JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Brief Original Article

Mutations in reverse transcriptase region of HBV affect Hepatitis B surface antigen titers and its correlation with HBV DNA

Hongyu Shen[1,2], Chunhua Chen[1,2], Chunyan Ye[2,3], Hongyu Zhang[1,2], Shuangxiong Hang[1,2], Min Chen[1,2], Zhen Zhu[1,2], Yuan Xue[2,3], Longgen Liu[2,3]

[1] Department of Clinical Laboratory, the Third peoples' Hospital of Changzhou, Changzhou, Jiangsu, China
[2] Institute of Hepatology, the Third People's Hospital of Changzhou, Changzhou, Jiangsu, China
[3] Department of Liver Diseases, the Third People's Hospital of Changzhou, Changzhou, Jiangsu, China

Abstract

Introduction: The impact of mutations in the reverse transcriptase region of HBV on serum HBsAg titer and its correlation with HBV DNA is largely unknown.

Methodology: A total of 644 patients, with a history of lamivudine or adefovir dipivoxil resistance who underwent genotypic resistance tests, were enrolled in this study. Serum HBsAg, hepatitis B e antigen and HBV DNA were quantified, and the HBV RT region was sequenced and analyzed. Then, the patients were divided into five sub-groups, including M204I/V, L180M+M204I/V, A181T/V, N236T and A181T/V+N236T according to the mutation spectra.

Results: HBsAg was lower in the wild-type and A181T/V+N236T groups as compared to the M204I/V, L180M+M204I/V and N236T groups. HBsAg was positively correlated with HBV DNA levels in the wild-type group ($r = 0.322$, $p < 0.01$), as well as in the M204I/V, L180M+M204I/V, A181T/V, and N236T subgroups, while no correlation was found in the A181T/V+N236T subgroup ($r = 0.159$, $p = 0.217$). Moreover, for patients with N236T mutation, HBsAg was positively correlated with HBV DNA level in the HBeAg negative group ($r = 0.435$, $p = 0.016$), but not in the HBeAg positive group ($r = 0.105$, $p = 0.594$). For patients with A181T/V or N236T mutation, HBsAg was positively correlated with HBV DNA in older patients (≥ 40 years), but not in younger patients (< 40 years).

Conclusions: Serum HBsAg titer and its correlation with HBV DNA may be affected by mutations in the reverse transcriptase region of HBV, that should be re-evaluated in patients with antiviral resistance.

Key words: hepatitis B virus; hepatitis B surface antigen; mutation; drug resistance.

J Infect Dev Ctries 2019; 13(11):1062-1067. doi:10.3855/jidc.11447

(Received 14 March 2019 – Accepted 01 October 2019)

Introduction

Hepatitis B virus (HBV) infection remains a challenging public health problem worldwide, and the number of HBV-related deaths increased by 33% during the past few decades [1,2]. Due to the limited chances of achieving functional cure, long-term or indefinite treatment with nucleos(t)ide analogs (NAs) is usually recommended in clinical practice [3,4].

HBsAg quantification, which is a valuable parameter to evaluate HBV replication, immune control and disease progression, has improved the management of chronic hepatitis B (CHB) [2,5]. However, HBsAg titers vary significantly with HBV genotypes, age of the patient, as well as different phases of HBV infection [6,7]. A study reported that serum HBsAg could predict HBV DNA levels in HBV e antigen (HBeAg) negative CHB patients [8], while another study showed a poor correlation between HBsAg and HBV DNA in treated and untreated patients [9]. Recently, Yang et al. reported significant positive correlation between HBsAg and HBV DNA in HBeAg positive patients, but not in younger (< 40 years old) HBeAg negative patients. Thus, the relationship between HBsAg and HBV DNA remains controversial [6-10].

NAs with low genetic barrier to induce resistance, including lamivudine (LAM) and adefovir dipivoxil (ADV), are being widely used in the last decade, and have resulted in prevalence of drug-resistant mutants. Theoretically, the open reading frames of HBV genomes overlap, and mutations in the reverse transcriptase (RT) region may affect HBsAg levels, infectivity as well as virion production. It has been reported that rtA181T mutation leads to sW172* substitution in the S domain, which is a stop codon and has a negative effect on HBsAg production [11,12]. Drug-resistance mutation rtM204I/sW196* was also

reported to be a stop codon in S gene [13]. HBV wild-type strains coexist with drug-resistant strains, and can rescue the production of HBV virions during LAM treatment [12]. The wild-type and drug-resistant strains may complement each other, and generate synergistic or complementary effects [12,14]. However, the impact of mutations in the RT region on HBsAg level is largely unknown.

In the present study, we analyzed the HBsAg level and its correlation with HBV DNA in a large cohort of CHB patients with different mutations in the RT region. These findings may extend our understanding of the relationship between HBsAg and HBV DNA, and re-evaluation of HBsAg levels may be necessary in patients with mutant HBV infection.

Methodology

Patients

A total of 644 patients with CHB were retrospectively recruited between June 2013 and May 2018 at the Third People's Hospital of Changzhou, China. Patients with chronic necroinflammatory liver disease caused by HBV infection were diagnosed with CHB according to the Chinese guidelines for prevention and treatment of CHB (2015 version) [15]. Patients with CHB, who had a history of LAM or ADV treatment for more than one year, and were confirmed as drug-resistant by genotypic resistance test, were recruited. Patients with autoimmune diseases, alcoholic liver disease, liver cirrhosis or cancer, immunodeficiency diseases, or co-infection with other hepatitis virus, were excluded. Among those patients, 239 were HBeAg negative while 405 were HBeAg positive. Another 644 treatment-naïve CHB patients infected with wild-type HBV strains were enrolled as controls (wild-type). The two groups were paired according to the HBeAg and age, with insignificant differences in the two parameters between the mutant and wild-type groups. The study was approved by the Ethics Committee of the Third People's Hospital of Changzhou according to the Declaration of Helsinki, 1975.

Laboratory tests

Demographic and clinical data, including HBsAg, HBeAg and HBV DNA, were collected within a week after the RT mutations were confirmed. Serum HBsAg and HBeAg levels were quantified using chemiluminescent immunoassay (Abbott Laboratories, Abbott Park, IL, USA), and HBV DNA was tested with Cobas Taqman HBV test (Roche Diagnostic, Basel, Switzerland). Serum HBV DNA was extracted using the QIAamp blood mini-kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The HBV RT region, spanning amino acids 110-288, was amplified by nested PCR, and the HBV strains were detected by direct Sanger sequencing. The details were described in our previous paper [16].

Statistical analysis

Continuous variables were expressed as mean ± standard deviation, and the differences were analyzed using one-way ANOVA (for normally distributed data). Pearson's correlation analysis was used to evaluate the correlation between quantitative HBsAg and HBV DNA levels. All analyses were performed using SPSS 17.0 software (Chicago, IL, USA). A two-sided $p < 0.05$ was considered as statistically significant.

Results

Comparison of HBsAg titers and HBV DNA levels between different groups

Of the 644 patients, 420 (65.2%) patients had a history of lamivudine (LAM) resistance, while the remaining 224 (34.8%) patients had adefovir dipivoxil (ADV) resistance. These patients were divided into five sub-groups, including M204I/V (n = 138), L180M+M204I/V (n = 282), A181T/V (n = 104), N236T (n = 58) and A181T/V+N236T (n = 62), according to the mutation spectra. Moreover, there was no significant difference in the frequencies of mutations among genotypes B and C ($p > 0.05$).

As shown in Figure 1A, HBsAg was lower in the wild-type and A181T/V+N236T groups as compared to the M204I/V, L180M+M204I/V and N236T groups. The HBV DNA levels had no significant difference between the wild-type group and mutant sub-groups (Figure 1B).

The patients were further divided according to their age and HBeAg levels. For the wild-type or M204I/V groups, HBsAg was higher in younger patients (< 40 years) as compared to the older patients (≥ 40 years) (Figure 2A). HBsAg level was higher in HBeAg (+) patients than in HBeAg (-) patients, except for the M204I/V and A181T/V+N236T groups (Figure 2B).

Figure 1. Comparison of HBsAg levels in patients with wild-type and drug-resistant mutations. Differences were analyzed using one-way ANOVA, followed by post hoc tests to compare the differences between the six groups. Line segment referred to the comparison between two groups. (a) HBsAg was lower in the wild-type group and A181T/V+N236T groups as compared to the M204I/V, L180M+M204I/V and N236T groups. (b) The HBV DNA levels had no significant difference between the wild-type group and mutant sub-groups. *, $p < 0.05$; **, $p < 0.01$, ns, no significance.

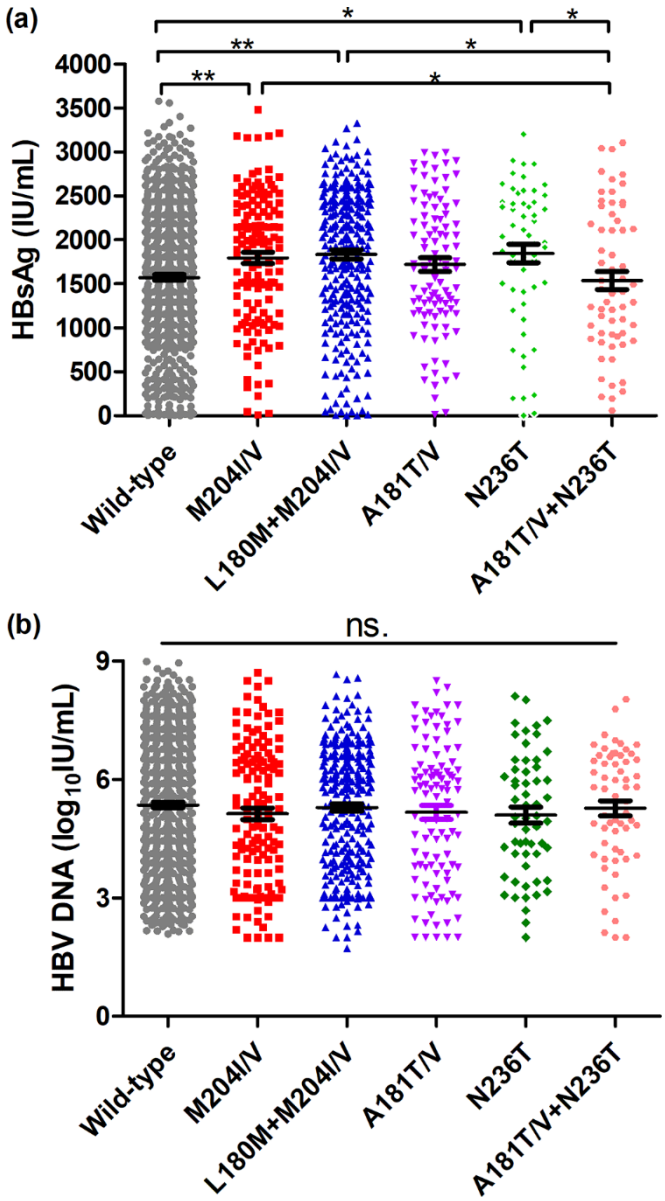
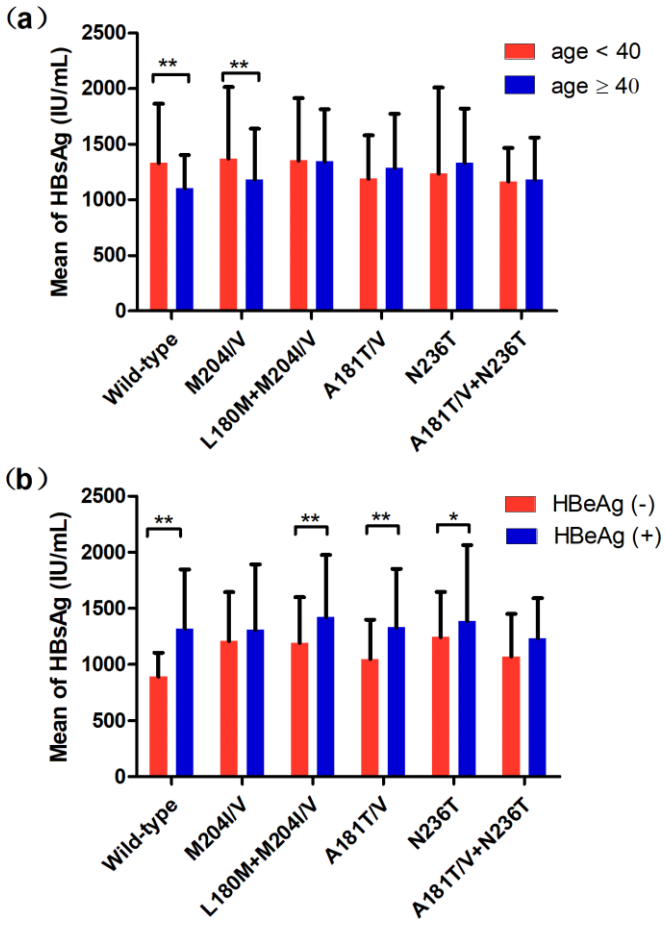


Figure 2. Comparison of HBsAg titers and HBV DNA levels in patients with different age and HBeAg status. (a) For the wild-type or M204I/V groups, HBsAg was higher in younger patients (< 40 years) as compared to the older patients (≥ 40 years). (b) HBsAg level was higher in HBeAg (+) patients than in HBeAg (-) patients, except for the M204I/V and A181T/V+N236T groups. Data are expressed as mean ± standard deviation. *, $p < 0.05$, **, $p < 0.01$.



Correlation between HBsAg and HBV DNA levels in the wild-type and mutant groups

HBsAg was positively correlated with HBV DNA levels in the wild-type group ($r = 0.322$, $p < 0.001$) (Figure 3a), as well as in the M204I/V ($r = 0.380$, $p < 0.001$) (Figure 3b), L180M+M204I/V ($r = 0.284$, $p < 0.001$) (Figure 3c), A181T/V ($r = 0.258$, $p = 0.008$) (Figure 3d), and N236T subgroups ($r = 0.369$, $p = 0.004$) (Figure 3e), but not in the A181T/V+N236T subgroup ($r = 0.159$, $p = 0.217$) (Figure 3f).

Correlation between HBsAg and HBV DNA levels in HBeAg positive and negative groups

For the wild-type group, there was a positive correlation between HBsAg and HBV DNA level in the HBeAg positive group ($r = 0.202$, $p < 0.001$), but not in the HBeAg negative group ($r = -0.011$, $p = 0.868$) (Table 1). For the M204I/V and L180+204I/V groups, HBsAg was positively correlated with HBV DNA levels in the HBeAg positive and negative groups (both $p < 0.05$). Moreover, no significant correlation was found in the A181T/V and A181T/V+N236T groups for both HBeAg positive and negative patients (all $p > 0.05$). For patients with N236T mutation, there was a positive correlation between HBsAg and HBV DNA level in the HBeAg negative group ($r = 0.435$, $p = 0.016$), but not in the HBeAg positive group ($r = 0.105$, $p = 0.594$).

Correlation between HBsAg and HBV DNA levels in patients aged < 40 and ≥ 40 years

As shown in Table 2, HBsAg was positively correlated with HBV DNA level in the wild-type, M204I/V and L180M+M204I/V groups, regardless of the age of patients (all $p < 0.05$). For patients with A181T/V or N236T mutation, there was a positive correlation between HBsAg and HBV DNA in older patients (≥ 40 years), but not in younger patients (< 40 years). Furthermore, the correlation between HBsAg and HBV DNA was not significant in the A181T/V+N236T group, regardless of the age (both $p > 0.05$).

Discussion

In the present study, HBsAg titers, and the correlation between HBsAg and HBV DNA were analyzed in CHB patients with different drug-resistant mutations. HBsAg was higher in the M204I/V, L180M+M204I/V and N236T groups than in the wild-type group. Moreover, HBsAg titers were positively correlated with HBV DNA levels in the wild-type and mutation groups, except for the A181T/V+N236T group.

HBsAg titers can be affected by mutations in the HBV genome, including preS, precore, and basal core promotor region [17-19]. A strong relationship between HBsAg and basal core promotor 1762/1764 mutations was previously reported [18]. Recently, G1896A

Figure 3. Correlation between HBsAg and HBV DNA levels in the wild-type and drug-resistant mutation groups. HBsAg was positively correlated with HBV DNA levels in the wild-type group (a), as well as in the M204I/V (b), L180M+M204I/V (c), A181T/V (d), and N236T subgroups (e), but not in the A181T/V+N236T subgroup (f).

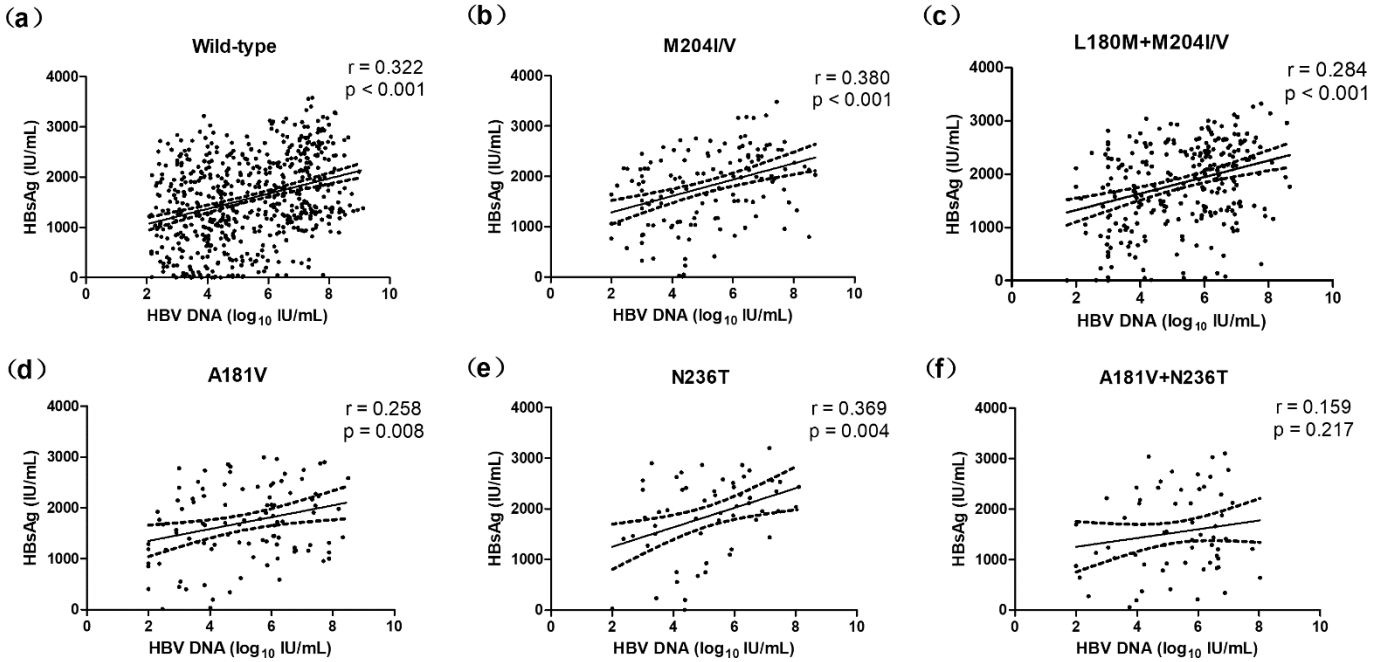


Table 1. Correlation between HBsAg and HBV DNA in HBeAg (-) and (+) patients.

HBV DNA (Log_{10}IU/mL)		HBsAg (IU/mL)	
		r	*P*
Wild type	HBeAg- (n = 239)	-0.011	0.868
	HBeAg+ (n = 405)	0.202	0.000
M204I/V	HBeAg- (n = 53)	0.465	0.000
	HBeAg+ (n = 85)	0.275	0.011
L180M+M204I/V	HBeAg- (n = 104)	0.414	0.000
	HBeAg+ (n = 178)	0.169	0.024
A181T/V	HBeAg- (n = 30)	0.225	0.231
	HBeAg+ (n = 74)	0.161	0.172
N236T	HBeAg- (n = 30)	0.435	0.016
	HBeAg+ (n = 28)	0.105	0.594
A181T/V+N236T	HBeAg- (n = 22)	0.357	0.103
	HBeAg+ (n = 40)	0.036	0.825

mutation in the precore region of HBV was reported to be associated with HBsAg clearance in HBV and HIV co-infected patients [19]. Although NA-resistance has become a manageable issue, persistent NA treatment is needed because covalently closed circular DNA (cccDNA) in the nucleus cannot be eliminated. Mutations in the RT region can impact HBV reverse transcript, and affect the relaxed circular DNA (rcDNA) formation and its conversion to cccDNA [14]. RtA181T and rtM204I mutations resulting in sW172* and sW196* substitutions in the S domain, serve as stop codons and have negative effects on HBsAg secretion [11-13]. In addition, HBV exists as quasispecies. Wild-type strains coexist with drug-resistant strains and rescue virion production of the mutants under NA treatment [12]. Therefore, the effects of drug-resistant mutations on HBsAg during long-term therapy remain a concern.

Consistent with previous studies [10], serum HBsAg level was higher in HBeAg positive patients as compared to HBeAg negative patients, and was positively correlated with HBV DNA level in the mutation groups, except for the A181T/V+N236T group. Su et al. reported that hotspot mutations in HBV RT/S overlapping sequence were correlated with lower serum HBsAg levels in HBeAg (+) CHB patients [20]. Our data showed that HBsAg titers may be affected by drug-resistant mutations in the RT region, which extends our understanding of HBsAg evaluation during antiviral treatment. Moreover, these data also suggested that the correlation between HBsAg and HBV DNA may be affected by the HBeAg status and age of patients.

A limitation to the present study is that mutations in HBV S region are not performed. Moreover, mutations in the HBV RT region may affect the HBsAg titers and its correlations with HBV DNA, while the mechanisms remain unknown.

Conclusion

Serum HBsAg titer and its correlation with HBV DNA may be affected by mutations in the RT region in HBV genome. The correlation between HBsAg and

Table 2. Correlation between HBsAg and HBV DNA in CHB patients (< 40 and ≥ 40 years old).

HBV DNA (Log_{10}IU/mL)		HBsAg (IU/mL)	
		r	*P*
Wild type	< 40 (n = 239)	0.202	0.002
	≥ 40 (n = 405)	0.326	0.000
M204I/V	< 40 (n = 58)	0.405	0.002
	≥ 40 (n = 80)	0.399	0.000
L180M+M204I/V	< 40 (n = 60)	0.256	0.048
	≥ 40 (n = 222)	0.292	0.000
A181T/V	< 40 (n = 28)	0.228	0.244
	≥ 40 (n = 76)	0.275	0.016
N236T	< 40 (n = 8)	0.255	0.542
	≥ 40 (n = 50)	0.398	0.004
A181T/V+N236T	< 40 (n = 12)	0.408	0.188
	≥ 40 (n = 50)	0.104	0.472

HBV DNA should be re-evaluated during NA treatment, especially for patients with NA-resistance.

Funding

This work was supported by grants from the Natural Science Foundation of Jiangsu Province (BK20180183), the Science and Technology project of Changzhou (CJ20180023) and the Chinese Foundation for Hepatitis Prevention and Control-WBE Liver Fibrosis Foundation (WBE20161013).

Authors' contributions

Shen HY and Liu LG conceived and designed the study. Shen HY, Chen CH, Ye CY, Zhang HY, Hang SX, Chen M and Zhu Z performed the experiments. Shen HY, Chen CH and Xue Y collected the data. Shen HY and Xue Y performed the analysis and drafted the manuscript. All authors read and approved the final manuscript. Shen HY and Chen CH contributed equally to this work.

References

1. Stanaway JD, Flaxman AD, Naghavi M, Fitzmaurice C, Vos T, Abubakar I, Abu-Raddad LJ, Assadi R, Bhala N, Cowie B, Forouzanfour MH, Groeger J, Hanafiah KM, Jacobsen KH, James SL, MacLachlan J, Malekzadeh R, Martin NK, Mokdad AA, Mokdad AH, Murray CJL, Plass D, Rana S, Rein DB, Richardus JH, Sanabria J, Saylan M, Shahraz S, So S, Vlassov VV, Weiderpass E, Wiersma ST, Younis M, Yu C, El Sayed Zaki M, Cooke GS (2016) The global burden of viral hepatitis from 1990 to 2013: findings from the Global Burden of Disease Study 2013. Lancet 388: 1081-1088.
2. Zhou K, Contag C, Whitaker E, Terrault N (2019) Spontaneous loss of surface antigen among adults living with chronic hepatitis B virus infection: a systematic review and pooled meta-analyses. Lancet Gastroenterol Hepatol 4: 227-238.
3. Papatheodoridi M, Papatheodoridis G (2019) Can we stop nucleoside analogues before HBsAg loss? J Viral Hepat 26: 936-941.
4. Jeng WJ, Hsu YC, Su TH, Chen CH (2019) Deliberations more than a cut-off hepatitis B surface antigen value for nucleos(t)ide analogue cessation. Hepatology 70: 1488-1489.
5. European Association for the Study of the Liver (2017) EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol 67: 370-398.
6. Jang JW, Yoo SH, Kwon JH, You CR, Lee S, Lee JH, Chung KW (2011) Serum hepatitis B surface antigen levels in the natural history of chronic hepatitis B infection. Aliment Pharmacol Ther 34: 1337-1346.
7. Celik M, Arabul M, Cekic C, Vatansever S, Ipek S, Aslan F, Unsal B (2014) Clinical utility of hepatitis B surface antigen levels during the natural history and treatment of chronic hepatitis B infection. Prz Gastroenterol 9: 164-167.
8. Li J, Sun X, Fang J, Wang C, Han G, Ren W (2017) Analysis of intrahepatic total HBV DNA, cccDNA and serum HBsAg level in chronic hepatitis B patients with undetectable serum HBV DNA during oral antiviral therapy. Clin Res Hepatol Gastroenterol 41: 635-643.
9. Wiegand J, Wedemeyer H, Finger A, Heidrich B, Rosenau J, Michel G, Bock CT, Manns MP, Tillmann HL (2008) A decline in hepatitis B virus surface antigen (hbsag) predicts clearance, but does not correlate with quantitative hbeag or HBV DNA levels. Antivir Ther 13: 547-554.
10. Yang N, Feng J, Zhou T, Li Z, Chen Z, Ming K, Liang G, Lei XX, Xu BL (2018) Relationship between serum quantitative HBsAg and HBV DNA levels in chronic hepatitis B patients. J Med Virol 90: 1240-1245.
11. Yeh CT (2010) Development of HBV S gene mutants in chronic hepatitis B patients receiving nucleotide/nucleoside analogue therapy. Antivir Ther 15: 471-475.
12. Wu C, Li B, Zhang X, Zhao K, Chen Y, Yuan Y, Liu Y, Chen R, Xu D, Chen X, Lu M (2019) Complementation of wild-type and drug-resistant hepatitis B virus genomes to maintain viral replication and rescue virion production under nucleos(t)ide analogs. Virol Sin 34: 377-385.
13. Colledge D, Soppe S, Yuen L, Selleck L, Walsh R, Locarnini S, Warner N (2017) Stop codons in the hepatitis B surface proteins are enriched during antiviral therapy and are associated with host cell apoptosis. Virology 501: 70-78.
14. McDaniel YZ, Patterson SE, Mansky LM (2019) Distinct dual antiviral mechanism that enhances hepatitis B virus mutagenesis and reduces viral DNA synthesis. Antiviral Res 170: 104540.
15. Tang CM, Yau TO, Yu J (2014) Management of chronic hepatitis B infection: current treatment guidelines, challenges, and new developments. World J Gastroenterol 20: 6262-6278.
16. Zhang HY, Liu LG, Ye CY, Chen CH, Hang SX, Zhu Z, Shen HY, Huang ZY, Chen WY, Xue Y (2018) Evolution of drug-resistant mutations in HBV genomes in patients with treatment failure during the past seven years (2010-2016). Virus Genes 54: 41-47.
17. Kuhnhenn L, Jiang B, Kubesch A, Vermehren J, Knop V, Susser S, Dietz J, Carra G, Finkelmeier F, Grammatikos G, Zeuzem S, Sarrazin C, Hildt E, Peiffer KH (2018) Impact of HBV genotype and mutations on HBV DNA and qHBsAg levels in patients with HBeAg-negative chronic HBV infection. Aliment Pharmacol Ther 47: 1523-1535.
18. Cheng PN, Chiu YC, Tsai HW, Wang RH, Chiu HC, Wu IC, Chang TT (2014) HBsAg expression of liver correlates with histological activities and viral replication in chronic hepatitis B. Ann Hepatol 13: 771-780.
19. Boyd A, Moh R, Maylin S, Abdou Chekaraou M, Mahjoub N, Gabillard D, Anglaret X, Eholie SP, Delaugerre C, Danel C, Zoulim F, Lacombe K (2018) Precore G1896A mutation is associated with reduced rates of HBsAg seroclearance in treated HIV hepatitis B virus co-infected patients from Western Africa. J Viral Hepat 25: 1121-1131.
20. Su M, Xiang K, Li Y, Deng J, Xu X, Yan L, Zhuang H, Li T (2016) Higher detection rates of amino acid substitutions in HBV reverse transcriptase/surface protein overlapping sequence is correlated with lower serum HBV DNA and HBsAg levels in HBeAg-positive chronic hepatitis B patients with subgenotype B2. Infect Genet Evol 40: 275-281.

Corresponding author

Longgen Liu, Ph.D
Institute of Hepatology, the Third People's Hospital of Changzhou
Address: No. 300 Lanling North Road, Changzhou, Jiangsu, China
Tel: +86 0519 82008326.
FAX: +86 0519 82009999.
E-mail: czsygbk@163.com

Conflict of interests: No conflict of interests is declared.