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Original Article

Functional Cure of Chronic Hepatitis B with Antiviral Treatment in Children having High-level Viremia and Normal or Mildly Elevated Serum Aminotransferase

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Abstract

Background and Aims: There is a lack of data supporting the notion that antiviral treatments can benefit children with chronic hepatitis B (CHB) having high viremia and normal or mildly elevated serum alanine aminotransferase (ALT) levels. We aimed to analyze the efficacy of antiviral treatments in children with CHB and explore the factors associated with functional cure. ***Methods:*** Forty-eight children with CHB having high viremia and normal or mildly elevated serum ALT levels were screened in this real-world study. Thirty-two children received either interferon-alpha (IFN-α) monotherapy, IFN-α therapy with a nucleoside analog (NA) add-on, or IFN-α and NA combination therapy. The 16 children in the control group did not receive antiviral treatment. All 48 children were available for follow-up assessments for the entire 36-month study period. We identified a functional cure with respect to hepatitis B virus (HBV) DNA loss, loss /seroconversion of circulating hepatitis B e antigen (HBeAg), and loss of hepatitis B surface antigen (HBsAg) with or without seroconversion. Cox regression analysis was employed to evaluate the factors that may have influenced the functional cure. ***Results:*** After 36 months, the cumulative functional cure rate was 56.25% (18/32) in the treated group and 0% (0/16) in the control group (p<0.001). In the treated group, the serum HBV DNA levels declined rapidly at the end of a 6-month visit and the cured children achieved a loss rate of 100% (18/18) within 16 months of beginning treatment, compared with 64.29% (9/14) of the uncured children (p<0.001). The rates of HBeAg seroconversion were significantly higher among the cured children than among the uncured children (p<0.001). All 16 children in the control group maintained high levels of serum HBV DNA and were positive for both serum HBeAg and HBsAg during the entire 36 months of the study period. Functional cure was associated with younger ages (1–6 vs. 7–14 years, p=0.013), $CD8^+$ T lymphocyte counts (p=0.013), and B lymphocyte counts (p=0.003). No serious adverse events were observed. ***Conclusions:*** Antiviral treatment achieved a functional cure of CHB in a high proportion of children having high-level viremia and normal or mildly elevated ALT levels. Younger age and high peripheral lymphocyte counts were associated with this functional cure.

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Introduction

Infection with the hepatitis B virus (HBV) presents a worldwide threat to human health. Based on virus-host interactions and clinical progression, the natural course of chronic HBV infection is divided into four chronological phases: immune-tolerant (IT), immune-active (IA), low-replicative, and reactivation.[1] Previous studies have indicated that the cure rate in patients with chronic hepatitis B (CHB) at IA phase with antiviral treatment is extremely low, while a great deal of attention has been paid recently to functional cure,[2] which is defined as HBV DNA loss, loss or seroconversion of circulating hepatitis B e antigen (HBeAg), and loss of hepatitis B surface antigen (HBsAg), with or without seroconversion. It is virtually impossible to achieve a functional cure in adult patients with CHB in the IT phase (IT-CHB) using the current antiviral drugs.[3] The IT phase of CHB is characterized by viremia, high HBeAg and HBsAg levels, and normal or mildly

Keywords: Child; HBV; Chronic hepatitis B; Antiviral therapy; Functional cure.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHB, chronic hepatitis B; CHC, chronic hepatitis C; CI, confidence interval; ETV, entecavir; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HR, hazard ratio; IA, immune-active; IFN-α, interferon-alpha; IT, immune-tolerant; IT-CHB, chronic hepatitis B in the immune-tolerant phase; LAM, lamivudine; NA, nucleoside analog; NK, natural killer.

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elevated serum alanine aminotransferase (ALT) level.[1]

Globally, approximately 2 million children under 5 years of age are perinatally infected with HBV each year through mother-to-infant (horizontal) transmission, and 90–95% of the children suffer from CHB.[4] Studies have reported that antiviral treatments for children with IT-CHB have minimal efficacy in achieving sustained loss of HBV DNA and HBeAg.[5–8] Therefore, current guidelines recommend initiating antiviral treatment in children with CHB in the IA phase[9] rather than the IT phase.[1,10,11] In addition, the Asian Pacific Association for the Study of the Liver 2015 guidelines suggest that in the absence of any evidence of active liver inflammation or a family history of hepatocellular carcinoma (HCC) or cirrhosis, IT-CHB patients under 30 years of age generally do not require immediate antiviral therapy.

In the IT phase, histological activity is typically dormant, and the risk of disease progression is low;[10–12] however, a significant proportion of children with IT-CHB suffer from hepatic inflammation and fibrosis.[13] Thus, young children with CHB can be in either the IA or the IT phase. Active HBV replication in pediatric patients often generates large quantities of virions and viral antigens. Meanwhile, HBV DNA can be easily integrated into the genome of proliferative hepatocytes, which may not only increase the complexity of antiviral treatment but also increase the risk of progression to cirrhosis and primary HCC in children with CHB when they grow up.[3]

To avoid a poor prognosis, studies have investigated the safety and efficacy of antiviral treatment in children with IT-CHB. In 2005, Artan *et al*.[14] reported that lamivudine (LAM) monotherapy did not completely achieve HBV DNA loss in children with IT-CHB. Several studies have since reported similar findings in children and adolescents with IT-CHB, bolstering the notion that antiviral treatments do not alter the disease outcome in children with IT-CHB.[3,5–8] However, other studies have suggested that antiviral treatment may achieve a functional cure in children with IT-CHB.[15–17] Zhu *et al*.[18] found that 22% of children with IT-CHB and 1–16 years of age achieved a functional cure after 1.5–2 years of antiviral treatment,[18] suggesting that timely antiviral intervention may prevent unfavorable outcomes in pediatric patients.[19]

The reasons for the inconsistencies in the research outcomes pertaining to the efficacy of antiviral treatment in children with IT-CHB remain unclear. Notably, there is no consensus or generally accepted definition of the IT phase of CHB in children. In other words, the inclusion criteria for children with IT-CHB, especially the cutoff values for serum ALT level to be considered normal or mildly elevated (typically ≤60 U/L) were inconsistent in the above studies.

In this real-world observational study, we identified and enrolled children with CHB as those having high-level viremia, circulating HBeAg, and either normal or mildly elevated serum ALT levels to avoid confusion regarding the concept of the IT phase. Our aim was to investigate whether antiviral treatment could positively impact the functional cure of children with CHB. The influencing factors of functional cure in children with CHB were also analyzed.

Methods

Ethical standards

Our study complied with the principles of the revised 2013 Declaration of Helsinki and was approved by the Medical Ethics Committee of the Fifth Medical Center of Chinese People's Liberation Army General Hospital (Ethical approval no. 2020048D). The first-line antiviral drugs used for children with CHB included interferon-alpha (IFN-α) and nucleoside analog (NA), which suppress viral replication. IFN-α treatment regimens were based on both antiviral treatments for children with chronic hepatitis C (CHC)[20,21] and our own previous clinical experience.[18] IFN-α is considered safe and effective for the treatment of CHC and CHB in children.[22,23]

The children enrolled in this study completed a careful preclinical evaluation at our clinic, and our physicians informed the children's parents or guardians of the adverse events associated with IFN-α treatment and highlighted the possibility that some children may not achieve a functional cure of their CHB. For that reason, written consent has been obtained from those parents or guardians wishing their children to receive antiviral treatment. We also respected the choice of the parents or guardians who opted out of the antiviral treatment for their child but requested that they complete the necessary follow-up visit.

Study participants

Forty-eight HBeAg-positive children aged 1–14 years who were diagnosed with CHB and admitted to our hospital between June 2014 and September 2021 were enrolled in this study. All the children met the inclusion and exclusion criteria outlined in Figure 1. The inclusion criteria were: (1) HBsAg positivity for >6 months; (2) HBeAg-positivity and anti-HBe-negativity; (3) serum HBV DNA levels of $>10^7$ IU/mL and serum ALT level of <60 U/L (upper limit of normal, 40 U/L) for two consecutive times within a period of at least 3 months; and (4) no history of antiviral therapy. The exclusion criteria were: (1) coinfection with hepatitis A, C, D, or E viruses, Epstein-Barr virus, cytomegalovirus, or human immunodeficiency virus; (2) coexistence of other liver disease, such as autoimmune hepatitis, drug-induced liver injury, or Wilson's disease; (3) liver transplantation or HCC; (4) a history of mental disorders; and (5) other severe comorbidities, such as severe thyroid disease or retinopathy. As there is no consensus on the cutoff value of serum ALT level in determination of the IT phase in children, we chose an ALT value of <60 U/L for this study, based on the findings of published research and our previous study.[15,17,18]

Antiviral regimens

Children whose parents or guardians opted for antiviral treatment received either IFN-α monotherapy, IFN-α with an NA add-on, or IFN-α and NA combination therapy. Thirteen patients were initially treated with IFN-α monotherapy. After 6 months, the treatment regimen were modified as follows:[13,18] (1) if a patient who initially received IFN-α monotherapy exhibited a decrease in the log10 serum HBV DNA level >2 (using real-time, quantitative polymerase chain reaction [PCR] and a cut-off value 40 IU/mL), IFN-α monotherapy was continued for another 18 months; and (2) if a patient exhibited a decrease in the log10 serum HBV DNA level of <2, either LAM (3 mg/kg/day) or entecavir (ETV) (0.015 mg/kg/day) was added to the regimen. Appositionally, 19 patients began with combination therapy of IFN-α and either LAM or ETV that was continued for the duration of the treatment period.

In this study, IFN-α (30–50 μg/m^2) was administered intramuscularly or subcutaneously to examine the participants once every other day. The IFN-α dose was calculated based on individual body surface area.[24] For the NA treatment component, ETV (0.015 mg/kg/day) was administered to children >2 years of age,[9] and LAM (3 mg/kg/day) was administered to children <2 years of age.[25]

There were two criteria for the discontinuation of antiviral

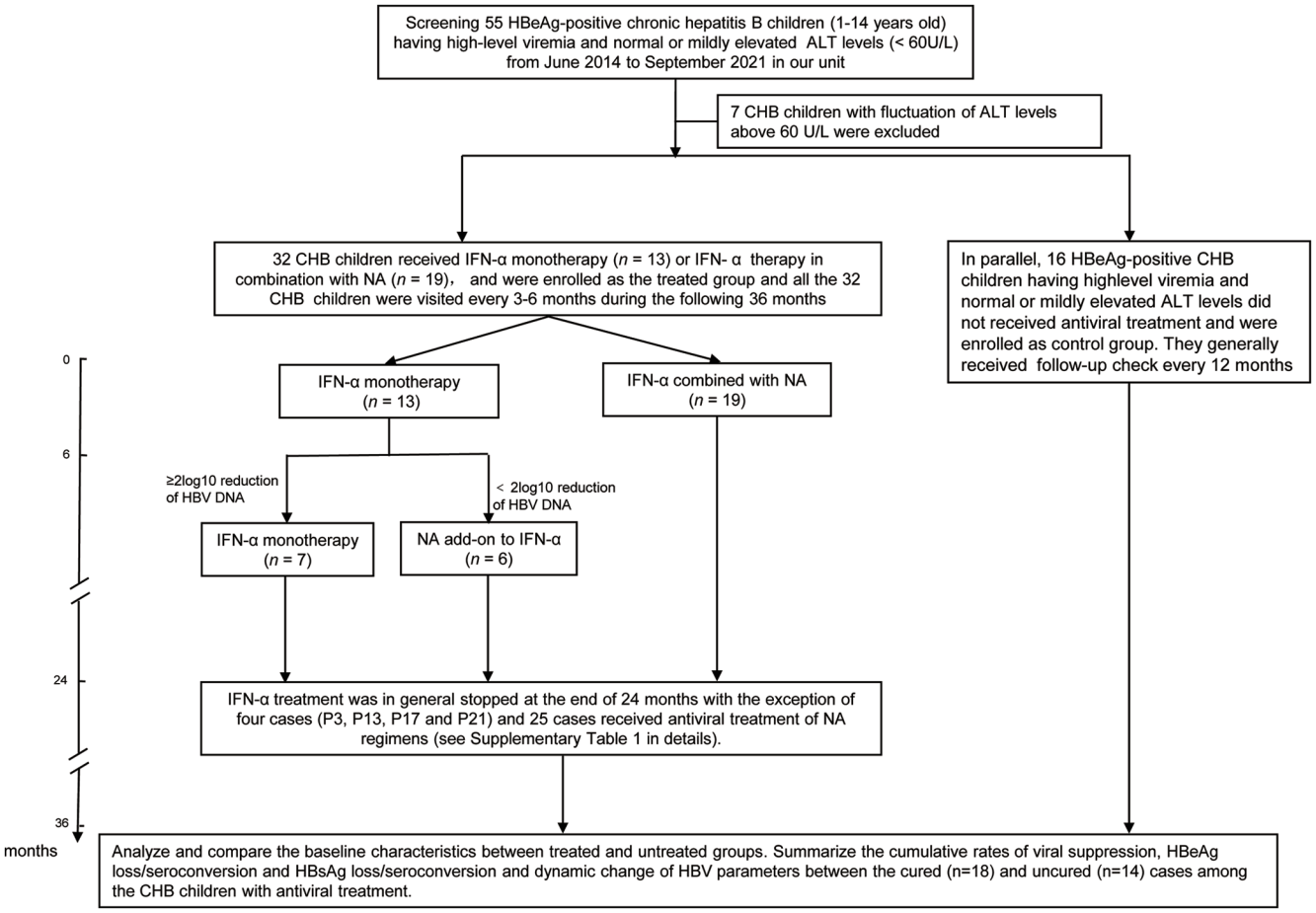

Fig. 1. Flow diagram of antiviral course of treatment in children with CHB having high-level viremia and normal or mildly elevated serum ALT levels. Fifty-five children 1–14 years of age) were screened, and 48 patients who met the inclusion/exclusion criteria were included in this study. Thirty-two patients received antiviral treatment consecutively and the remaining 16 made up the untreated control group. In the treated group, the duration of IFN-α monotherapy among the cured children depended on the time point when serum HBsAg loss and anti-HBs positivity occurred. The study data, including antiviral regimens, are shown in detail in Table 1. We analyzed the rate of functional cure in children with CHB in the immune-tolerant phase at 36 months. ALT, alanine aminotransferase; CHB, chronic hepatitis B; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; IFN-α: interferon-α; NA, nucleoside analog.

treatment.[25] First, the maximum duration of IFN-α monotherapy was 24 months regardless of whether a patient achieved HBsAg seroconversion. In some special settings, children with CHB who achieve HBsAg seroconversion within 18 months of beginning IFN-α treatment receive a consolidation course of IFN-α for an additional 6 months. Second, for children receiving IFN-α and NA combination therapy, the aforementioned duration of IFN-α monotherapy was adhered to. While the duration of NA treatment is not defined,[26] the maximum duration in this study was 36 months. For example, if children with CHB achieved a functional cure within 30 months of starting antiviral treatment, then they continued with consolidation treatment with NA for an additional 6 months. The 16 children enrolled in the control group did not receive any antiviral therapy. However, they completed follow-up visits over the 36-month course of the study, as shown in Figure 1. The baseline characteristics, blood serology, and clinical outcomes of the study participants in the treated and control groups are shown in Table 1.

Serological assays

The serological and clinical biochemical test data for the study participants undergoing antiviral treatment were recorded at baseline and every 3 months at the follow-up visits. The data included routine blood tests, biochemical liver function assessments, plasma HBV DNA quantification, and serological HBV marker evaluation. Quantitative HBsAg levels were assayed with a Roche Cobas HBsAg II-Q kit (Roche Diagnostics, Manheim, Germany), and HBV DNA levels were assayed by qPCR.

Histological analysis

Liver histology was performed in 40 of the 48 enrolled children with a one-second needle biopsy, following a standard protocol.[27] Liver samples were fixed, embedded, stained, and scored by a blinded senior pathologist, with the grade of inflammation and stage of fibrosis observed and evaluated with reference to previous studies.[28]

Analysis of peripheral lymphocyte subsets

The absolute peripheral lymphocytes and related subsets were analyzed with a lymphocyte detection kit (Beijing Tongsheng Shidai Biotechnology Co, Beijing, China) and monitored using a BD FACSCalibur system (Becton, Franklin Lakes, NJ, USA), following the manufacturers' instructions and as described in our previous study.[29]

Table 1. Baseline characteristics and outcome of children with chronic hepatitis B (CHB) having high-level viremia and normal or mildly elevated serum alanine aminotransferase (ALT) levels in treated and control groups

Parameters	Treated group	Control group	*p*-value
n	32	16	
Age (y)	3.35 (2.77, 5.53)	5.15 (3.60, 9.25)	0.058
≥1 and <3	9 (28.13)	2 (12.50)	0.207
≥3 and <7	19 (59.38)	9 (56.25)	
7–14	4 (12.50)	5 (31.25)	
M:F	18:14	10:6	0.918
qHBsAg, $\log_{10}$ IU/mL	4.58 (4.07, 4.84)	4.79 (4.64, 5.04)	0.124
HBV DNA, $\log_{10}$ IU/mL	7.99 (7.60, 8.55)	8.53 (8.16, 8.66)	0.185
Neutrophil count, 10^9/L	2.73 (1.99, 3.59)	2.47 (2.10, 3.01)	0.726
$CD4^+$ T lymphocyte count, cells/μL[a]	1,466.00 (1,084.50, 1,929.75)	1,026.00 (831.50, 1,934.00)	0.320
$CD8^+$ T lymphocyte count, cells/μL[a]	862.50 (594.25, 1,219.00)	855.00 (702.00, 948.00)	0.915
B lymphocyte count, cells/μL[a]	1,008.00 (544.25, 1,369.25)	603.00 (451.00, 1,042.50)	0.145
NK cell count, cells/μL[a]	334.50 (196.50, 447.75)	329.00 (180.00, 585.50)	0.957
HBV genotype			0.663
B genotype	5 (15.63)	2 (12.50)	
C genotype	20 (62.50)	12 (75.00)	
Undetected	7 (21.88)	2 (12.50)	
AST, U/L	48.00 (44.50, 56.25)	46.50 (31.50, 51.50)	0.175
ALT, U/L	43.00 (38.75, 49.00)	39.00 (21.25, 50.25)	0.599
Range (min, max)	(18, 57)	(16, 59)	
Patients w/ normal level	9 (28.13)	8 (50.00)	0.241
Patients w/ 1–1.5× ULN	23 (71.88)	6 (50.00)	
Inflammation grade[b]			0.023[c]
G0	2 (7.14)	5 (41.67)	
G1	18 (64.29)	6 (50.00)	
G2	8 (28.57)	1 (8.33)	
Fibrosis stage[b]			0.113
S0	4 (14.29)	1 (8.33)	
S1	15 (53.57)	11 (91.67)	
S2	8 (28.57)	0 (0)	
S3	1 (3.57)	0 (0)	
Family history of HBV infection			0.592
Mother w/ HBsAg positivity	26 (81.25)	14 (87.50)	
Both parents w/ HBsAg positivity	2 (6.25)	0 (0)	
Neither parent w/ HBsAg positivity	4 (12.50)	2 (12.50)	
Clinical outcome			
HBV DNA loss	27 (84.38)	0 (0)	<0.001
HBeAg loss	19 (59.38)	0 (0)	<0.001
HBeAg seroconversion	16 (50.00)	0 (0)	0.002
HBsAg loss	18 (56.25)	0 (0)	0.001
HBsAg seroconversion	18 (56.25)	0 (0)	0.001

[a]Peripheral lymphocyte subsets were tested in 35/48 enrolled children. [b]Liver histological analyses were performed with 28/32 children in the treatment group and 12/16 children in the control group using a one-second needle biopsy sample. [c]Some children in the control group with biopsy inflammation grade G0; whether this contributed to the statistical difference in hepatic inflammation between the treated and control groups requires investigation. Categorical variables expressed as number (*n*) and percentage (%), and continuous variables as median (interquartile range). AST, aspartate aminotransferase; ALT, alanine aminotransferase; CHB, chronic hepatitis B; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; NK, natural killer; qHBsAg, quantitative hepatitis B surface antigen; ULN, upper limit of normal.

Assessment of efficacy and safety of antiviral treatment

In keeping with previous studies,[30] the lower limit of HBV DNA detection was 20 IU/mL and measured by qPCR (Roche Cobas AmpliPrep Test) and was defined as the HBV DNA clearance limit. HBeAg seroconversion was defined as the loss of HBeAg and the appearance of anti-HBe. Using a lower limit of detection of 0.05 IU/mL, HBsAg loss was defined as a serum HBsAg level of <0.05 IU/mL, and was assayed with a Roche Cobas HBsAg II-Q kit. The primary metric used to determine the functional cure among the study participants was the rate of HBsAg loss after a 36-month period. Secondary measurements included HBV DNA loss and HBeAg seroconversion. For the safety evaluation, adverse events, laboratory test results, vital signs, and anthropometric parameters (e.g., height and weight), were recorded throughout the 36-month study period. In addition, specific assays for LAM and ETV genetic resistance were performed.

Statistical analysis

The statistical analysis was performed with R Foundation for Statistical Computing software (v. 4.1.1; Vienna, Austria; http://www.r-project.org/). Categorical variables were reported as numbers and percentages. Continuous variables were reported as medians and interquartile range. Comparisons between the treated and control groups were conducted using either chi-squared or Fisher's exact tests for categorical variables, and Mann-Whitney *U* tests for continuous variables. The Kaplan-Meier method was used to calculate cumulative rates, with differences compared using log rank tests. Generalized estimating equations were used to compare the dynamic changes in five serological indicators between the two groups. Univariate and multivariate Cox regression analysis were to screen for independent influencing factors associated with functional cure. Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated. All tests were two-sided, and the results were considered statistically significant at $p<0.05$.

Results

Baseline data of study participants

The 48 children enrolled in the study were divided into treated ($n=32$) and control ($n=16$) groups. Except for higher liver inflammation grades in the treated group, there were no significant differences in the baseline parameters between the two groups (Table 1). In the treated group, 28 children (87.50%) had been infected with HBV perinatally. The remaining four had been infected by unknown routes (Table 2). Ten of the 32 children in the treated group were conventionally vaccinated against HBV, but mother-to-infant transmission occurred nonetheless. The majority of participants in the treatment group carried HBV genotype C ($n=20$, 62.50%) or B ($n=5$, 15.63%). Twenty-eight patients in the treated group underwent liver biopsy, and most had mild hepatic inflammation (G1 or G2) and/or fibrosis (S1 or S2). Two (7.14%) had no hepatic inflammation, and four (14.29%) had no hepatic fibrosis. The baseline characteristics of the 16 children in the control group were comparable to those of the children in the treated group. Twelve of the children in the control group underwent liver biopsy (Table 1).

The 32 children in the treated group received antiviral treatment consecutively and visited the clinic for follow-up every 3 months for 36 months (Fig. 1).[18] Response-guided treatment was followed in the therapeutic regimens. Thirteen of the 32 patients in the treatment group were initially treated with IFN-α monotherapy, and 19 received combination therapy of IFN-α and NA (either LAM or ETV). The therapeutic regimens and discontinuation standards for each antiviral treatment are shown in Fig. 1. At 36-months, children in the treated group were categorized as either cured (exhibiting HBsAg loss) or uncured (HBsAg-positive).

Efficacy analysis of antiviral treatment

Overall, of the 32 children who received antiviral treatment, 27 (84.38%) achieved HBV DNA loss, 16 (50%) exhibited HBeAg seroconversion, and 18 (56.25%) exhibited HBsAg seroconversion 36 months after the initiation of antiviral treatment (Supplementary Fig. 1). Using the serum HBsAg loss criterion, 18 children (56.25%) were designated cured and 14 (43.75%) were identified as uncured (Table 2). There were no significant differences in baseline HBV DNA load, HBsAg quantities, serum ALT levels, hepatic inflammation grade, or liver fibrosis stage between the cured and uncured patients. The baseline parameters that differed between the two groups were age and peripheral $CD4^+$ T, $CD8^+$ T, and B-cell counts (Table 2). Compared with the 18 cured children who achieved sustained viral suppression and loss of both HBeAg and HBsAg in their peripheral blood, only 9 of the 14 uncured children (64.29%) achieved a durable HBV DNA loss, and only one exhibited HBeAg seroconversion (Table 2). Notably, the serum HBV DNA, HBeAg, and HBsAg levels were not only positive but also high in all 16 untreated children throughout the 36-month follow-up period. Any dynamic changes in serum HBV DNA, HBeAg, and HBsAg loss or seroconversion between the cured and uncured children, as well as the clinical parameters and potential associated factors influencing functional cure, were characterized and compared.

Rapid HBV DNA loss in the cured group

Antiviral therapy effectively suppressed viral replication in both the cured and the uncured children. The cumulative loss rate of serum HBV DNA increased more rapidly in the cured patients than in the uncured patients after 6–15 months of antiviral treatment (Fig. 2A). In addition, there was a rapid, dynamic reduction in viral load from baseline and almost complete viral suppression among the cured children at six months after the initiation of the antiviral treatment (Fig. 2B). The viral load of the uncured children decreased slowly in comparison. At 36 months, the serum HBV DNA loss rate of the was 100% in cured children and 64.29% (9/14) in the uncured children ($p<0.001$; Fig. 2A). Three of the five remaining HBV DNA positive patients in the uncured group continued to have low levels of serum HBV DNA (1.3×10^2–4.69×10^3 IU/mL) after 36 months of antiviral treatment. Notably, the 18 patients who were cured, with complete viral suppression, did not experience viral rebound during the 36-month follow-up period. The data clearly indicate that antiviral treatment in children with CHB results in sustained viral DNA, HBeAg, and HBsAg loss. In contrast, a high level of serum HBV DNA was present among the untreated children throughout the study period.

Significantly higher hepatitis B e antigen loss and seroconversion in the cured group

In CHB patients undergoing antiviral treatment, HBeAg loss and seroconversion often occur before HBsAg loss. As shown in Figure 3A, the cumulative rates of HBeAg loss from baseline in the cured children at 6, 12, 24, and 36 months were 11.11% (2/18), 61.11% (11/18), 83.33% (15/18), and 100% (18/18), respectively, compared with 0% (0/14),

Table 2. Characteristics of children with chronic hepatits B (CHB) having high-level viremia and normal or mildly elevated serum alanine aminotransferase (ALT) levels in cured and uncured groups at the end of a 36-month follow-up period

Characteristic	Total	Cured group	Uncured group	*p*-value
n	32	18	14	
Age (y)	3.35 (2.77, 5.53)	2.95 (1.68, 3.65)	4.40 (3.42, 7.48)	0.002
≥1 and <3	9 (28.13)	9 (50)	0 (0)	0.002
≥3 and <7	19 (59.38)	9 (50)	10 (71.43)	
7–14	4 (12.50)	0 (0)	4 (28.57)	
M:F	18:14	8:10	10:4	0.243
qHBsAg, $\log_{10}$ IU/mL	4.58 (4.07, 4.84)	4.46 (3.94, 4.70)	4.64 (4.42, 4.94)	0.230
HBV DNA, $\log_{10}$ IU/mL	7.99 (7.60, 8.55)	7.73 (7.50, 8.45)	8.21 (7.81, 8.81)	0.138
Neutrophil count, 10^9/L[a] (*n*=24)	2.73 (1.99, 3.59)	2.35 (1.80, 3.49)	2.87 (2.61, 3.63)	0.271
$CD4^+$ T lymphocyte count, cells/μL[a] (*n*=24)	1,466.00 (1,084.50, 1,929.75)	1,656.00 (1,441.50, 2,361.75)	1,221.50 (813.00, 1,430.25)	0.040
$CD8^+$ T lymphocyte count, cells/μL[a] (*n*=24)	862.50 (594.25, 1,219.00)	955.00 (851.00, 1,310.50)	568.50 (468.25, 807.25)	0.008
B lymphocyte count, cells/μL[a] (*n*=24)	1,008.00 (544.25, 1,369.25)	1,314.50 (965.50, 1,679.25)	545.50 (457.00, 801.00)	0.002
NK cell count, cells/μL[a] (*n*=24)	334.50 (196.50, 447.75)	347.00 (199.00, 510.75)	309.50 (200.50, 431.75)	0.725
HBV genotype				0.434
B genotype	5 (15.63)	4 (22.22)	1 (7.14)	
C genotype	20 (62.50)	11 (61.11)	9 (64.29)	
Undetected	7 (21.88)	3 (16.67)	4 (28.57)	
AST, U/L	48.00 (44.50, 56.25)	51.00 (46.25, 58.75)	46.00 (39.25, 50.75)	0.060
ALT, U/L	43.00 (38.75, 49.00)	44.50 (41.50, 49.75)	42.00 (31.75, 45.25)	0.090
Range (min, max)	(18, 57)	(30, 57)	(18, 54)	
Patients w/ normal level	9 (28.13)	3 (16.67)	6 (42.86)	0.216
Patients w/ 1–1.5× ULN	23 (71.88)	15 (83.33)	8 (57.14)	
Inflammation grade[b] (*n*=28)				0.237
G0	2 (7.14)	0 (0)	2 (16.67)	
G1	18 (64.29)	11 (68.75)	7 (58.33)	
G2	8 (28.57)	5 (31.25)	3 (25)	
Fibrosis stage[b] (*n*=28)				0.461
S0	4 (14.29)	1 (6.25)	3 (25)	
S1	15 (53.57)	9 (56.25)	6 (50)	
S2	8 (28.57)	5 (31.25)	3 (25)	
S3	1 (3.57)	1 (6.25)	0 (0)	
Family history of HBV infection				0.943

(continued)

Table 2. *(continued)*

Characteristic	Total	Cured group	Uncured group	*p*-value
Mother w/ HBsAg positivity	26 (81.25)	15 (83.33)	11 (78.57)	
Both parents w/ HBsAg positivity	2 (6.25)	1 (5.56)	1 (7.14)	
Neither parent w/ HBsAg positivity	4 (12.50)	2 (11.11)	2 (14.29)	
Postdelivery HBV vaccination				0.712
Yes (failed to block transmission)	10 (31.25)	5 (27.78)	5 (35.71)	
No	22 (68.75)	13 (72.22)	9 (64.29)	
Antiviral treatment				0.376
IFN-α monotherapy	7 (21.87)	5 (27.78)	2 (14.29)	
IFN-α combined w/ NA	19 (59.38)	11 (61.11)	8 (57.14)	
IFN-α add-on w/ NA	6 (18.75)	2 (11.11)	4 (28.57)	

[a]Peripheral lymphocyte subsets were tested in 24/32 enrolled children; [b]Liver histological analyses were performed in 28/32 children in the treatment group using a one-second needle biopsy sample. Categorical variables expressed as number (*n*) and percentage (%), and continuous variables as median (interquartile range). The cured group is defined as exhibiting HBsAg loss, and the uncured group is defined as exhibiting HBsAg positivity. AST, aspartate aminotransferase; ALT, alanine aminotransferase; CHB, chronic hepatitis B; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IFN-α: interferon-α; NA: nucleotide analog; NK, natural killer; ULN, upper limit of normal.

7.14% (1/14), 7.14% (1/14), and 7.14% (1/14) in the uncured children. The cumulative rates of HBeAg seroconversion in the cured children at the same time points were 11.11% (2/18), 50% (9/18), 61.11% (11/18), and 83.33% (15/18), respectively. The rates were significantly higher in the cured group than in the uncured group, in which only one individual (7.14%) exhibited HBeAg seroconversion ($p<0.001$; Fig. 3B). As expected, HBeAg loss occurred significantly more rapidly in the cured children than in the uncured children (Fig. 3C). As noted above, a similar result was found for serum HBV DNA loss. Anti-HBe was detected sooner in the cured children than in the uncured children (Fig. 3D). Overall, there were significantly higher rates of HBeAg loss and seroconversion among the cured children, and sustained control of HBeAg loss was achieved in this group.

Characteristics of hepatitis B surface antigen loss and associated factors

Interestingly, the cumulative rates of HBsAg loss among the cured children were 16.67% (3/18), 61.11% (11/18), 83.33% (15/18), and 100% (18/18) at 6, 12, 24, and 36 months, respectively (Fig. 4A). Concurrently, the cumulative rates of HBsAg seroconversion in this group at the same times were 11.11% (2/18), 50% (9/18), 83.33% (15/18), and 100% (18/18), respectively (Fig. 4B). Longitudinal analysis (Fig. 4C, D) indicated a rapid decrease in serum HBsAg load in the cured children. Among the 18 cured patients, 11 (61.11%) exhibited HBsAg loss at 12 months, and nine had serum anti-HBs. At 36 months, 14 of the 18 cured patients had high titers of serum anti-HBs (500–1,000 IU/L). In contrast, the serum HBsAg levels remained high in the uncured children, but decreased slow following treatment (Fig. 4C, D). In addition, we did not find a significant difference in inflammation grade between the cured and uncured groups, possibly because of insufficient sample size (Supplementary Fig. 2A, B). Only one cured child had an ALT flare (>200 U/L) during IFN-α monotherapy, with a level of 224 U/L.

To further investigate whether baseline age was an independent influencing factor of antiviral therapy efficacy, we analyzed the association between age and HBsAg loss. The baseline age distribution differed significantly between the cured and uncured groups ($p=0.002$), as shown in Figure 5A. Among the 18 cured patients, nine were <3 years of age, eight were 3–6 years of age, and one was >6 years of age (Supplementary Fig. 3A). The cured group was significantly younger than the uncured group, which included nine children 3–6 years of age and five 6–13 years of age (Fig. 5A). In other words, nine children under 3 years of age achieved a functional cure. Among the 19 patients 3–6.4 years of age in the treatment group, a functional cure was achieved in nearly half (9/19), but four patients >7 years of age were not cured, even with a 36-month course of antiviral treatment. Furthermore, the cumulative rate of treatment gradually, but significantly, decreased with an increase in baseline age at the initiation of treatment (Supplementary Fig. 3B). Cox regression analysis indicated that the baseline age at treatment initiation was closely correlated with HBsAg loss ($p=0.013$, Supplementary Table 1), corroborating the findings of our previous study involving infants with CHB and elevated ALT levels.[28]

Interestingly, the baseline lymphocyte counts differed significantly between the cured and the uncured patients (Fig. 5B, C, D). In addition to the differences in age distribution, the $CD4^+$ T ($p=0.040$), $CD8^+$ T ($p=0.008$), and B cell counts ($p=0.002$) were significantly higher in the cured group than in the uncured group (Table 2). Univariate analysis indicated that high $CD8^+$ T cell ($p=0.013$) and B cell counts ($p=0.003$)

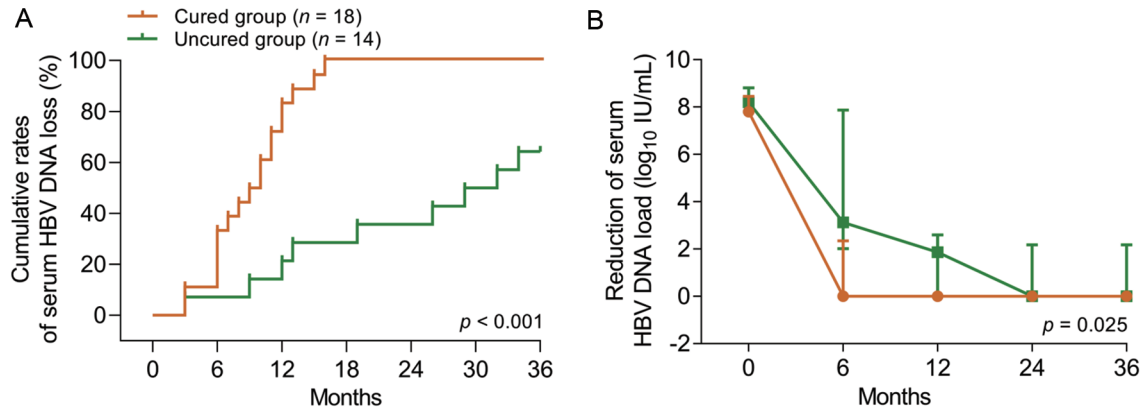

Fig. 2. Cumulative rate of HBV DNA loss and reduction of viral load in cured and uncured children during antiviral therapy. Comparison of dynamic changes in cumulative rates of serum HBV DNA loss (A) log rank test, $p<0.001$) and (B) viral load, generalized estimating equation, $p=0.025$) in cured ($n=18$, orange line) vs. uncured ($n=14$, green line) children on antiviral treatment. HBV, hepatitis B virus.

were significantly correlated with HBsAg loss. However, there was no correlation between $CD4^+$ T cells and HBsAg loss (Supplementary Table 1). Multivariate analysis found no correlation between lymphocyte number and HBsAg loss.

Safety evaluation of adverse events

Serious adverse events were not observed in any of the 32 children who received antiviral treatment during the 36-month study period. However, mild or moderate adverse events occurred without the special clinical treatment. The adverse events that were recorded are shown in Table 3. Fever (24/32) and fatigue (16/32) were the most common adverse events observed during the IFN-α treatment. Eleven, eight, and six children experienced nausea, alopecia,

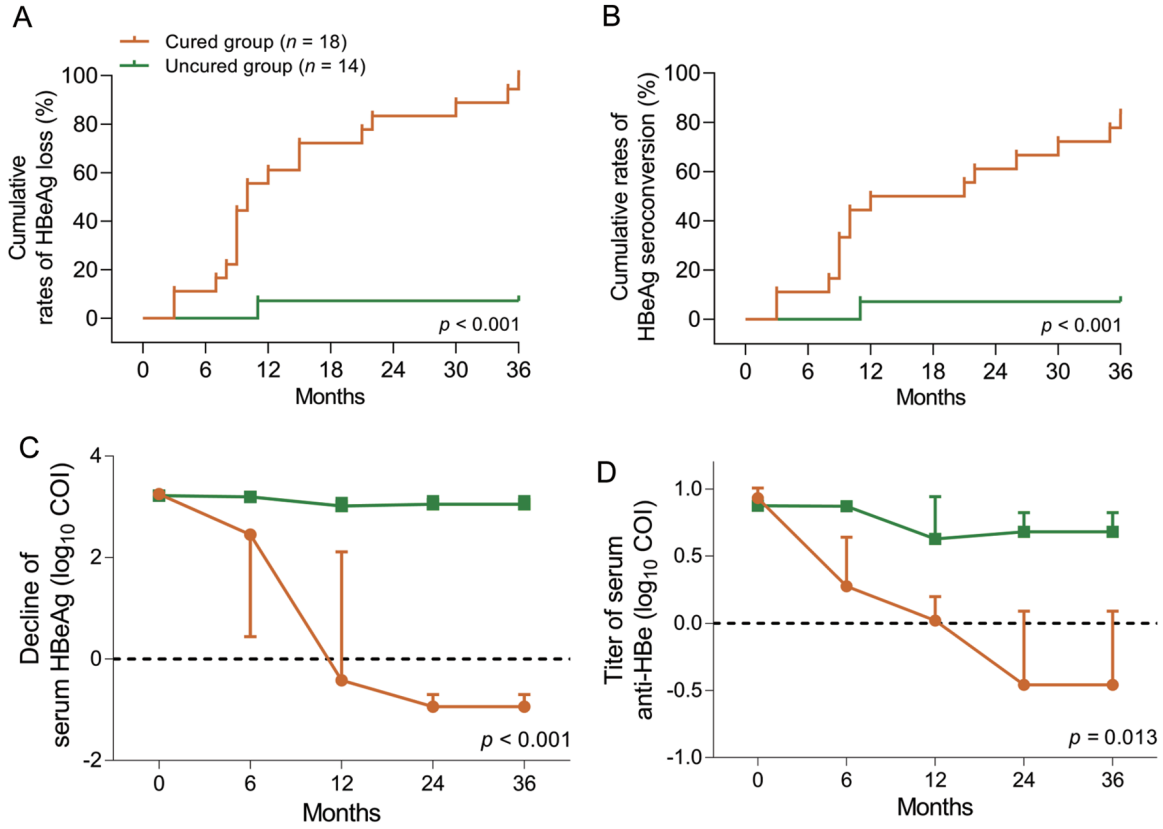

Fig. 3. Cumulative rate of HBeAg loss/seroconversion and other dynamic changes in the blood serology of the cured and uncured children receiving antiviral therapy. Comparison of dynamic changes in (A) cumulative rates of serum HBeAg loss (log rank test, $p<0.001$) and (B) seroconversion (log rank test, $p<0.001$), (C) serum HBeAg, and (D) anti-HBe (generalized estimating equation, $p<0.001$, $p=0.013$) in cured ($n=18$, orange line) vs. uncured ($n=14$, green line) children on antiviral therapy. Dotted lines in graphs C and D indicate the threshold cutoff values for HBeAg loss (<1 cutoff index (COI) or 0 $\log_{10}$ COI and anti-HBe positivity (<1 or 0 $\log_{10}$ COI). HBeAg, hepatitis B e antigen.

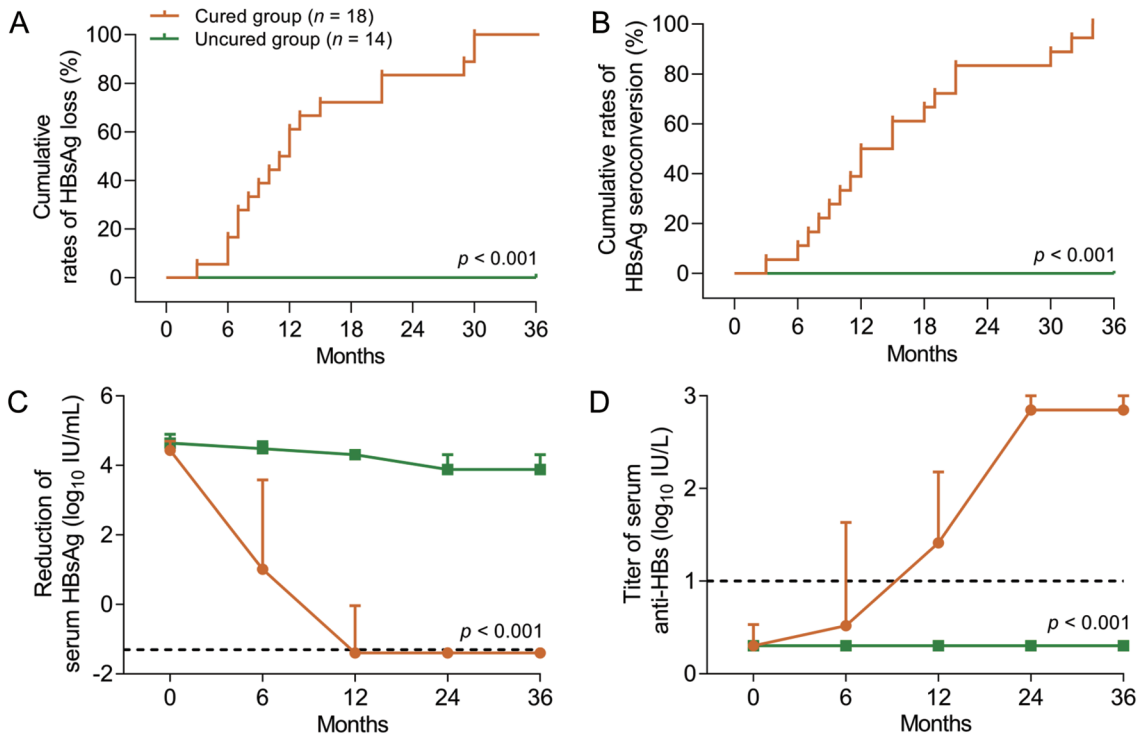

Fig. 4. Cumulative rate of HBsAg loss/seroconversion and other dynamic changes in the blood serology of the cured and uncured children receiving antiviral therapy. Comparison of dynamic changes in (A) cumulative rates of serum HBsAg loss and (B) seroconversion (log rank test, $p<0.001$, $p<0.001$, respectively), (C) serum HBsAg loads, and (D) anti-HBs levels (generalized estimating equation, $p<0.001$, $p<0.001$, respectively) in cured ($n=18$, orange line) vs. uncured ($n=14$, green line) children on antiviral therapy. Dotted lines in graphs C and D indicate the threshold cutoff value for HBsAg loss (<0.05 IU/mL or −1.3 $\log_{10}$ IU/mL) and anti-HBs positivity (>1 $\log_{10}$ IU/L or 10 IU/L). HBsAg, hepatitis B surface antigen.

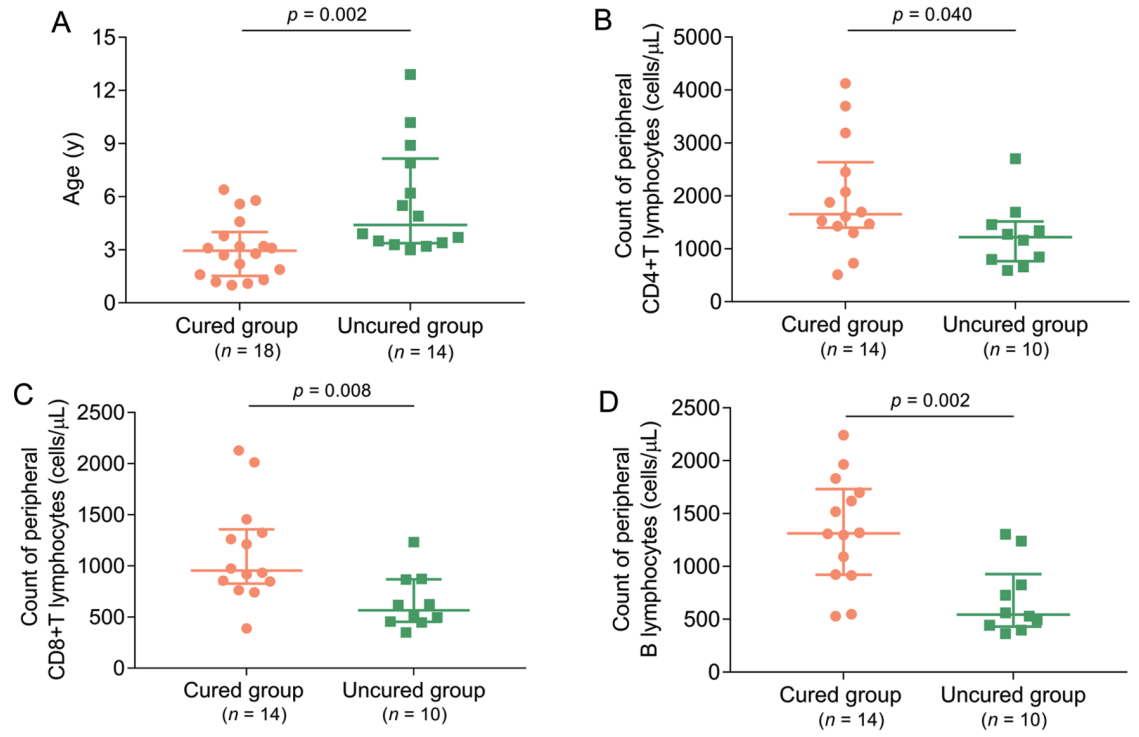

Fig. 5. Comparison of baseline pediatric age and peripheral lymphocyte counts in cured vs. uncured children receiving antiviral therapy. (A) Age distribution and distribution of (B) $CD4^+$ T, (C) $CD8^+$ T, and (D) B cell counts in the treated group.

Table 3. Cumulative adverse events among the 32 pediatric patients with CHB receiving IFN-α treatment

Type	*n*	Grade[a]	Treatment	Outcome
Fever	24	1 (*n*=22); 2 (*n*=2)	acetaminophen or no treatment	relief/spontaneous remission
Fatigue	16	1	none	self-relief
Nausea	11	1	none	self-relief
Alopecia	8	1	none	self-relief
Leukocytopenia	6	1	none	relief after discontinuation of IFN-α therapy
Headache	3	1	none	self-relief
Arthralgia	2	1	none	self-relief
Rash	2	1	none	self-relief
Thrombocytopenia	1	1	none	relief after discontinuation of IFN-α therapy
Hypothyroidism	1	1	none	relief after discontinuation of IFN-α therapy
Growth suppression	1	1	none	growth restoration 3–6 months after discontinuation of IFN-α therapy

[a]Grading basis: Common Terminology Criteria for Adverse Events, v. 5.0 (Published Nov. 27, 2017 by the U.S. Department of Health and Human Services). CHB, chronic hepatitis B; IFN-α, interferon-α.

and leukopenia, respectively. Other adverse events included headaches (3/32), arthralgia (2/32), rash (2/32), thrombocytopenia (1/32), hypothyroidism (1/32), and growth suppression (1/32). No viral breakthrough, rebound after treatment discontinuation, or drug resistance was observed during the 36-month follow-up period.

Discussion

It is well known that spontaneous HBsAg loss occurs rarely in children with CHB, with an annual rate of 1% per year.[31] In this study, we analyzed the functional cure rate of CHB in children having high-level viremia and normal or mildly elevated ALT levels following empirical antiviral treatment. It was found that antiviral therapy led to sustained HBsAg loss and seroconversion with good safety and tolerability in children with CHB. In contrast, none of the 16 patients in the control group, which did not receive the antiviral treatment, achieved HBeAg loss/seroconversion or a functional cure during the 36-month study period. High cure rates, similar to those in recent reports,[18] were achieved. The rates differed from other studies that implemented similar antiviral regimens in relatively older patients.[15,17] Our findings suggest that the antiviral therapy is likely to result in a functional cure in some children with CHB with normal or mildly elevated ALT levels, which may improve the disease prognosis.

The findings of this study are expected to support the notion that youth may be an associated determinant of functional cure with successful antiviral treatment, but the underlying mechanisms of this cure remain unclear. A relatively low diversity of HBV quasispecies in younger children with CHB may enhance their response to antiviral treatment.[32] In addition, large quantities of liver and peripheral blood HBsAg in IT-CHB patients are believed to induce an immune tolerance specific to HBV infection in hosts, which also represents the lack of an HBV-specific T cell immune response to control active HBV replication.[3] By comparison, recent studies have demonstrated that children may harbor functionally active HBV-specific T cells.[33,34] These T cells may proliferate and produce more cytokines in children than in adult patients with CHB.[34] Significantly, the relatively short duration of viral antigen, for example, hepatitis B core-related antigen (HBcrAg) and HBsAg, exposure means HBV-specific T cells in younger children are less exhausted and depleted.[35,36] Those factors may be responsible for the higher cure rates observed in young children. In addition, compared with adults, children have higher proportions and absolute numbers of peripheral T and B lymphocytes, with peripheral lymphocyte subsets in children experiencing a significant transformation at around 4–6 years of age, considered a "physiological turning point" (Supplementary Fig. 4). However, whether these age-related factors contribute to the antiviral responses in patients with CHB requires further investigation.

This study had a number of limitations. First, owing to a lack of consensus on antiviral treatment in children with CHB having high-level viremia and normal or mildly elevated ALT levels, few parents or guardians want their children to receive antiviral treatment. Thus, enrolling more pediatric patients in a 3-year study period is difficult. Second, most children enrolled in this study had viral genotype B or C. As those genotypes are most common among Asian patients, the study findings may not be applicable to children in other ethnic populations and should be replicated worldwide. Third, while the antiviral regimens used in the present study were complex, their safety profiles in children have been confirmed by our previous experience and other clinical studies.[20–23] Finally, there was a lack of multiple cohorts

of children with CHB in this study. Thus, we decided to carry out this preliminary study at our own hospital. Despite the limitations, the real-world data reported here indicate that antiviral therapy is safe and efficacious in the treatment of children with CHB having high-level viremia and normal or mildly elevated ALT levels. While a previous study confirmed that IFN-α treatment may be more effective in younger children with CHB,[37] including different regimens within this real-world study (IFN-α monotherapy, IFN-α combined with NA, IFN-α add-on with NA) failed to draw a conclusion regarding which treatment regimen is the most effective. Conducting follow-ups every 3 months for the 16 pediatric patients who did not receive routine antiviral treatment was difficult, and visiting them was inconvenient, as they lived outside of Beijing. Therefore, they were only monitored for at most five serum HBV markers and HBV DNA at three times, baseline, at 12 or 24 months, and at the end of the 36-month study period).

In conclusion, our study demonstrated that children with CHB having high viremia and either normal or mildly elevated ALT levels may achieve a functional cure following antiviral treatment. In addition, our data suggest that the child's age and immune characteristics may influence the functional cure in the case of CHB infection. However, it is necessary to conduct a prospective, multicenter, randomized controlled trial with a larger sample size to further evaluate the efficacy of antiviral therapy in CHB. In addition, the immunological and virological mechanisms underlying the functional cure of CHB with antiviral treatments need to be clarified.

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Conflict of interest

FSW has been an executive associate editor of *Journal of Clinical and Translational Hepatology* since 2013. The other authors have no conflict of interests related to this publication.

Author contributions

Conception and design of the study (MZ, FSW), collection of the data (JL, PY), analysis and interpretation of data (PY, JL, ZQ, YD, FW, WG, JF, YJ, JG, LL, CZ, SS), writing of the manuscript (JL, PY, FSW) and revise the manuscript (FSW, MZ).

Ethical statement

Our study complied with the principles of the revised 2013 Declaration of Helsinki and was approved by the Medical Ethics Committee of the Fifth Medical Center of Chinese People's Liberation Army General Hospital (Ethical approval no. 2020048D).

Data sharing statement

All data are available upon request.

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