionally, each sample was tested twice through the above method and the average value was taken for further comparison.

Statistical analysis

All statistical analyses were performed by using SPSS, version 20.0 (SPSS, Inc., Chicago, IL, USA). Continuous variables were expressed in mean ± standard deviation or median (minimal value, maximal value), as appropriate. A logarithmic transformation was applied to HBV-DNA loads. Qualitative differences between subgroups were analyzed using R × C χ^2 tests. One way ANOVA test, Student t-test, Kruskal-Wallis test and Mann-Whitney U Test were adopted appropriately. A two-sided *P* value < 0.05 was considered to be statistically significant.

Results

Demographic and clinical characteristics

A total of 326 CHB patients with concurrent hepatitis B e antigen and antibody were finally analyzed, including 248 males (76.1%). The mean age was 34 ± 12 year. The mean titer of HBeAg, anti-HBe and HBsAg was 1.48 ± 2.85 PEIU/mL, 0.53 ± 0.29 S/CO and 3118.15 ± 11867.20 IU/mL, respectively. HBV DNA were positively detected in 287 patients (88.0%), and the mean HBV DNA level was 5.15 ± 1.38 log10 IU/mL. The mean level of ALT was 213.91 ± 398.38 U/L. Additionally, there was no statistical difference between the treated group and control group at baseline. All demographic, clinical and serological characteristics are shown in **Table 1**.

Table 2. Treatment efficacy among PEG-IFN α-2a group, NAs group and control group [n (%)] at week 48

	PEG-IFN α-2a group (n = 42)	NAs group (n = 123)	Control group (n = 161)	*P* value	*P*1	*P*2
Undetectable HBV DNA rate	24 (57.1)	95 (77.2)	31 (19.3)	< 0.001	0.012	< 0.001
HBsAg seroclearance rate	6 (14.3)	4 (3.3)	1 (0.6)	< 0.001	0.010	0.007
HBeAg seroconversion rate	27 (64.3)	49 (39.8)	14 (8.7)	< 0.001	0.006	< 0.001
ALT normalization rate	36 (85.7)	104 (84.6)	38 (23.6)	< 0.001	0.856	< 0.001

ALT, alanine aminotransferase. *P* value for the overall groups; *P*1 value for PEG-IFN α-2a group vs NAs group; *P*2 value for the treated group vs the control group.

Subgroup analysis was carried out on the treated group: a total of 165 patients (50.6%) accepted antiviral treatment, among whom 128 were males and 37 were females. Furthermore, the mean age was 34 ± 11 years. The duration of therapy ranged from 24.71 to 244.21 weeks. The patients were divided into two groups: PEG-IFN α-2a group (n = 42, 71.4% males) and NAs group (n = 123, 79.7% males). There was no statistical difference between these two groups among all investigated indexes except age (24 ± 11 vs 37 ± 11; t = -6.283, $P < 0.001$).

Antiviral treatment group achieved better biochemical and serological responses

At week 48, the treated group achieved higher ALT normalization rate, undetectable HBV DNA rate, HBeAg seroconversion rate, and HBsAg seroclearance rate (84.8% vs 23.6%; 46.1% vs 8.7%; 6.1% vs 0.6%; and 72.1% vs 19.3%, all $P < 0.05$) (**Table 2**).

Subgroup analysis was carried out in the treated group: 27 (64.3%) and 6 (14.3%) patients achieved HBeAg seroconversion and HBsAg seroclearance in PEG-IFN α-2a group, which were significantly higher than NAs group ($\chi^2 = 7.532$, $P = 0.006$ & $\chi^2 = 6.695$, $P = 0.010$). And the NAs group has a higher undetectable HBV DNA rate (57.1% vs 77.2%; $\chi^2 = 6.287$, $P = 0.012$). However, there was no statistical difference of the ALT normalization rate between these two groups (85.7% vs 84.6%; $\chi^2 = 0.033$, $P = 0.856$).

Concurrent patients have a lower HBeAg/anti-HBe affinity index

A total of 56 samples (the mean titer of HBeAg was 0.13 S/CO) were chosen for the preliminary experiment. When the titer of anti-HBe was less than 0.5 S/CO, the 5 M urea elution method has a higher positive elution rate than the total positive elution rate (over 60% vs 44.6%). As a result of this, it was decided to choose the samples with the titer of anti-HBe less than 0.5 S/CO for further experiments (unpublished data).

Having successfully tested 59 HBeAg(-)/Anti-HBe(+) serum samples and 79 HBeAg(+)/Anti-HBe(+) serum samples, all were tested for at least twice through the above method and the average value was taken for further comparison. The concurrent patients were younger than the HBeAg(-)/Anti-HBe(+) patients (30 ± 10.1 year vs 35 ± 12.8 year, $P = 0.015$), while the concurrent patients had a higher titer of anti-HBe (0.21 ± 0.13 S/CO vs 0.12 ± 0.13 S/CO, $P < 0.001$). However, the mean affinity index (AI) of the concurrent patients was 95.42 ± 36.26, which was significantly lower than the HBeAg(-)/Anti-HBe(+) patients 122.10 ± 46.10, $P < 0.001$. All data are shown in **Table 3** and **Figure 2**.

Discussion

A previous study shown that the prevalence of concurrent HBeAg/anti-HBe detection was common (10.4%) compared with the HBeAg/anti-HBe dual negative patients (< 1%), and this phenomenon could sustain for a few months to years [8]. However, little is known about the clinical outcomes and the possible mechanisms leading to this unique serological pattern of the e-system, especially antiviral treatment-naive patients. In this large sample (n = 326) cross-sectional observation cohort, we found that: 1. Concurrence of HBeAg and anti-HBe was correlated with pronounced liver disease; 2. It is suitable to treat the concurrent patients, and PEG-IFN is preferred; 3. Decreased affinity

Table 3. Clinical characteristics and affinity index of the patients by HBeAg and Anti-HBe Status

	HBeAg(+)/anti-HBe(+) (n = 79)	HBeAg(-)/anti-HBe(+) (n = 59)	Test statistics (t/χ^2/U)	*P* value
Gender (male/female)	66/13	48/11	0.113	0.737
Age (year)	30 ± 10.1	35 ± 12.8	2.455	0.015
HBeAg (S/CO)	3.94 ± 4.58	0.42 ± 0.15	-5.897	< 0.001
Anti-HBe (S/CO)	0.21 ± 0.13	0.12 ± 0.13	-4.171	< 0.001
AI (%)	95.42 ± 36.26	122.10 ± 46.10	3.805	< 0.001

AI: affinity index.

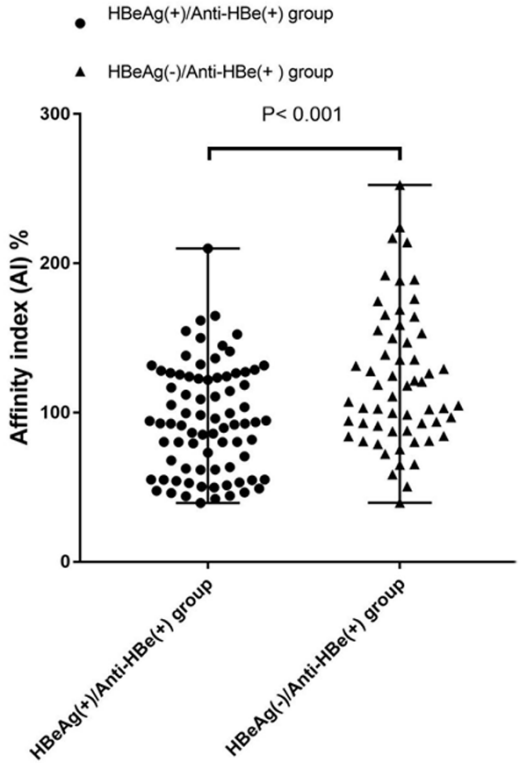

Figure 2. The mean affinity index (AI) of the 59 HBeAg(-)/Anti-HBe(+) serum samples and 79 HBeAg(+)/Anti-HBe(+) serum samples.

between HBeAg and anti-HBe is a probably explanation for this concurrent pattern.

Elevated ALT, HBeAg and anti-HBe titers closing to the cutoff value are the two main clinical characteristics of antiviral treatment-naive patients with concurrent HBeAg/anti-HBe detection. In line with the previous study [8], high ALT levels were also found in the concurrent treatment-native patients, indicating severe immune-mediated liver injury, which in turn might result in a reduction in viral replication [12-14], as a high percentage of patients with undetectable HBV-DNA was also observed in this study. Additionally, the mean titers of HBeAg and anti-HBe were 1.48 PEIU/mL and 0.53 S/CO, respectively, closing to their respective cut-off values. The mutations in pre-core and/or basal core promoter could be the reason for reduction or abrogation of HBeAg expression at the transcriptional or translational phases [15-20]. Additionally, this could also be the result of strong immune clearance [21].

It is worth mentioning that during the natural history of chronic HBV infection, higher serum ALT and TBiL are associated with a higher rate of HBeAg seroconversion, after which viral replication and liver inflammation activity would significantly reduce [22-27]. Furthermore, patients with a low titer of baseline HBeAg were also more likely to have HBeAg seroconversion [28, 29]. Therefore, to quickly and effectively suppress viral replication and control liver inflammation activity, antiviral treatment is recommended for the concurrent patients. About 50.6% (165) of the enrolled patients were willing to accept antiviral treatment, among whom 42 received PEG-IFN α-2a therapy and 123 received NAs. Up to Jan 2018, the treated group (PEG-IFN α-2a group and NAs group, n = 165) has higher HBsAg negative conversion rate, HBeAg seroconversion rate, HBV DNA negative conversion rate and ALT normalization rate compared with the control group (n = 161). For further study, sub-group analysis was carried out on the treatment group. The PEG-IFN α-2a group had significantly higher HBeAg seroconversion and HBsAg negative conversion rate than NAs group, which was basically identical to previous studies. Additionally, both of PEG-IFN α-2a group and NAs group had higher HBeAg seroconversion rate in concurrent patients (64.3% & 39.8%) than the HBeAg(+)/Anti-HBe(-) patients (about 40% in PEG-IFN α-2a group and 20% in NAs group). Loss of HBeAg and the development of antibody to hepatitis

B e antigen (anti-HBe) (HBeAg seroconversion) is associated with undetectable serum HBV DNA, normalization in alanine aminotransferase (ALT) levels with improvement in liver histology, which is viewed as a significant milestone and endpoint in the treatment of HBeAg-positive patients with CHB. Therefore, it is recommended that PEG-IFN be preferentially used for these patients considering the above evidence.

Decreased affinity between HBeAg and anti-HBe is a probably explanation for this concurrent pattern [30, 31]. During the nature history of CHB, two different protein composition of HBeAg in sera were found: the large HBeAg polypeptides are presented as immune-complexes while the small polypeptides are presented as free-state [2, 3, 21]. During progression of HBeAg seroconversion, there will be a period that HBeAg has disappeared while anti-HBe could not yet be detected, which is called as window period [32-35]. It's because when the epitopes of HBeAg was closed by anti-HBe, HBeAg and anti-HBe would be presented as immune-complexes. Theoretically, the antigen-antibody reaction is a reversible chemical reaction: antigen + anti-body ⇋ antigen-antibody complex. Therefore, the concurrent pattern of HBeAg and anti-HBe might be due to the low affinity of anti-HBe. To further explore the possible mechanisms, an improved 5 mol/L urea elution method was used to measure the relative affinity of anti-HBe. Consistent with our speculation, the mean affinity index (AI) of the concurrent patients was significantly lower than the HBeAg(-)/Anti-HBe(+) patients. Therefore, special tests such as the surface plasma resonance technology could be used to verify this in the future.

There are three limitations of the current study. First, the HBeAg-positive and anti-HBe-positive group were not included for comparison, though the clinical and virological characteristics of these groups have been well studied before. Second, Liver histology was not assessed as few patients agreed with liver biopsy. Additionally, the method using to measure the relative affinity of anti-HBe is relatively rough and not so accurate, though we have indirectly reflected that decreased affinity between HBeAg and anti-HBe is a probably explanation for the concurrent pattern.

In conclusion, the prevalence of concurrent HBeAg/anti-HBe detection is often accompanied by severe liver inflammation. To effectively suppress viral replication and achieve HBeAg seroconversion, Peg-IFN is preferred. In addition, this study has indirectly verified that decreased affinity between HBeAg and anti-HBe is a probably explanation for the concurrent of HBeAg and anti-HBe.

Acknowledgements

This study was partly supported by Science and Technology Planning Project of Guangdong Province, China (61130697010). The funding agency had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosure of conflict of interest

None.

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References

[1] Jaroszewicz J, Calle SB, Wursthorn K, Deterding K, Schlue J, Raupach R, Flisiak R, Bock CT, Manns MP, Wedemeyer H, Cornberg M. Hepatitis B surface antigen (HBsAg) levels in the natural history of hepatitis B virus (HBV)-infection: a European perspective. J Hepatol 2010; 52: 514-522.

[2] Thomas DL, Di Bisceglie AM, Alter HJ, Terrault NA. Understanding the natural history of chronic HBV and HCV infections. J Fam Pract 2010; 59: S17-S22.

[3] Wang L, Zou ZQ, Wang K, Yu JG, Liu XZ. Role of serum hepatitis B virus marker quantitation to differentiate natural history phases of HBV infection. Hepatol Int 2016; 10: 133-138.

[4] Fung J, Cheung KS, Wong DK, Mak LY, To WP, Seto WK, Lai CL, Yuen MF. Long-term outcomes and predictive scores for hepatocellular carcinoma and hepatitis B surface antigen seroclearance after hepatitis B e-antigen seroclearance. Hepatology 2018; 68: 462-472.

[5] Qu LS, Zhu J, Chen H, Jin F, Ni RZ, Lu CH. Effects of hepatitis B e-antigen on recurrence of hepatitis B-related hepatocellular carcinoma after curative resection: A meta-analysis. Hepatol Res 2013; 43: 347-354.

[6] Cheng Y, Guindon S, Rodrigo A, Lim SG. Increased viral quasispecies evolution in

HBeAg seroconverter patients treated with oral nucleoside therapy. J Hepatol 2013; 58: 217-224.
[7] Hsu YS, Chien RN, Yeh CT, Sheen IS, Chiou HY, Chu CM, Liaw YF. Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. Hepatology 2002; 35: 1522-1527.
[8] Wang J, Zhou B, Lai Q, Wang Y, Shen G, Wang Z, Chen J, Hou J. Clinical and virological characteristics of chronic hepatitis B with concurrent hepatitis B E antigen and antibody detection. J Viral Hepat 2011; 18: 646-652.
[9] Chu CM, Sheen IS, Lin SM, Liaw YF. Sex difference in chronic hepatitis B virus infection: studies of serum HBeAg and alanine aminotransferase levels in 10,431 asymptomatic Chinese HBsAg carriers. Clin Infect Dis 1993; 16: 709-713.
[10] Lampertico P, Manzin A, Rumi MG, Paolucci S, Del NE, Clementi M, Colombo M. Hepatitis B virus precore mutants in HBeAg carriers with chronic hepatitis treated with interferon. J Viral Hepat 1995; 2: 251-256.
[11] Marinos G, Torre F, Gunther S, Thomas MG, Will H, Williams R, Naoumov NV. Hepatitis B virus variants with core gene deletions in the evolution of chronic hepatitis B infection. Gastroenterology 1996; 111: 183-192.
[12] Alam MM, Mahtab MA, Akbar SM, Kamal M, Rahman S. Hepatic necroinflammation and severe liver fibrosis in patients with chronic hepatitis B with undetectable HBV DNA and persistently normal alanine aminotransferase. Bangladesh Med Res Counc Bull 2014; 40: 92-96.
[13] Ke WM, Xie SB, Li XJ, Zhang SQ, Lai J, Ye YN, Gao ZL, Chen PJ. There were no differences in serum HBV DNA level between HBeAg-positive and HBeAg-negative chronic hepatitis B with same liver histological necroinflammation grade but differences among grades 1, 2, 3 and 4 apportioned by the same hepatic parenchyma cell volume. J Viral Hepat 2011; 18: 637-645.
[14] Xu Z, Shen J, Pan X, Wei M, Liu L, Wei K, Liu L, Yang H, Huang J. Predictive value of serum Golgi protein 73 for prominent hepatic necroinflammation in chronic HBV infection. J Med Virol 2018; 90: 1053-1062.
[15] Buckwold VE, Xu Z, Chen M, Yen TS, Ou JH. Effects of a naturally occurring mutation in the hepatitis B virus basal core promoter on precore gene expression and viral replication. J Virol 1996; 70: 5845-5851.
[16] Chen CY, Crowther C, Kew MC, Kramvis A. A valine to phenylalanine mutation in the precore region of hepatitis B virus causes intracellular retention and impaired secretion of HBe-antigen. Hepatol Res 2008; 38: 580-592.
[17] Cui XJ, Cho YK, Song BC. Influence of the basal core promoter and precore mutation on replication of hepatitis B virus and antiviral susceptibility of different genotypes. J Med Virol 2015; 87: 601-608.
[18] Suppiah J, Mohd ZR, Bahari N, Haji NS, Saat Z. G1896A Precore mutation and association with HBeAg status, genotype and clinical status in patients with chronic hepatitis B. Hepat Mon 2015; 15: e31490.
[19] Marrone A, Zampino R, Luongo G, Utili R, Karayiannis P, Ruggiero G. Low HBeAg serum levels correlate with the presence of the double A1762T/G1764A core promoter mutation and a positive response to interferon in patients with chronic hepatitis B virus infection. Intervirology 2003; 46: 222-226.
[20] Geng Y, Wang X, Lu X, Wu X, Xu N, Han L, Xu J. Mutation changes in the preC/Core promoter in HBeAg-positive patients with chronic hepatitis B during interferon therapy. Medicine (Baltimore) 2016; 95: e2657.
[21] Perrillo RP. Acute flares in chronic hepatitis B: the natural and unnatural history of an immunologically mediated liver disease. Gastroenterology 2001; 120: 1009-1022.
[22] Yang J, Yan D, Guo R, Chen J, Li Y, Fan J, Fu X, Yao X, Diao H, Li L. Predictive value of serum ALT and T-cell receptor beta variable chain for HBeAg seroconversion in chronic hepatitis B patients during tenofovir treatment. Medicine (Baltimore) 2017; 96: e6242.
[23] You H, Ma H, Liu T, Cong M, Wang P, Ou X, Wang X, Ren J, Li H, Wang B, Jia J. Different models of HBeAg seroconversion predicated by on-treatment ALT and HBV DNA profiles. J Viral Hepat 2009; 16: 876-882.
[24] Lee CM, Ong GY, Lu SN, Wang JH, Liao CA, Tung HD, Chen TM, Changchien CS. Durability of lamivudine-induced HBeAg seroconversion for chronic hepatitis B patients with acute exacerbation. J Hepatol 2002; 37: 669-674.
[25] van Nunen AB, Hansen BE, Suh DJ, Lohr HF, Chemello L, Fontaine H, Heathcote J, Song BC, Janssen HL, de Man RA, Schalm SW. Durability of HBeAg seroconversion following antiviral therapy for chronic hepatitis B: relation to type of therapy and pretreatment serum hepatitis B virus DNA and alanine aminotransferase. Gut 2003; 52: 420-424.
[26] Yu W, Wang Y, Shen C, Ji R, Zhang L, Zhao X, Su M, Zhang Y, He W, Cao J, Hao Y, Chen S, Zhao C. Predictors of HBeAg seroconversion after long-term nucleos(t)ide analogues treatment for chronic hepatitis B: a multicenter study in real clinical setting. Braz J Infect Dis 2017; 21: 213-218.
[27] Yuen MF, Yuan HJ, Hui CK, Wong DK, Wong WM, Chan AO, Wong BC, Lai CL. A large population study of spontaneous HBeAg seroconver-

sion and acute exacerbation of chronic hepatitis B infection: implications for antiviral therapy. Gut 2003; 52: 416-419.

[28] Gao YH, Meng QH, Zhang ZQ, Zhao P, Shang QH, Yuan Q, Li Y, Deng J, Li T, Liu XE, Zhuang H. On-treatment quantitative hepatitis B e antigen predicted response to nucleos(t)ide analogues in chronic hepatitis B. World J Hepatol 2016; 8: 1511-1520.

[29] Thompson AJ, Nguyen T, Iser D, Ayres A, Jackson K, Littlejohn M, Slavin J, Bowden S, Gane EJ, Abbott W, Lau GK, Lewin SR, Visvanathan K, Desmond PV, Locarnini SA. Serum hepatitis B surface antigen and hepatitis B e antigen titers: disease phase influences correlation with viral load and intrahepatic hepatitis B virus markers. Hepatology 2010; 51: 1933-1944.

[30] Galati G, De Vincentis A, Vespasiani-Gentilucci U, Gallo P, Vincenti D, Solmone MC, Dell'Unto C, Picardi A. Coexistence of HBsAg and HBsAb in a difficult-to-treat chronic hepatitis B: loss of HBsAg with entecavir plus tenofovir combination. BMC Gastroenterol 2014; 14: 94.

[31] Liu W, Hu T, Wang X, Chen Y, Huang M, Yuan C, Guan M. Coexistence of hepatitis B surface antigen and anti-HBs in Chinese chronic hepatitis B virus patients relating to genotype C and mutations in the S and P gene reverse transcriptase region. Arch Virol 2012; 157: 627-634.

[32] Liaw YF, Chu CM, Huang MJ, Sheen IS, Yang CY, Lin DY. Determinants for hepatitis B e antigen clearance in chronic type B hepatitis. Liver 1984; 4: 301-306.

[33] Xing T, Xu H, Cao L, Ye M. HBeAg seroconversion in HBeAg-positive chronic hepatitis B patients receiving long-term nucleos(t)ide analog treatment: a systematic review and network meta-analysis. Plos One 2017; 12: e169444.

[34] Bozkaya H, Ayola B, Lok AS. High rate of mutations in the hepatitis B core gene during the immune clearance phase of chronic hepatitis B virus infection. Hepatology 1996; 24: 32-37.

[35] Takano T, Tajiri H, Etani Y, Miyoshi Y, Tanaka Y, Brooks S. Natural history of chronic hepatitis B virus infection in childhood and efficacy of interferon therapy. Scand J Gastroenterol 2015; 50: 892-899.