Original article

Factors associated with serum hepatitis B surface antigen levels and its on-treatment changes in patients under lamivudine therapy

Jinjun Chen¹, Zhanhui Wang¹, Bin Zhou¹, Yanjun Wang¹, Jinlin Hou*¹

¹Institute of Hepatology, Department of Infectious Diseases, Key Laboratory for Organ Failure Research, Nanfang Hospital, Southern Medical University, Guangzhou, PR China

*Corresponding author e-mail: jlhousmu@yahoo.com.cn

Background: We aimed to investigate factors associated with serum hepatitis B surface antigen (HBsAg) levels and its kinetics under lamivudine treatment in chronic hepatitis B patients.

Methods: HBsAg levels were measured with the Architect HBsAg assay (Abbott laboratories, Abbott Park, IL, USA) in genotype B (HBV/B) or C (HBV/C) patients (n=218). Early HBsAg kinetics in 86 hepatitis B e antigen (HBeAg)-positive patients and long-term HBsAg changes in 45 patients with rapid and sustained viral suppression were further analysed.

Results: Mean HBsAg levels were higher in male (n=181) than in female (n=37) patients (3.59 versus 3.23 log₁₀ IU/ml; P=0.036), and higher in 121 HBV/B than in 97 HBV/C patients (3.68 versus 3.34 log₁₀ IU/ml; P=0.006). In addition to HBV DNA loads (P<0.001), male gender (P=0.012) and HBV/B infection (P=0.035) were independently associated with higher HBsAg levels in antiviral-naive patients. HBsAg increases (0.00–0.87 log₁₀) were found in 28/86 patients who obtained viral suppression under 12 weeks of lamivudine therapy. Higher baseline HBsAg levels (P=0.046), HBV/B infection (P=0.007) and faster HBV DNA declines (P=0.006) independently contributed to greater HBsAg decreases under 12 weeks treatment. An apparent dissociation between HBsAg and HBV DNA changes were found in 14/45 patients with rapid and sustained viral suppression, who had low baseline HBsAg levels and predominant HBV/C infection.

Conclusions: HBV/B and male gender were associated with higher HBsAg levels in antiviral-naive patients. Higher baseline HBsAg levels and HBV/B infection contributed to greater early HBsAg declines in HBeAg-positive patients, and might correlate with discordance between HBsAg and HBeAg or HBV DNA under long-term lamivudine treatment.

Introduction

Serum hepatitis B surface antigen (HBsAg) results from HBV virions and subviral particles, and the latter exceeds the former by a variable factor of 10²–10⁵. Serum HBsAg is taken as a hallmark of HBV infection and HBV DNA loads indicate viral replication capacity [1]. Automated assays newly developed for HBsAg quantitations facilitate multifaceted studies of factors correlated with serum HBsAg in cohorts of HBV carriers with or without antiviral treatment(s), including investigations of the relationships between HBsAg and HBV DNA.

Correlation between serum HBsAg levels and HBV DNA loads is complex, and has been found to be strong in hepatitis B e antigen (HBeAg)-positive patients [2–5], but weak in HBeAg-negative subjects [2–4] or lost in inactive carriers [2]. A European study shows that such correlation exclusively exists in genotype D, rather than A, patients [6]. Factors additional to viral replication and infection phase were speculated as contributing to such complicated correlations.

Correlation between HBsAg and HBV DNA kinetics is also complex. We have observed discordant kinetics of these two parameters in HBeAg-positive patients under adefovir or pegylated interferon treatment [7]. Approximately 30% of patients did not obtain an HBsAg decrease along with HBV DNA suppression under pegylated interferon or telbivudine treatment [8,9]. Stable HBsAg levels are found in patients with spontaneous HBeAg seroconversion accompanied with significant HBV DNA declines [4]. It is assumed that factors other than HBV DNA suppression exist and contribute to HBsAg kinetics under antiviral therapy.
Our study aimed to search for the clinical and/or virological factors associated with serum HBsAg levels in chronic hepatitis B patients prior to antiviral therapy, and to explore HBsAg kinetics in HBeAg-positive cases with profound and sustained HBV DNA suppressions under short- or long-term lamivudine treatment.

Methods

Treatment-naive cohort

To investigate factors associated with serum HBsAg levels, an immunoactive cohort was established based on the following criteria: chronic patients >14 years old admitted to our Hepatology Unit (Southern Medical University, Guangzhou, PR China) from July 2003 to December 2006, serum HBV DNA loads >4.0 log_{10} copies/ml measured by the LightCycler assay (Roche Diagnostics, Mannheim, Germany) with a lower limit of detection of 3.0 log_{10} copies/ml, and no antiviral therapy with either nucleoside/nucleotide analogues or standard/pegylated interferon-α administrated prior to this investigation. Patients with HCV, hepatitis D virus or HIV infection were excluded. Serum samples drawn within 1 week prior to or after admission were able to be recovered from cryostoration at -30°C. Patients infected with HBV genotype B (HBV/B) or genotype C (HBV/C), which was determined by PCR-based restricted fragment length polymorphism [11], were eligible for this retrospective investigation.

Lamivudine treatment cohort

The early kinetics of serum HBsAg were further investigated in HBeAg-positive patients from the treatment-naive cohort who received 12 weeks lamivudine (100 mg/day; GlaxoSmithKline, Suzhou, China) therapy. Clinical data at treatment week 12 were collected and serum HBsAg quantitations were performed. To observe the HBsAg changes under long-term antiviral treatment, further investigation was carried out in patients who achieved undetectable serum HBV DNA at week 24 (rapid viral suppressions) and remained undetectable onwards throughout 3 years lamivudine treatment (sustained viral suppressions). Frequent visits with 3–6 month intervals were documented with alanine aminotransferase (ALT) levels, HBeAg status and HBV DNA loads. Serum HBsAg quantitations were performed in samples drawn at treatment week 24, and treatment years 1.0, 1.5, 2.0, 2.5 and 3.0, with up to 2 weeks variation found in some patients’ visit schedules. A preliminary analysis of this long-term treatment cohort has been published elsewhere [12]. All serum samples were collected at each visit and cryostored at -30°C. This retrospective investigation was approved by the Ethics Committee in Nanfang Hospital (Guangzhou, PR China).

HBsAg quantification

Serum HBsAg levels were quantified with Architect HBsAg assay (Abbott Laboratories, Abbott Park, IL, USA). The dynamic range for this assay is between 0.05–250.00 IU/ml and the vast majority of samples were retested with a 1:500 dilution. The manual dilution strategy was stringently performed according to the manufacturer’s recommendation, and the intraassay variation with manual dilution ranged between 2.59% and 9.74%.

Viral genotype determinations

After DNA extraction [10], nested amplification of HBV surface gene was performed. The primers were Pres2 (nt2820-2837; forward, 5′-ggg aca cca tat tct tgg-3′) paired with reverse primer Pol2 [10], and forward primer Bs1 [10] paired with P36 (nt990-971; reverse, 5′-aca tac ttt cca atr ag-3′) for the first and second round amplifications, respectively. Direct sequencing with Bs1 and P36 as the sequencing primers and genotyping (nt56-990) was performed as reported [13].

Clinical routine tests

ALT levels with the upper limit of normal at 40 U/l were routinely determined. Serum HBeAg was detected by the Abbott Assym (Abbott Laboratories) assay for the treatment-naive cohort or the Abbott Architect (Abbott Laboratories) assay for serial samples from the treatment cohort. The cutoff values were 1.0 signal/cutoff (S/CO) for both assays.

Statistical analyses

The χ^2 test, Fisher’s exact test, Student’s t-test, Spearman’s correlation, and Mann–Whitney U test were used when appropriate. Repeated measurement was used to compare HBsAg and HBV DNA levels at varied time points in the treatment cohort. Linear regression analysis was performed to investigate factors associated with serum HBsAg levels (treatment-naive cohort) and HBsAg changes (as a continuous variable) from baseline to treatment week 12 (treatment cohort). Binary logistic analysis was adopted to investigate the factors associated with trends or patterns of serum HBsAg changes. Survival analysis was done by Kaplan–Meier method with the log rank test. All statistical analyses were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) and P<0.05 was considered significant.

Results

Factors associated with HBsAg levels in treatment-naive patients

Overall, 218 patients naive to antiviral treatment were enrolled into this retrospective investigation

J Chen et al.
HBsAg levels and on-treatment changes

Antiviral Therapy 17.1

73

Male patients had higher serum HBsAg levels than female patients (mean ± sd 3.59 ± 0.93 versus 3.23 ± 0.91 log10 IU/ml; P = 0.036), and HBeAg-positive patients had higher levels than negative cases (mean ± sd 3.68 ± 0.93 versus 3.12 ± 0.81 log10 IU/ml; P < 0.001). HBV/B patients had higher HBsAg levels than HBV/C (mean ± sd 3.68 ± 1.00 versus 3.34 ± 0.80 log10 IU/ml; P = 0.006), whereas such differences disappeared in either HBeAg-negative (Figure 1A) or female subgroups of patients (Figure 1B).

Serum HBsAg levels were positively correlated with serum HBV DNA loads and negatively with patients’ ages, but not with ALT levels (Table 1).

Table 1. Factors associated with serum HBsAg levels in antiviral treatment-naive patients with univariate or multivariate analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value*</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female gender</td>
<td>181/37</td>
<td>t = 2.11 4</td>
<td>0.036</td>
</tr>
<tr>
<td>Positive/negative HBeAg status</td>
<td>160/58</td>
<td>t = 4.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Genotype B/C</td>
<td>121/97</td>
<td>t = 2.79 5</td>
<td>0.006</td>
</tr>
<tr>
<td>ALT, ULN</td>
<td>4.58 ± 4.68</td>
<td>r = 0.129</td>
<td>0.060</td>
</tr>
<tr>
<td>HBV DNA, log10 copies/ml</td>
<td>7.78 ± 2.07</td>
<td>r = 0.486</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age, years</td>
<td>33 ± 11</td>
<td>r = 0.339</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Total n=218. *Data are shown as patient n for categorized variables (gender, hepatitis B e antigen [HBeAg] status and genotype) or mean ± sd for continuous variables (alanine aminotransferase [ALT], HBV DNA and age). Statistics adopted in univariate analysis include Student’s t-test and correlation coefficients from Spearman’s correlation analysis. Regression coefficients derived from linear regression analysis. CI of regression coefficients b. ULN, upper limit of normal.

Figure 1. Comparison of serum HBsAg levels between patients infected with HBV genotype B and those with genotype C

Data are shown as median (range). Results are stratified according to (A) hepatitis B e antigen (HBeAg) status or (B) gender. HBsAg, hepatitis B surface antigen.

Factors associated with absolute HBsAg changes under 12 weeks lamivudine treatment

To observe early HBsAg kinetics, 86 HBeAg-positive patients treated with 12 weeks lamivudine were investigated. HBV DNA loads declined significantly from baseline to week 12 (mean ± sd 8.59 ± 1.51 versus 4.25 ± 1.34 log10 copies/ml; P < 0.001), as did HBsAg (mean ± sd 3.76 ± 0.76 versus 3.42 ± 0.41 log10 IU/ml; P < 0.001; Figure 2A).
HBsAg or HBV DNA changes from baseline to treatment week 12 were taken as continuous variables. Median HBsAg changes were significantly greater in HBV/B patients than in HBV/C (0.43 versus 0.03 log\(_{10}\); \(P<0.001\)), and seemed greater in male than in female patients (0.31 versus 0.05 log\(_{10}\); \(P=0.088\)).

HBsAg changes were positively correlated with baseline HBV DNA loads (\(r_s=0.355\); \(P<0.001\)) and baseline HBsAg levels (\(r_s=0.506\); \(P<0.001\)). Correlation between HBsAg and HBV DNA changes existed in 86 patients (\(r_s=0.311\); \(P=0.004\)), and became stronger in HBV/B (Figure 2B), but was lost in HBV/C cases (Figure 2C).

A linear regression analysis (\(R^2=0.266\)) showed that HBV genotype (regression coefficient \(\beta=0.279\); \(P=0.007\)), baseline HBsAg levels (\(\beta=0.241\); \(P=0.046\)) and HBV DNA decline (\(\beta=0.386\); \(P=0.006\)) were independently associated with absolute HBsAg changes.

Factors associated with trends of HBsAg changes under 12 weeks lamivudine treatment

HBV DNA decline occurred in all 86 patients (0.75–7.78 log\(_{10}\)), and 25 (29.1%) obtained undetectable HBV DNA at week 12. However, HBsAg in 28 (32.6%) patients was simultaneously augmented
HBsAg reductions <0.1 log<sub>10</sub> occurred in 12 patients, and declines >0.5 log<sub>10</sub> were found in 26 patients (Figure 2A).

Compared to patients who had no or <0.1 log<sub>10</sub> reductions (n=40), baseline HBsAg levels, baseline HBV DNA loads and its reductions were significantly greater in those with HBsAg decreases >0.5 log<sub>10</sub> (n=26). In addition, the latter group included more HBV/B or male patients than the former (Table 2).

HBV/C infection (relative risk 7.565, 95% CI 1.753, 32.639; P=0.007) and low baseline HBsAg level (relative risk 0.318, 95% CI 0.107, 0.940; P=0.038) were associated with HBsAg changes <0.1 log<sub>10</sub> while HBV DNA decline was of borderline significance (P=0.053).

General profiles of patients with rapid and sustained viral suppressions

To observe HBsAg changes under long-term antiviral treatment, 45 patients who obtained rapid and sustained viral suppressions were further studied and preliminarily described elsewhere [12]. Briefly, 34 patients were male and 21 were infected with HBV/B. There were 21/45 (46.7%) patients with undetectable HBV DNA at week 12, from which HBeAg loss emerged in 32/45 (57.8%) at the end of 3 years treatment [12].

From baseline to week 24, HBsAg drops from 0.62 to 0.5 log<sub>10</sub> in those with HBsAg decreases >26. In addition, HBsAg changes <0.1 log<sub>10</sub> were significant. Compared to week 24, HBsAg reductions were mean ±SD 0.10 ±0.10 (year 1; P=0.354), 0.17 ±0.29 (year 1.5; P=0.562), 0.18 ±0.29 (year 2; P=0.542), 0.24 ±0.30 (year 2.5; P=0.421) and 0.25 ±0.30 (year 3; P=0.414) log<sub>10</sub>, respectively. Such HBsAg change was defined as a biphasic pattern, which was initiated with a rapid decline in the early phase from baseline to week 24, and followed by no significant reduction in the late phase from week 24 to week 156 (Figure 3B).

HBsAg declines <0.1 log<sub>10</sub>, which occurred in 18 patients at week 24, were persistently observed throughout 3 years therapy in 14 (77.8%) cases (Figure 3A). HBsAg changes between baseline and varied on-treatment time points, which were composed of reductions as much as 0.09 log<sub>10</sub> and elevations up to 1.03 log<sub>10</sub>, were found in these 14 patients during 3-year treatment; such HBsAg change was identified as a stable pattern (Figure 3C).

Comparisons between biphasic and stable pattern of HBsAg changes

Compared to patients with stable HBsAg changes, more HBV/B infections and higher baseline HBV DNA loads were found in patients with a biphasic pattern (Table 2).

Mean HBsAg levels at baseline were higher in patients with a biphasic than a stable pattern (Table 2), which were comparable both at week 12 (3.55 versus 3.49 log<sub>10</sub> IU/ml; P=0.761) and 24 (3.28 versus 3.55 log<sub>10</sub> IU/ml; P=0.215). Along with sustained viral suppressions, HBsAg levels became lower in biphasic versus stable patterns at year 1.0 (3.58 versus 3.59 log<sub>10</sub> IU/ml; P=0.016), year 1.5 (3.80 versus 3.65 log<sub>10</sub> IU/ml; P=0.016), year 2.0 (3.72 versus 3.72 log<sub>10</sub> IU/ml; P=0.003) and year 2.5 (3.70 versus 3.70 log<sub>10</sub> IU/ml; P=0.003), respectively (Figure 3B and 3C). With the exception of one case who obtained HBsAg loss at week 76, mean HBsAg level at end of treatment was 3.04 log<sub>10</sub> IU/ml, ranged from 2.02 to 4.01 log<sub>10</sub> IU/ml.

Definition of HBsAg pattern in patients with rapid and sustained viral suppressions

HBsAg levels persisted at lower levels throughout the treatment period in 22/23 patients (95.7%) after a decline >0.5 log<sub>10</sub> from baseline to week 24 (Figure 3A). For these 22 patients, HBsAg reductions from baseline to week 12 (mean ±SD 1.11 ±0.11 log<sub>10</sub>; P<0.001) or week 12 to week 24 (mean ±SD 0.27 ±0.11 log<sub>10</sub>; P=0.028) were both significant. Compared to week 24, HBsAg reductions were mean ±SD 0.10 ±0.10 (year 1; P=0.354), 0.17 ±0.29 (year 1.5; P=0.562), 0.18 ±0.29 (year 2; P=0.542), 0.24 ±0.30 (year 2.5; P=0.421) and 0.25 ±0.30 (year 3; P=0.414) log<sub>10</sub>, respectively. Such HBsAg change was defined as a biphasic pattern, which was initiated with a rapid decline in the early phase from baseline to week 24, and followed by no significant reduction in the late phase from week 24 to week 156 (Figure 3B).

HBsAg declines <0.1 log<sub>10</sub>, which occurred in 18 patients at week 24, were persistently observed throughout 3 years therapy in 14 (77.8%) cases (Figure 3A). HBsAg changes between baseline and varied on-treatment time points, which were composed of reductions as much as 0.09 log<sub>10</sub> and elevations up to 1.03 log<sub>10</sub>, were found in these 14 patients during 3-year treatment; such HBsAg change was identified as a stable pattern (Figure 3C).

Comparisons between biphasic and stable pattern of HBsAg changes

Compared to patients with stable HBsAg changes, more HBV/B infections and higher baseline HBV DNA loads were found in patients with a biphasic pattern (Table 2).

Mean HBsAg levels at baseline were higher in patients with a biphasic than a stable pattern (Table 2), which were comparable both at week 12 (3.55 versus 3.49 log<sub>10</sub> IU/ml; P=0.761) and 24 (3.28 versus 3.55 log<sub>10</sub> IU/ml; P=0.215). Along with sustained viral suppressions, HBsAg levels became lower in biphasic versus stable patterns at year 1.0 (3.18 versus 3.58 log<sub>10</sub> IU/ml; P=0.025), year 1.5 (3.11 versus 3.65 log<sub>10</sub> IU/ml; P=0.016), year 2.0 (3.10 versus 3.72 log<sub>10</sub> IU/ml; P=0.016) and year 2.5 (3.04 versus 3.70 log<sub>10</sub> IU/ml; P=0.003), respectively (Figure 3B and 3C). With the exception of one case who obtained HBsAg loss at week 76, mean HBsAg level at end of treatment was 3.04 log<sub>10</sub> IU/ml, ranged from 2.02 to 4.01 log<sub>10</sub> IU/ml.

Table 2. Patients with distinct HBsAg changes under 12 weeks or 3 years lamivudine treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HBsAg decrease within 12 weeks of treatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Patterns of HBsAg change within 3 years of treatment&lt;sup&gt;b&lt;/sup&gt;</th>
<th>-value</th>
<th>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;0.1 log&lt;sub&gt;10&lt;/sub&gt;</td>
<td>&gt;0.5 log&lt;sub&gt;10&lt;/sub&gt;</td>
<td>P-value</td>
<td>Stable</td>
</tr>
<tr>
<td>Patients, n</td>
<td>40</td>
<td>26</td>
<td>-</td>
<td>14</td>
</tr>
<tr>
<td>Age, years</td>
<td>27 ±5</td>
<td>29 ±8</td>
<td>0.365</td>
<td>28 ±7</td>
</tr>
<tr>
<td>Male/Female gender, n</td>
<td>32/8</td>
<td>26/0</td>
<td>0.018</td>
<td>8/6</td>
</tr>
<tr>
<td>Baseline ALT, ULN</td>
<td>3.92 ±3.17</td>
<td>4.79 ±3.13</td>
<td>0.276</td>
<td>5.4 ±6.90</td>
</tr>
<tr>
<td>Baseline HBV DNA, log&lt;sub&gt;10&lt;/sub&gt; copies/ml</td>
<td>9.14 ±0.60</td>
<td>8.06 ±1.77</td>
<td>0.001</td>
<td>7.60 ±1.24</td>
</tr>
<tr>
<td>Baseline HBsAg, log&lt;sub&gt;10&lt;/sub&gt; IU/ml</td>
<td>4.12 ±0.86</td>
<td>3.46 ±0.66</td>
<td>0.001</td>
<td>3.41 ±0.36</td>
</tr>
<tr>
<td>Genotype B/C, n</td>
<td>12/28</td>
<td>19/7</td>
<td>0.001</td>
<td>4/10</td>
</tr>
<tr>
<td>HBV DNA decrease, log&lt;sub&gt;10&lt;/sub&gt;</td>
<td>5.02 ±1.43</td>
<td>3.89 ±1.67</td>
<td>0.006</td>
<td>-</td>
</tr>
</tbody>
</table>

Data are mean ±SD unless indicated otherwise. *Change in hepatitis B surface antigen (HBsAg) or HBV DNA within 12 treatment weeks is calculated as the value at baseline minus the corresponding value at week 12. A stable pattern of HBsAg change within 3 years of lamivudine treatment was defined as HBsAg (IU/ml) decrease at any visit compared with baseline that was <0.1 log<sub>10</sub>, and a biphasic pattern was defined as those decreases >0.5 log<sub>10</sub> at week 24 and onwards. ULN, upper limit of normal.
Figure 3. Change in serum HBsAg, HBeAg and HBV DNA in patients who achieved rapid and sustained viral suppressions under 3 years of lamivudine treatment.
in patients with biphasic pattern, and was lower than patients with a stable pattern 3.71 (3.09–4.25 log10 IU/ml); P=0.002).

Decreased HBeAg titres, paralleled with viral suppressions, were found in all 45 patients with biphasic or stable HBsAg pattern (Figure 3B and 3C). In patients without HBeAg loss, HBeAg titres at the end of the investigation were comparable between patients with a biphasic pattern (1.10–38.64 S/CO) and those with stable pattern (1.02–7.89 S/CO; P=0.535).

Cumulative HBeAg loss was comparable (59.1% versus 57.1%; P=0.774) between patients with biphasic and stable patterns (Figure 3D). At week 12, HBeAg loss occurred in 1/22 and 3/14 patients, respectively, and was comparable (P=0.153).

Discussion

Our results showed that serum HBsAg levels in antiviral-naïve chronic hepatitis B patients were associated with patients’ gender, viral genotype and viral replication. Further investigation found that viral genotype and viral suppressions contributed to HBsAg kinetics in HBeAg-positive patients under lamivudine treatment. Apparent discordant kinetics were found between HBsAg and other viral markers, for example, HBeAg and HBV DNA in patients with rapid and sustained viral suppressions.

We found that the male gender was independently associated with higher HBsAg levels. Gender-dependent HBsAg expressions have been observed in transgenic mice after puberty, and further experiments validated that HBsAg expression was up-regulated by testosterone [14,15] via the ligand-stimulated androgen receptor pathway [16], and down-regulated by oestrogen via inhibition of viral messenger RNA (mRNA) synthesis [17]. The synergistic effects of sex hormones on HBsAg expression could be responsible for the gender-associated HBsAg levels.

Our results showed that HBV/B patients had higher HBsAg levels than HBV/C, consistent with results from a large cohort [9], but contrary to a study by Nguyen et al. [2]. Further stratified analysis with our data showed that genotypic (B/C) effects on HBsAg levels existed exclusively in male patients. Comparable HBsAg levels between HBV/B and HBV/C patients were possibly due to predominant female cases in the cohort of Nguyen et al. [2]. In vitro experiments have presented higher HBsAg mRNA transcriptions from HBV/B strains than from HBV/C [18].

HBsAg mRNA transcription is driven by a surface promoter, whereas pregenomic mRNA is driven by a core promoter then reverse transcribed into HBV DNA. Different transcriptional regulation of HBsAg and HBV DNA could permit the weak correlation between them, even when covalently closed circular DNA is taken as the same transcriptional template [2,3]. Differential regulations of HBsAg mRNA transcription are believed to be the underlying mechanism for gender and genotypic disparity in HBsAg levels.

HBV/B infection was associated with profound early HBsAg drop and correlated with a biphasic pattern of HBsAg changes under long-term lamivudine treatment. Recent data have shown that HBsAg changes are also genotype-dependent in HBeAg-positive patients treated with telbivudine [9] or HBeAg-negative patients under pegylated interferon-α2a treatment [19,20]. HBV genotype was believed to have one novel biological role in viral life cycle; that is, affecting baseline HBsAg levels and its early kinetics under antiviral treatment.

With rapid and sustained viral suppressions under long term lamivudine treatment, HBsAg exhibited an obvious dissociation with HBeAg in 14/45 patients, in which HBeAg titre achieved continuous decline while HBsAg presented a stable pattern. Increased or fluctuated HBsAg levels have also been observed in patients with sustained viral suppressions under tenofovir treatment [21]. HBeAg titres at the end of observation in patients without HBeAg loss were low (1.02–38.64 S/CO), indicating the closer correlation between HBeAg titre and HBV replication [3,22]. The two patterns (stable or biphasic) had no association with HBeAg loss throughout 3-year treatment, consistent with the telbivudine treatment cohort [9], implying that HBeAg quantitations could be superior to HBsAg for prediction of HBeAg loss/seroconversion under antiviral therapies [23]. HBsAg kinetics had no benefit in predicting HBeAg loss for patients treated with lamivudine or telbivudine, and frequent HBsAg quantitations were suboptimal for such a purpose.

With significant HBV DNA declines, HBsAg drops <0.1 log10 were found in 46/86 patients under 12 weeks lamivudine treatment, and stable HBsAg levels could be predicted in 14/18 patients along with rapid and sustained viral suppressions. This further confirmed that the elevations or slight reductions of HBsAg from baseline to week 12 or 24 were not due to the delayed responses to viral suppressions. Similar discordance between on-treatment changes of HBsAg and HBV DNA have been found in 10/31 (32.3%) patients treated with adefovir [7], 56/162 (34.6%) with telbivudine [9] and >60% of HIV–HBV-coinfected patients under efficient tenofovir treatment [21]. Frequent serum HBsAg monitoring would not help to predict viral responses for such cases, which accounted for >30% of patients under long term oral antiviral therapy.

Two distinct phases of serum HBsAg changes in patients with a biphasic pattern were found: a rapid reduction phase along with a dramatic viral suppression (baseline to week 24) and a steady phase concurrent
with the appearance of inactive viral replication (week 24 and onwards). This was consistent with the biphasic dynamics of viral loads under nucleotide/nucleoside analogue therapy [24–27]. The biphasic pattern implied the dependency of HBsAg decreases upon viral suppression in the rapid reduction phase, whereas the steady phase might indicate the slow turnover of HBsAg-positive hepatocytes [28].

HBsAg levels and its kinetics under antiviral treatment were associated with multiple factors, especially viral genotype. Frequent monitoring of HBsAg kinetics in >30% of HBsAg-positive patients under oral antiviral therapy had no benefit for clinical management. An algorithm for clinical practice regarding HBsAg that was quantitatively derived from homogeneous patient cohort was preferred [29]; otherwise, such algorithms would be cohort-specific [8,20], and therefore suboptimal for general clinical practice.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (30730082 and 30901271), National Grand Program on Key Infectious Disease (number 2008ZX10002-004), and the Guangdong Natural Science Foundation (10451051501005787). The design, collection, analysis and interpretation of the data and writing of the present report are independent of any sponsor. We appreciated the reviewers’ critical comments for this manuscript.

Disclosure statement

JH has acted as a consultant for Novartis, GlaxoSmithKline and Bristol-Myers Squibb. All other authors declare no competing interests.

References

HBsAg levels and on-treatment changes


