Numerical Diagnoses of Superinfection in Chronic Hepatitis B Viral Dynamics

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Key Words

Chronic hepatitis B • HBV DNA load • Covalently closed circular DNA • Spontaneous fluctuation • Superinfection • Mathematical model

Abstract

Objectives: Fluctuation profile has been observed in chronic hepatitis B patients who are untreated or interrupt therapy. A mathematical model and its parameters could be used to diagnose the assumption of superinfection of hepatocytes and to understand the causes for the spontaneous fluctuation pattern of HBV DNA loads in chronically infected patients. **Methods:** We propose a new conceptual model in terms of chemical kinetics, which is based on the assumption that hepatocytes can be superinfected with hepatitis B virus (HBV). Minimizing the sum of squares of the deviations, we fitted the model to the HBV DNA trajectories from clinical data and obtained the model parameters. **Results:** The model with the fitted parameters can capture the tendency of HBV DNA trajectories. The mean value of the fitted number of virions that enter a single hepatocyte at the beginning stage of an invasion is 2.10 ± 0.18 . The dynamics patterns may correlate with the clinical phenotypes of patients and the value of clinical parameters, such as α -fetoprotein, hepatitis B e-antigen, hepatitis B e-antibody, total bilirubin and

alanine transaminase. **Conclusions:** The superinfection scenario is possible in HBV infection and it may induce HBV DNA fluctuation in the host.

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Introduction

Hepatitis B is an enormous challenge to global public health and it is caused by the hepatitis B virus (HBV). Roughly 400 million people are infected with HBV worldwide [1]. Currently, antiviral treatment with interferon- α and nucleotide analogs is the main approved therapy for chronic HBV infection [2].

During antiviral therapy, the HBV DNA level is detected regularly to be used as a measurement of the antiviral effect of hepatitis B. In fact, some complex decay profiles of HBV DNA (flat partial response, biphasic, triphasic and stepwise decay) have been observed in treated patients [3, 4]. However, for 35 untreated patients with chronic HBV infection, Chun et al. [5] reported that spontaneous fluctuations of HBV DNA load were observed and there was no determinate correlation between the HBV DNA loads and the levels of alanine transaminase (ALT). In our previous study [6], serum was collected consecutively from 3 untreated patients with different

Fig. 1. HBV DNA profiles observed in patients and results of fitting the model 2.6 to each patient's HBV DNA data: the best fit of the model (dashed line) to HBV DNA patient data (open circles on solid line). Experimental data are represented by open circles and the solid line is used to emphasize plausible phase of viral load. Here **A**, **B** and **C** are untreated patients; **D**, **E** and **F** are therapy-

interrupting patients. The parameters are listed in equations 3.1 and 3.2 as well as table 1; the initial number of uninfected cells, infected cells and free virus particles are 5.0×10^7 , 1.5×10^7 and the number of free virus particles at zero time. The SSD are 0.176 (**A**), 0.796 (**B**), 4.377 (**C**), 0.099 (**D**), 8.076 (**E**) and 3.532 (**F**).

clinical phenotypes, and HBV DNA was measured by fluorescent real-time quantitative PCR. The results showed that there were spontaneous fluctuations of HBV DNA load for patients A and C, but the HBV DNA load of patient B ultimately tended to stabilize after some small fluctuations (fig. 1A–C). Recently, in a long-term tracking study for 3 chronic hepatitis B patients who stopped anti-virus medicine, we also found spontaneous fluctuations in their HBV dynamics (fig. 1D–F).

Viral DNA is converted into a single covalently closed circular DNA (cccDNA) molecule when HBV infects a cell, and there are up to 50 copies of cccDNA in an infected cell [7]. However, Zeuzem et al. [8] indicated that virus uptake by hepatocytes previously uninfected (de nova infection) or infected (superinfection) cannot be discriminated, and transformation of noninfected into virus-producing hepatocytes cannot be measured in vivo. Ciupe et al. [9] also indicated that the amplification is caused either

by synthesis pathways or multiple infection events. What are the mechanisms of superinfection, e.g. do multiple viruses infect a single hepatocyte simultaneously or one by one? To detect the existence of superinfection, Ciupe et al. [9] first proposed a mathematical model under the assumption that multiple copies of cccDNA result from multiple infection events (superinfection of hepatocytes) one by one. It looks as if the results support the biological relevance of a superinfection scenario.

In this paper, we propose a different mathematical model in terms of chemical kinetics, which is based on the assumption that hepatocytes can be superinfected with HBV simultaneously. According to the numerical simulations, we find that the observed HBV DNA profiles (fig. 1) can be captured by our model and the fitted parameters have clinical significance. Hence, the superinfection scenario is possible in HBV infection and it may induce HBV DNA fluctuation in the host.

Materials and Methods

Clinical Data

In an earlier work [6], we reported the HBV DNA load of 3 untreated patients (fig. 1A–C). Recently, for 3 HBV patients who stopped anti-virus medicine, we collected their HBV DNA load by fluorescent real-time quantitative PCR in a long-term tracking study (fig. 1D–F). The first detection time, i.e. the starting point to count was labeled as zero, the period during which patients D, E and F received lamivudine monotherapy was from –1,120 to –54 days, from –1,279 to –730 days and from –387 to –51 days, respectively. The detailed HBV DNA load and correlative clinical information of each patient are listed in the appendix. In these cases, we found there is a fluctuation profile of HBV, which is different from those reported in the literature [3, 4].

Model Description

Basic models of viral dynamics usually contain 3 variables [10, 11], which are functions of time (t), the populations of uninfected cells $x(t)$, infected cells that produce virus $y(t)$ and free virus particles $v(t)$. Concretely, the framework of the basic model is as follows:

$$\begin{cases} \frac{dx}{dt} = f(x, y, v) - g(x, y, v), \\ \frac{dy}{dt} = g(x, y, v) - ay, \\ \frac{dv}{dt} = ky - uv. \end{cases} \quad (2.1)$$

As for HBV infection, 2 assumptions underlying equation 2.1 in Nowak et al. [10, 11] are:

assumption H1: hepatocytes are born at a constant rate λ and die at a density-dependent rate dx ; therefore, the growth function of hepatocytes is $f(x, y, v) = \lambda - dx$; and

assumption H2: hepatocytes are infected in a simple mass action process [12]; therefore, the incidence function of hepatocytes is $g(x, y, v) = \beta xv$.

Equation 2.1 has been repeatedly modified to be used to simulate the real process of HBV infection. On one hand, assumption H1 assumes that healthy hepatocytes are replenished through ‘re-seeding’ from an outside source, e.g. bone marrow. However, it is well established that liver recovery following injury is facilitated by widespread hepatocyte proliferation [13]. Thus, several conceptual models have been worked out by introducing a logistic function of healthy hepatocyte growth [4, 9, 14, 15]. On the other hand, a fundamental problem with model 2.1 on assumptions H1 and H2 is that it predicts a biologically unlikely relationship between liver size and susceptibility to HBV infection, which may lead to a false understanding that an individual with a smaller liver should be more resistant to the virus infection than an individual with a larger one [15–18]. As stated in [18], a typical patient chronically infected with HBV has a total serum daily production rate of about 2×10^{11} to 3×10^{12} virions [19]. An average human liver consists of billions of liver cells. These large numbers suggest that a more plausible HBV model should employ a standard incidence function [15–18] instead of the mass action incidence used in assumption H2. In brief, the main modifications to the above-mentioned assumptions are:

assumption H1': a logistic function for healthy hepatocyte growth is introduced, i.e. $f(x, y, v) = rx(1 - (x + y)/K)$; and

assumption H2': the mass action process is replaced with a standard incidence function, i.e. $g(x, y, v) = \beta xv/(x + y)$.

During the process of chronic HBV infection, there is usually a quasi-homeostasis between hepatocytes (x), free virions (v) and infected hepatocytes (y). In chemical reaction notation, this can be written as:

where $n \geq 1$ approximates the average number of virions that enter a single hepatocyte simultaneously at the beginning stage of an invasion and $n > 1$ corresponds to the superinfection assumption in Zeuzem et al. [8] and Ciupe et al. [9].

From Rossotti and Rossotti [20], the equilibrium constant α of equation 2.2 can be defined by:

$$\alpha = \frac{[y]}{[x] \cdot [v]^n}, \quad (2.3)$$

where $[x]$ is the number of the species x , etc. Let $h(v) = [y]/([x] + [y])$. Then the number of infected hepatocytes is proportional to $h(v)$, and the number of uninfected hepatocytes is proportional to $1 - h(v)$. Equation 2.3 can be rewritten as:

$$\alpha = \frac{h(v)}{(1 - h(v)) \cdot [v]^n}. \quad (2.4)$$

From equation 2.4, we can obtain the following proportional function of infected hepatocytes:

$$h(v) = \frac{\alpha[v]^n}{1 + \alpha[v]^n}. \quad (2.5)$$

From a biological point of view, the proportion of infected hepatocytes in the quasi-homeostasis is also a measurement of the virus virulence, i.e. the average rate of infection per susceptible host. Thus, supposing superinfection exists and referring to equation 2.5, we can modify assumption H2 as follows:

assumption H2'': the mass action process is replaced with a Holling type functional response, i.e. $g(x, y, v) = \beta xv^n / (1 + \alpha v^n)$.

Furthermore, as there is a cytokine-mediated ‘cure’ of infected cells during HBV infection [3, 4, 9, 21], the hepatocyte growth function could be modified to be:

assumption H1'': $f(x, y, v) = rx(1 - (x + y)/K) + \rho y$.

The combination of assumptions H1'' and H2'' about HBV infection yields the following modified model:

$$\begin{cases} \frac{dx}{dt} = rx \left(1 - \frac{x + y}{K} \right) + \rho y - \frac{\beta xv^n}{1 + \alpha v^n}, \\ \frac{dy}{dt} = \frac{\beta xv^n}{1 + \alpha v^n} - (a + \rho) y, \\ \frac{dv}{dt} = ky - uv, \end{cases} \quad (2.6)$$

Table 1. Estimates for the parameters in model 2.6

Patient	r d ⁻¹	n vir	β ml d ⁻¹ vir ⁻¹	α ml vir ⁻¹	a d ⁻¹	k vir cell ⁻¹ d ⁻¹	ρ d ⁻¹
A	0.20	2.01	1.28×10^{-16}	3.88×10^{-16}	0.47	16.73	1.36×10^{-2}
B	0.99	1.91	3.01×10^{-12}	2.15×10^{-12}	0.48	12.94	6.22×10^{-3}
C	0.68	2.07	6.95×10^{-12}	2.96×10^{-12}	0.26	0.01	3.39×10^{-4}
D	0.89	1.84	1.17×10^{-14}	8.78×10^{-15}	0.16	304.35	1.44×10^{-4}
E	0.61	1.77	2.72×10^{-10}	2.52×10^{-10}	0.27	49.18	3.31×10^{-5}
F	0.67	2.97	3.00×10^{-20}	3.33×10^{-20}	0.24	22.58	1.46×10^{-5}

where parameter r is the intrinsic growth rate of healthy hepatocytes, and K is the carrying capacity of hepatocytes. Infected hepatocytes die at a constant rate a per cell and are possibly ‘cured’ by noncytolytic processes at a constant rate ρ per cell. Hepatocytes become infected at an incidence rate of $g(x, y, v)$ in assumption H2'', and n is a measurement of the superinfection assumption in Zeuzem et al. [8] and Ciupe et al. [9]. Infection rate functions of the general form $\beta x v^m / (1 + \alpha v^n)$ were studied by Liu et al. [22] and more recently by other researchers for special values of n and m in epidemiology [23, 24]. Free virions are released from infected hepatocytes at the rate of k and die at the rate of u .

Results

Parameter Estimation

A human liver contains approximately 2×10^{11} hepatocytes and a human has about total 3,000 ml plasma [11]. Usually, tested virus loads are recorded in copies/ml. Consequently, we can first estimate the carrying capacity of hepatocytes by the following formula:

$$K = \frac{2 \times 10^{11}}{3000} \approx 6.7 \times 10^7. \quad (3.1)$$

Since free virus has a mean half-life of at most 1.2 ± 0.6 days [25], similar to Eikenberry et al. [16], we can then fix the death rate for free virions as:

$$u = 0.693 \text{ d}^{-1}. \quad (3.2)$$

To estimate the remaining parameters $r, \rho, n, \beta, \alpha, a$ and k , we fitted model 2.6 to the patients' HBV DNA data by minimizing the sum of squares of the deviations (SSD). Using the software MATLAB, we numerically solved model 2.6 for the baseline parameter values mentioned earlier and the corresponding initial conditions for each patient. Furthermore, `fminsearch()` function was used to minimize the objective function SSD and to find the appropriate parameters. The results are listed in table 1.

Parameter Analysis

Model 2.6 with the fitted parameters provided a good trend of HBV DNA trajectories (fig. 1). There were periodic fluctuations of HBV DNA load for patients A, C, D, E and F, but the HBV DNA load of patient B ultimately tended to stabilize after small fluctuations, which is consistent with the results in Deng et al. [6].

We have noticed that all the fitted parameters n , the average number of virions that enters a single hepatocyte at the beginning stage of an invasion, are greater than 1 in table 1 and the mean is 2.10 ± 0.18 . This means that on average, about 2 virions enter a single hepatocyte at the beginning stage of an invasion simultaneously. Thus, the superinfection assumption of hepatocytes in Zeuzem et al. [8] and Ciupe [9] is verified by these numerical diagnoses.

The value of the fitted parameter r (intrinsic growth rate of healthy hepatocytes) of patient B is the largest ($r = 0.99$), which is consistent with the fact that patient B had the highest level of α -fetoprotein among the 6 patients. As the clinical diagnosis of patient A was HBV-related liver cirrhosis, his hepatic regeneration was weakened and the number of live hepatocytes decreased. As a result, patient A had the lowest intrinsic growth rate of healthy hepatocytes ($r = 0.20$) and a higher mortality rate of the infected hepatocytes ($a = 0.47$). Among the 6 patients, the mean of total bilirubin (TBIL) in patient D was the smallest, i.e. patient D's level of liver injury is slight, which may have induced a relatively high intrinsic growth rate of healthy hepatocytes ($r = 0.89$). For the other patients, hardly any difference was noticed in their hepatocyte regeneration rates.

Comparing the fitted viral production rate (k) with hepatitis B e-antigen (HBeAg) and hepatitis B e-antibody (HBeAb), we found that the virion production rates of patients with high expression levels of HBeAg and HBeAb (patients D, E and F) were significantly higher than those of patients with low expression levels (patients

Table 2. Local sensitivity coefficients with respect to parameters

Patient	Change range	LSC_r^*	LSC_n^*	LSC_β^*	LSC_α^*	LSC_a^*	LSC_k^*	LSC_ρ^*
A	+5%	6.26	108.28	31.41	4.07	1.09	0.06	0.008
	-5%	66.52	277.83	4.31	43.17	1.05	0.24	0.009
B	+5%	1.04	20.76	1.59	3.29	2.31	0.15	0.014
	-5%	1.83	20.69	3.39	2.08	3.31	0.16	0.014
C	+5%	3.87	976.24	365.76	4.81	0.25	357.19	0.009
	-5%	409.86	441.28	4.33	213.99	0.33	3.84	0.0005
D	+5%	260.63	259.19	137.78	241.89	620.52	3.77	21.26
	-5%	329.68	377.17	319.98	140.73	739.68	5.14	27.23
E	+5%	15.47	13.27	43.12	20.19	60.40	0.14	0.54
	-5%	38.60	14.77	26.19	35.85	30.05	0.24	0.49
F	+5%	24.67	47.03	70.57	34.36	42.67	0.17	0.01
	-5%	70.22	24.54	33.95	82.90	53.05	0.15	0.0005

A, B and C). Since higher expression levels of HBeAg and HBeAb correspond to higher reproduction status and lower clearance status of viruses, the serum titer of HBeAg and HBeAb may correlate with viral production rate. Although the expression level of HBeAg in patient B was relatively high, it decreased gradually over time, and there was a significant increase of α -fetoprotein and total bilirubin. In addition, the clinical diagnosis of patient B was severe chronic hepatitis B. Thus, patient B's hepatocyte regeneration was enhanced and a large number of infected hepatocytes were cleared via inflammation and necrosis, resulting in a high intrinsic growth rate of healthy hepatocytes and a low virion production rate ($k = 12.94$).

For the patients with spontaneous fluctuations (patients A, C, D, E and F), we can conclude that there was a positive correlation between fluctuation amplitude and ALT level. A higher ALT value indicates an increased inflammatory activity, which leads to greater immune pressure for HBV and more interference with HBV DNA. As a result, patients with high ALT (patients E and F) displayed high fluctuation amplitude (almost 2 log). However, the amplitude of patient D was also almost 2 log although his ALT level was not significantly higher than those of patients A and C. We guess that is because a positive correlation also exists between the fluctuation amplitude and the history of antiviral therapy with lamivudine since the administration time of patient D was the longest (about 3 years) among those interrupting therapy, i.e. patients D, E and F.

From table 1, we know the magnitude of equilibrium constant α of equation 2.2 changes from 10^{-10} to 10^{-20} . This indicates that the progress of viral infection varies

from patient to patient. The magnitude of the fitted cure rate (ρ) of infected hepatocytes changes from 10^{-2} to 10^{-5} , which suggests that little cytokine-mediated 'cure' of infected hepatocytes happen during HBV infection. Thus, it may be reasonable to take it as zero as in Dahari et al. [4].

Sensitivity Analysis

In the sensitivity analysis of our parameters, local sensitivity coefficients (LSC) were used. In Xue and Xie [26], the LSC is defined as the variation degree of the design function $F(x)$ under small changes of each design variable x_i , which can be calculated by:

$$LSC_i = \left| \frac{\partial F(x)}{\partial x_i} \right|. \quad (3.3)$$

Since each parameter has different dimensions, in order to compare the sensitivity coefficients of each parameters, we can standardize equation 3.3 as:

$$LSC_i^* = \left| \frac{\partial F(x)}{\delta} \cdot \frac{1}{F(x)} \right|, \quad (3.4)$$

where δ is the change range of parameter.

Taking SSD as the design function, and increasing or decreasing 5% of each parameter, we can obtain the corresponding LSC, which are listed in table 2. From table 2, we know the mean of LSC_n^* is 215.09 ± 82.34 , which is the largest in all parameters. Since a larger sensitivity coefficient corresponds to higher sensitivity degree, we can conclude that parameter n , the average number of virions that enters a single hepatocyte at the beginning stage of an invasion, is an important parameter for chronic HBV kinetics.

Discussion

During antiviral therapy, Lewin et al. [3] and Dahari [4] detected complex decay profiles of HBV. However, for patients untreated or interrupting therapy, a fluctuation profile was observed by Chun et al. [5] and Deng et al. [6] and in our recent long-term tracking study on some patients (fig. 1). To interpret the fluctuation, we introduced model 2.6 of HBV infection. In the context of the model, we define a parameter of the average number of virions that enter a single hepatocyte at the beginning stage of an invasion, which is inspired by the superinfection assumption of Zeuzem et al. [8] and Ciupe et al. [9] in terms of chemical kinetics.

The results of numerical diagnoses (tables 1, 2) show that the superinfection scenario in HBV infection is possible, because the model with the fitted parameters can capture the tendency of HBV DNA trajectories (fig. 1) and the parameter of the average number of virions that enter a single hepatocyte at the beginning stage of an invasion is the most sensitive parameter. Therefore, besides immune circadian rhythms [27], delayed immune response [28], logistic hepatocyte growth and standard incidence function [15], we believe that superinfection of hepatocytes can also give rise to viral oscillation in the host.

The clinical diagnoses of patients A, B and C were liver cirrhosis, severe chronic hepatitis B and asymptomatic HBV carriers, respectively, in Deng et al. [6]. Thus, patients A and C were in an immune stable stage, and their viral loads displayed variations with relatively low amplitude. Patient B represented a typical process of immune clearance, during which a large number of hepatocytes were lysed, and viruses decayed in a huge range. Therefore, there are correlations between HBV DNA loads and clinical phenotypes of untreated patients.

Since patients D, E and F were therapy-interrupting patients and their HBV DNA loads also exhibited spontaneous fluctuations, we can conclude that the fluctuation of HBV DNA was likely intrinsic. After long-term suppression of antiviral therapy, the viral loads in patients returned to certain levels and then restarted natural fluctuation. This may explain why all HBV DNA loads of the 35 patients were spontaneous fluctuation in Chun et al. [5]. However, different from Chun et al. [5], we find that there are some correlations between spontaneous fluctuation and clinical parameters, such as α -fetoprotein, HBeAg, HBeAb, total bilirubin and ALT. Moreover, the dynamic behavior of HBV DNA load may

also correlate with the frequency of lamivudine resistance, which changes with treatment time. It was observed that lamivudine-resistant mutations increased from 23% in year 1 to 65% in year 5 during the long-term lamivudine treatment [29]. These observations in patients D, E and F may have implications for the role of the frequency of lamivudine resistance along with treatment time in chronic hepatitis B patients [30–32], and suggests that future studies should work on determining the relationship between HBV DNA dynamics and drug resistance.

The fluctuation period is short, because the within-host half-life of HBV virions is about 24 h. However, it is difficult to sample patients daily. Thus, the oscillations obtained from the model, as expected, were highly periodic, whereas the data does not show such periodicity since the sampling time intervals of HBV DNA are irregular and some intervals are quite long in our clinical data. Our results could only simulate the tendency over time and more evidence is needed to prove the existence of the superinfection of hepatocytes. For example, more samples with regular long-term clinical data and detailed analytic analyses on model 2.6 are needed in future studies. Nevertheless, our new model can effectively capture the observed fluctuation pattern, and the fitted parameters have clinical significance.

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Appendix

Clinical Data of Patients

HBV DNA load and correlative clinical parameters of each patient

Pa-tient	Time days	HBV DNA log cp ml ⁻¹	HBeAg	HBeAb	ALT IU ml ⁻¹	TBIL μmol ml ⁻¹	AFP ng ml ⁻¹	Pa-tient	Time days	HBV DNA log cp ml ⁻¹	HBeAg	HBeAb	ALT IU ml ⁻¹	TBIL μmol ml ⁻¹	AFP ng ml ⁻¹
A	0	8.551	6.73	0.02	48	15.2	8.28	D	0	8.656	1,423	9.31	43	2.9	1.98
	4	8.616	18.58	0.04	39	26.2	7.13		25	8.981	1,452	9.46	28	7.8	2.14
	11	8.356	5.41	0.03	31	20.1	5.61		179	8.025	1,334	8.63	21	7.7	2.47
	34	7.410	13.93	0.03	14	20.3	5.49		364	7.888	1,315	8.51	32	8.5	1.96
	39	7.812	14.89	0.03	15	25.4	6.73		541	8.620	1,408	9.24	23	11.7	2.09
	46	8.349	13.74	0.03	20	16.1	9.65		724	7.130	1,336	8.27	29	5.0	2.29
	53	7.469	21.01	0.04	27	21.6	9.81								
B	0	9.390	134.90	0.53	127	407.3	459.6	E	0	9.534	1,023	6.35	221	14.3	1.99
	4	8.404	113.40	0.49	57	274.4	246.1		151	5.473	123.12	3.16	53	8.3	6.91
	8	8.608	70.19	0.38	59	186.5	170.8		254	4.852	21.64	3.67	66	10.6	NA
	11	8.188	45.95	0.30	54	151.3	121.1		354	5.124	52.34	2.37	108	18.6	NA
	15	6.707	16.36	0.26	47	129.0	73.25		619	5.155	63.26	1.48	30	8.3	2.37
	21	6.468	4.97	0.19	39	114.5	53.24		668	4.543	109.80	1.69	38	8.5	2.30
	22	5.847	4.23	0.18	42	117.2	53.72		759	8.405	1,055	7.29	155	12.7	NA
	28	6.371	2.70	0.16	34	74.7	38.21		865	5.667	17.52	0.31	36	14.8	55.83
	60	6.044	0.85	0.11	41	55.2	11.90		966	7.948	614.80	2.74	127	58.6	15.71
									1,022	4.844	4.20	0.23	36	36.7	60.96
C	0	5.140	2.01	0.55	24	8.9	2.35	F	0	7.540	1,501	9.92	73	8.8	2.05
	7	4.493	2.59	0.54	26	8.7	2.71		18	6.983	1,492	9.87	37	7.3	1.57
	14	3.960	3.10	0.51	23	9.7	2.65		113	7.364	1,526	9.62	38	16.6	1.95
	21	4.656	3.93	0.56	29	7.5	2.52		285	8.013	1,607	9.94	74	6.8	NA
	28	4.606	4.36	0.55	29	8.8	2.63		446	7.568	1,564	9.57	58	13.6	1.80
	35	5.634	5.15	0.55	28	8.8	2.59		552	8.173	1,629	9.89	125	13.7	1.62
	42	5.575	4.55	0.55	26	8.6	2.59		706	4.777	1,416	8.97	174	11.2	1.73
	49	5.840	5.39	0.52	25	10.0	2.64								
	56	5.555	4.47	0.54	35	7.2	2.67								
	63	5.328	5.22	0.53	34	8.8	2.39								
	70	4.405	5.09	0.50	43	8.8	2.89								
	77	5.671	5.82	0.53	29	10.5	2.68								

NA indicates absent data. HBeAg, HBeAb and α-fetoprotein were detected with chemiluminescence immunoassay kit on Elecsys 2010 system (Roche Diagnostics, Germany). For HBeAg, a numeric value above 1.0 means positive. For HBeAb, a numeric

value below 1.0 means positive. HBV DNA was detected with real-time fluorescent PCR kit on Roche LightCycler (Roche Diagnostics, Germany). ALT and TBIL were analyzed on a Hitachi 7020 automatic biochemistry analyzer.

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