Incidence and Predictors of Relapse After Stopping Antiviral Therapy in Pediatric Chronic Hepatitis B

Piyush Upadhyay, MD,* Bikrant Bihari Lal†, MD, DM,* Vikrant Sood, MD, DM,* Rajeev Khanna, MD,* Ekta Gupta, MD,† Archana Rastogi, MD,‡ and Seema Alam‡, MD*

Background: There are no definite end-points for stopping therapy in pediatric chronic hepatitis B (CHB). The study objective was to evaluate the incidence of relapse after stopping antiviral therapy and to identify its predictors.

Methods: All hepatitis B surface antigen (HbsAg) positive children presenting to our hospital, who had been on antivirals for at least 2 years with undetectable hepatitis B virus-deoxyribonucleic acid (HBV-DNA) and normal alanine aminotransferase (ALT) on 3 consecutive occasions over last 12 months were included. Antivirals were stopped if liver biopsy showed histological activity index <5 and fibrosis (Ishak) <3. Virological relapse was defined as the elevation of HBV-DNA (>2000 IU/mL) and biochemical relapse as a rise in ALT levels to >2 times the upper limit of normal. Those having biochemical relapse were started on pegylated interferon alpha-2b-based sequential therapy.

Results: Of the 114 children with CHB screened, 31 HBsAg-positive children fulfilled inclusion criteria and antivirals were stopped in them. Virological and biochemical relapse was seen in 12 (38.7%) and 5 (16.1%) children within 12 months of stopping antiviral treatment. On Cox regression, hepatitis B e antigen (HBeAg) positive status at the time of stopping antiviral therapy (HR: 6.208, 95% CI: 1.630–23.638) and longer time taken for HBV-DNA to become undetectable while on antivirals (HR: 1.027, 95% CI: 1.000–1.055) were the independent predictors of relapse.

Conclusion: Discontinuation of antiviral treatment in children with CHB resulted in relapse in one-third of the patients. Relapse was frequent in those who were HBeAg-positive at the time of stopping therapy and in those who required longer therapy for HBV-DNA to become undetectable.

Key Words: ccc-DNA, biochemical relapse, children, virological relapse, HBeAg, pediatric hepatitis B, pegylated interferon, stopping rule, repeat liver biopsy, antiviral therapy

The goal of antiviral therapy in patients with chronic hepatitis B (CHB) is to improve survival by preventing the progression of liver damage to cirrhosis, end-stage liver disease, hepatocellular carcinoma (HCC) and death. Most children are in the stage of hepatitis B e antigen (HBeAg)-positive CHB infection (previously known as the immune-tolerant stage) and do not require therapy. Spontaneous HBeAg seroconversion is reported to occur in less than 2% per year among those younger than 3 years and 4–5% per year in older children. Current guidelines recommend treatment for children with HBeAg-positive chronic hepatitis, characterized by persistently elevated alanine aminotransferase (ALT). Tenofovir is the recommended antiviral in children older than 12 years and entecavir in children 2–12 years old. There are clear guidelines for stopping antiviral therapy in adults. In absence of hepatitis B surface antigen (HBsAg) loss, most guidelines recommend stopping antivirals only after at least 12 months of HBeAg seroconversion; and the presence of undetectable hepatitis B virus-deoxyribonucleic acid (HBV-DNA) with persistently normal ALT levels. After discontinuation of antivirals in adults, virological relapse (VR) has been reported in 42%–68%, whereas biochemical relapse (BR) has been reported in 21.7%–63% of patients on follow-up. There is no ambiguity about the decision to stop antiviral therapy in children who achieve a functional cure, defined as loss of detectable serum HBsAg with or without seroconversion to hepatitis B surface antibody. However, only 1.7%–8.3% of children treated with antivirals achieve this target of functional cure. There is no consensus about the duration of antiviral therapy in the vast majority who continue to remain HBsAg-positive. Initiation of antivirals at a very young age in children, coupled with a lack of definitive endpoint for stopping therapy, often implies an extremely long cumulative dose and duration of exposure to antivirals, thereby increasing the risk of adverse events and drug resistance. An extensive literature search failed to show studies designed to evaluate incidence and predictors of relapse after stopping antivirals in children. Therefore, we planned this pilot research to determine the incidence and identify risk factors of early relapse on stopping antiviral therapy in children with CHB.

METHODS

This was a prospective interventional study conducted at a tertiary care pediatric hepatology center in New Delhi over a period of 2 years (from January 2019 to December 2020). Approval was obtained from the institutional ethical committee vide letter no IEC/2019/67/MA-06. The study was conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Informed, written consent was obtained from the parents/guardians of the children. The criteria for stopping antivirals in the study were: (1) children between the ages of 2 and 18 years with CHB; (2) having received oral antivirals for at least 24 months; (3) with persistently normal ALT levels over the last 12 months (at least 3 consecutive ALT <1.5 times the upper limit of normal (ULN)); (4) undetectable HBV-DNA on 3 consecutive occasions in the last 12 months; and (5) histological activity index (HAI) <5, Ishak fibrosis stage <3 on liver histology. Normal ALT levels were defined as per the cutoffs obtained from the ‘SAFETY study’, which has defined separate cutoffs in healthy boys (25.8 IU/L) and girls (22.1 IU/L).
free of viral hepatitis or fatty liver disease.15 Children with a fam-
ily history of cirrhosis, HCC or co-infection with hepatitis C virus
and HIV were excluded. Liver Biopsy was performed to evaluate
the HAI and fibrosis as per the modified Ishak system.20 The details
of the HAI score and fibrosis staging have been described in Text,
Supplemental Digital Content 1, http://links.lww.com/INF/E752.
Children who had become HBsAg-negative did not undergo repeat
liver biopsy at the time of stopping therapy. Ultrasound-guided
percutaneous liver biopsy was performed through an intercostal
approach in a mid-axillary line using a Tru-Cut liver biopsy needle
(BARD Biopsy Systems, Peripheral vascular Inc. Mexico) (18 G
size, 10 cm needle length). The need for short general anesthesia
was decided on a case-to-case basis in younger children. Addition-
ally, quantitative estimation of covalently closed circular DNA
(ccc-DNA) was also performed in the liver tissue. ccc-DNA lev-
eels were measured by real-time polymerase chain reaction (PCR)
based in-house assay on lightcycler 480 (LC480) (Roche Diagnos-
tics, Almere, Netherlands) instrument using following prim-
ers: Forward primer: 5’-CTCCCCGTCTGTGCCCTTCT-3’; Reverse
Primer: 5’-GCCCCAAAGCCACCCAAG-3’. This is a qualitative
real-time PCR-based assay using sybergreen probes for the target
detection.21 Every run included a negative plasma sample, water and
positive control with a known concentration of ccc-DNA (10^6 copy-
es/mg). The results were then analyzed with LightCycler 480 soft-
ware v1.5.0.39 (Roche Diagnostics). Calculations for the amount of
ccc-DNA were carried out based on the standard curve plotted
with the dilutions made by using positive control. The results were
expressed in copies/mg of the liver biopsy tissue received after its
weight measurement. HBV-DNA was measured in the plasma sam-
ple by using an automated real-time PCR kit, COBAS Amplicor/
COBAS TaqMan, v2.0 (Roche Diagnostics, GmbH, Mannheim,
Germany). This is an automated hepatitis B viral load quantitative
assay needing 650 µL of serum or plasma, with the linear range of
quantification being 1.3–8.23 log_{10} IU/ml in all of them. HBsAg
quantification was performed by the chemiluminescent immunoas-
say (CLIA) method (Abbott Laboratories, Chicago, IL).22 HBeAg
was detected using a semi-quantitative chemiluminescence-based
assay which gives results in a signal to cutoff (S/Co) ratio, the
value of which is directly proportional to the amount of analyte
in the sample. The higher the S/Co value higher is the amount of
analyte in the sample. A S/Co ratio greater than 1 is reported as
HBeAg detected by the assay. Similarly, anti-HBe was analyzed
and reported using a semi-quantitative assay with S/Co ratio less
than 0.9 reported as the presence of anti-HBe in serum.
After stopping antivirals, the patients were followed up at 1
month, 3 months and every 3 months thereafter (Fig. 1). Workup
at each follow-up included: ALT, quantitative HBV-DNA (qHBV-
DNA), quantitative HBsAg (qHBsAg), HBeAg, alfa-fetoprotein
(AFp) and ultrasonography of the abdomen. Virological relapse
was defined as an elevation of qHBV-DNA >2000 IU/mL. Bio-
chemical relapse was defined as an elevation of ALT >2 times ULN.
The primary outcome measure of the study was to determine the
incidence of relapse after stopping antiviral therapy. A secondary
exploratory objective was to identify the predictors of relapse after
stopping antiviral therapy. The predictors studied included clinical
parameters, antivirals used, HBeAg seroconversion status, time
taken for HBV-DNA to become undetectable on therapy, HBV gen-
otype, ccc-DNA levels, duration of normal ALT before stopping
therapy and qHBsAg at the time of stopping antivirals. Those with
only VR were continued on regular follow-up whereas those with
VR as well as BR were treated with combination therapy: 8 weeks
of antivirals (entecavir in those younger than 12 years; tenofovir in

![FIGURE 1. Study Design. AFP indicates alfa-fetoprotein; ALT: Alanine aminotransferase; HBeAg: Hepatitis B e antigen; HBV-
DNA: Hepatitis B virus-deoxyribonucleic acid; HCC: Hepatocellular carcinoma; ccc-DNA: Circular covalently closed DNA; F: Fibrosis; HAI: Histological activity index; NA: Nucleos(t)ide analogue; Peg-IFN: Pegylated interferon; qHBV-DNA: Quantitative
HBV-DNA; ULN: Upper Limit of Normal; USG: Ultrasonogram.](https://www.pidj.com)
those older than 12 years), followed by the combination of peg-IFN alpha 2b (60 mcg/m²/week) for 48 weeks. Figure 1 depicts the study design and follow-up protocol.

**STATISTICAL ANALYSIS**

All analyses were performed with SPSS 22.0 (SPSS, Chicago, IL). As this was a pilot study, all the children fulfilling the inclusion criteria during the study period were enrolled. Continuous variables were expressed as median and inter-quartile range (IQR), whereas categorical variables were expressed as proportions. Both categorical and continuous variables were analyzed using Cox analysis. For identifying predictors of VR, univariate analysis was followed by Cox regression analysis. Variables which were significant predictors of VR on univariate analysis and had clinical relevance were entered in the Cox regression multivariate analyses for predictors of VR. Several models were evaluated with different combinations of variables based on their significance in univariate analysis, confounding factors, their relevance based on existing literature and preventing multicollinearity at the same time. A maximum of 1 variable was included in the models for every 5 events. Cox regression was performed using the forward (likelihood ratio) method. The variables remaining in the equation at the last step of regression analysis were deemed independent predictors of relapse. The strength of association was represented as hazard ratio (HR) with its 95% confidence intervals (CI). Receiver operating characteristic curves were made for independent continuous variables and the area under the receiver operating characteristic curve (AUROC) was calculated. Youden’s index was used to determine the best cutoff of the particular variable and sensitivity as well as specificity were determined at that cutoff.

**RESULTS**

A total of 114 CHB children were screened during the study period, of which 39 (34.2%) children satisfied the biochemical and virological criteria for inclusion in the study. Eight of them were HBsAg-negative; whose treatment was stopped without a repeat liver biopsy. Parents of all remaining 31 HBsAg-positive children gave consent for inclusion in the study, liver biopsy and consideration of stopping antiviral treatment. All 31 children had HAI <5 and fibrosis <3 in liver biopsy performed at the time of enrollment and all their parents agreed for close follow-up. Table 1 depicts the baseline clinical, biochemical and virological characteristics of these children at the time of enrollment in the study. The cohort predominantly consisted of children with genotype D (75%). All these children had HBeAg-positive chronic hepatitis B at the time of initiation of their antiviral therapy. Majorities were males (96.8%) with a median age of 15 years (IQR: 11.5–17 years). Thirteen (41.9%) were persistently HBeAg-positive (no HBe seroconversion) at the time of enrollment. The median ccc-DNA in the liver histology was 4 log, copies/ml (IQR: 3.1 log,–5.4 log,). None had advanced fibrosis either on histology or on transient elastography (TE) (median TE: 5 kPa (4.4–5.4)). These children had received oral antivirals for a median duration of 60 months (IQR: 36–78 months). They had a normal ALT on therapy for a median of 30 months.

**Relapse After Stopping Antivirals**

All 31 HBsAg-positive children enrolled in the study were followed up for a minimum of 12 months. Twelve of these 31 children (38.7%) developed VR. Five children (16.1%) additionally had a BR. Most (58.3%) of the VR occurred within a month of antiviral stoppage (Fig. 2). The BR was more common in those developing VR at 3 months or beyond after stopping antivirals. In 4 children, VR, as well as BR, were detected at the same time-point during follow-up (Fig. 2). Of the 7 children who developed only VR, 6 (85.7%) developed it within 1 month after stopping antiviral treatment. None of the 8 children with HBsAg-negative status developed VR or BR until 12 months of follow-up.

**Risk Factors for Development of Relapse**

On univariate analysis, HBeAg-positive status at the time of stopping therapy (HR: 7.058, 95% CI: 1.825–27.29, P = 0.005), semi-quantitative HBeAg (HR: 1.002, 95% CI: 1.001–1.003, P < 0.001), semi-quantitative anti-HBe (HR: 1.025, 95% CI: 1.003–1.049, P = 0.028) and longer time taken for qHBV-DNA to become undetectable on antiviral treatment (HR: 1.024, 95% CI: 1.003–1.046, P = 0.028) were the significant predictors of VR after stopping antiviral therapy (Table 2). Various models were evaluated on Cox multivariate regression analysis using several combinations of variables which were either significant on univariate analysis (HBeAg-positive at the time of stopping therapy, semi-quantitative HBeAg, semi-quantitative anti-HBe and time taken for qHBV-DNA to become undetectable) or deemed clinically important based on literature review (quantitative HBsAg and ccc-DNA on liver tissue); while at the same time avoiding multicollinearity (Table 3). HBeAg-positive status and semi-quantitative HBeAg were not entered together in any model to prevent confounding. HBeAg-positive status at the time of stopping therapy was a strong independent

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**TABLE 1. Baseline Clinical and Laboratory Profile of the 31 HBsAg-Positive at the Time of Stopping Antiviral Treatment**

| Parameter | Value |
|-----------|-------|
| Age (median[IQR]) | 15 years(11.5–17) |
| Gender Male | 30 (96.8%) |
| | Female | 1 (3.2%) |
| HBeAg status at the time of enrollment Reactive | 13 (41.9%) |
| | Nonreactive | 18 (58.1%) |
| ALT at the time of enrollment | 30 IU/L (23–35) |
| Liver histology findings | |
| Histological activity index | 0–1 20 (64.5%), 2–3 11 (35.5%), >3 0 |
| Fibrosis | 1 14 (45.2%), 2 13 (41.9%), >2 4 (12.9%), >3 0 |
| Liver stiffness by transient elastography | 5 kPa (4.4–5.4) |
| Genotype (n = 24) | |
| Genotype D | 18 (75%) |
| Genotype A | 4 (16.7%) |
| Genotype C | 2 (8.3%) |
| Duration of antiviral therapy (median[IQR]) | 60 months(36–78) |
| Previous sequential therapy with peg–IFN | 13 (41.9%) |
| Duration of antiviral treatment postsequential therapy[median[IQR]] | 36 months(27–54) |
| Time taken for qHBV-DNA to become undetected during antiviral therapy[median[IQR]] | 32 months(16–49) |
| Duration of normal ALT on antiviral therapy [median[IQR]] | 30 months(15.8–31) |

ALT indicates alanine aminotransferase; ccc-DNA, covalently closed circular DNA; HBsAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; IQR, inter-quartile range; qHBV-DNA, quantitative HBV-DNA; Peg-IFN, pegylated interferon.
Treatment Withdrawal in Pediatric Hepatitis B

The predictor of relapse in all the models evaluated (Table 3). Time taken for qHBV-DNA to become undetectable was an independent predictor of VR in models 1 and 4 (Table 3). In model 3, we evaluated a semi-quantitative HBeAg along with semi-quantitative anti-HBe and time taken for HBV-DNA to become undetectable. In this model, only semi-quantitative HBeAg was the independent predictor of VR. qHBsAg, ccc-DNA in liver tissue and semi-quantitative anti-HBe were not significant predictors of VR.

**FIGURE 2.** Pattern of relapse after stopping antiviral therapy in children with chronic hepatitis B. HBeAg indicates Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen.

| Variable | Relapse (n = 12) | No Relapse (n = 19) | Hazard ratio (95% CI) | P Value |
|----------|-----------------|--------------------|-----------------------|---------|
| Age at enrollment (years) [median (IQR)] | 16 (13.5–17) | 13 (10–17) | 1.129 (0.96–1.326) | 0.140 |
| qHBsAg (Log10) [median (IQR)] | 3.84 (3.15–4.41) | 3.71 (2.92–4.19) | 1.465 (0.806–2.66) | 0.211 |
| HBeAg-positive at time of stopping therapy n (%) | 9 (75%) | 4 (21%) | 7.058 (1.825–27.29) | <0.001 |
| Semi-quantitative HBeAg (sample-cutoff ratio) | 785.3 (4.8–1431) | 0.47 (0.28–7.3) | 1.002 (1.001–1.003) | <0.001 |
| Semi-quantitative Anti-HBe (sample-cutoff ratio) | 40.7 (2.15–53.35) | 0.23 (0.02–10.8) | 1.002 (1.003–1.003) | 0.028 |
| Duration of antiviral treatment (months) [median (IQR)] | 60 (36–78) | 48 (32–72) | 0.960 (0.905–1.019) | 0.136 |
| History of sequential treatment, n (%) | 5 (41.6) | 8 (42.1) | 0.982 (0.227–4.251) | 1.000 |
| Duration of normal ALT on antivirals (months) [median (IQR)] | 16 (13.5–33) | 32 (19.5–48) | 0.979 (0.992–1.029) | 0.07 |
| Time taken for qHBV-DNA to become undetectable (months), [median (IQR)] | 46 (23–64) | 24 (11–39) | 1.024 (1.003–1.046) | 0.028 |
| Duration since HBeAg seroconversion (months) [median (IQR)] | 24 (12–28) | 24 (12–36) | 1.103 (0.417–2.919) | 0.843 |
| ccc-DNA in liver (log10) copies/ml, [median (IQR)] | 4.19 (3.5–6.99) | 3.2 (2.5–5.1) | 1.176 (0.893–1.550) | 0.248 |

ALT indicates alanine aminotransferase; ccc-DNA: Circular covalently closed circular deoxyribonucleic acid; CI, confidence interval; IQR, inter-quartile range; HBeAg, hepatitis B e antigen; qHBsAg, quantitative HBsAg; qHBV-DNA, quantitative hepatitis B virus-deoxyribonucleic acid.
on multivariate analysis. A longer time is taken (more than 22.5 months) for qHBV-DNA to become undetectable predicted VR with a sensitivity of 83.3% and specificity of 47.4% (AUROC: 0.737, 95% CI: 0.56–0.931, P = 0.029) (see Figure, Supplemental Digital Content 2; http://links.lww.com/INF/E753). Children who were HBeAg-positive at the time of stopping therapy were at significantly higher risk of relapse as compared to those who were HBeAg-negative (log-rank 10.705, P = 0.001) (see Figure, Supplemental Digital Content 3; http://links.lww.com/INF/E754). In the present study, 69.2% HBeAg-positive children relapsed within 12 months compared to 16.7% of those with HBeAg-negative status relapsed on antiviral withdrawal.

Most of the guidelines in adult hepatitis B recommend stopping antivirals after 12–36 months of HBe seroconversion with undetectable HBV-DNA and persistently normal ALT levels. There are no studies specifically designed to evaluate the timing and criteria for stopping antiviral therapy in children. Very low HBSAg serocconversion (1.7–8.3%) and HBeAg serocconversion (21–31.3%) rates observed in children on antivirals imply longer, often indefinite antiviral therapy. Longer duration of therapy increases the cumulative risk of adverse events as well as the risk of development of antiviral resistance. The present study provides some insights into patient selection for stopping antivirals in children who fail to achieve HBSAg serocconversion. VR rate of 38.7% and BR rate of 16.1% at 12 months observed in children in our study is consistent with relapse rates observed in adults after antiviral discontinuation. The VR rates reported have varied from 42% at 3 months to 68% at 6 years of follow-up, whereas BR rates have varied from 21.7% at 3 months to 63% at 6 years of follow-up after antiviral discontinuation.

HBeAg-positive status at the time of stopping therapy and the longer time taken for qHBV-DNA to become undetectable were the most important predictors of relapse in the present study. The optimal endpoint for stopping therapy would be the loss of HBSAg, often termed ‘functional cure’ but it is only rarely achievable in children. Short of it, HBeAg serocconversion, coupled with HBV-DNA loss and ALT normalization characterizes a state of good immune control with a low replicative phase of the disease. The high rates of early relapse (69.2%) seen in HBeAg-positive children in the present study suggest that treatment withdrawal should not be attempted in the absence of HBeAg serocconversion. The important predictors of relapse after stopping antivirals in adults include (1) higher qHBsAg level at the time of stopping the therapy; (2) shorter duration of consolidation therapy; (3) slower HBV-DNA decline while on therapy; (4) baseline pretreatment HBV-DNA; (5) hepatitis B core-related antigen (HBcAg); and (6) hepatitis B virus-ribonucleic acid (HBV-RNA). The maximum

**DISCUSSION**

VR was seen in 38.7% and BR in 16.1% of HBsAg-positive children within 12 months of stopping their antivirals. Most cases of relapse occurred within a month of stopping the therapy. None of the HBsAg-negative children developed relapse on follow-up. HBeAg-positive status at the time of stopping therapy and longer time taken for qHBV-DNA to become undetectable while on therapy were the independent predictors of relapse. While 69.2% of HBeAg-positive children developed VR; only 16.7% of those with HBeAg-negative status relapsed on antiviral withdrawal.

**TABLE 3.** Various Models Evaluated by Cox multivariate Analyses to Identify Risk Factors for Development of Virological Relapse after Stopping Antiviral Treatment in Children with Chronic Hepatitis B

| Variables | Beta coefficient | Wald | Significance | Hazard ratio | 95% CI for Hazard ratio |
|-----------|-----------------|------|--------------|--------------|------------------------|
| Model 1   |                 |      |              |              |                        |
| HBeAg-positive | 1.826 | 7.163 | 0.007 | 6.208 | 1.630 | 23.638 |
| Time taken for qHBV-DNA to become undetectable (months) | 0.027 | 3.936 | 0.047 | 1.027 | 1.000 | 1.055 |
| Model 2   |                 |      |              |              |                        |
| HBeAg-positive | 1.942 | 4.896 | 0.026 | 6.971 | 1.628 | 38.325 |
| Duration of normal ALT | 0.187 | 3.46 | 0.047 | Variable not in equation | Variable not in equation |
| Model 3   |                 |      |              |              |                        |
| Semi-quantitative HBeAg (sample-cutoff ratio) | 0.002 | 12.686 | <0.001 | 1.002 | 1.001 | 1.003 |
| Semi-quantitative anti-HBe (sample-cutoff ratio) | 0.488 | Variable not in equation | Variable not in equation |
| Time taken for qHBV-DNA to become undetectable(months) | 0.096 | Variable not in equation |
| Model 4   |                 |      |              |              |                        |
| HBeAg-positive | 1.826 | 7.163 | 0.007 | 6.208 | 1.630 | 23.638 |
| Quantitative HBsAg | 0.330 | Variable not in equation |
| Model 5   |                 |      |              |              |                        |
| HBeAg-positive | 1.994 | 6.335 | 0.012 | 7.345 | 1.555 | 34.698 |
| Log ccc-DNA in liver tissue | 0.757 | Variable not in equation |

ccc-DNA indicates Circular covalently closed circular deoxyribonucleic acid; CI, confidence interval; HBeAg, hepatitis B e antigen; qHBsAg, quantitative HBsAg; qHBV-DNA, quantitative hepatitis B virus-deoxyribonucleic acid. log, logarithm.

**FIGURE.** Treatment of Children with Relapse

Four (80%) of the 5 children with BR were treated with sequential therapy. The fifth child only had a transient BR; hence, he was treated with antivirals alone. Of the 4 children who were treated with sequential therapy, 3 were HBeAg-positive whereas 1 was HBeAg-negative. None of the 3 HBeAg-positive children showed HBeAg serocconversion at 1 year of therapy. Two of them achieved virological clearance (qHBV-DNA undetectable). All 4 achieved normalization of their ALT levels at 1 year of therapy. The rest of the patients with only VR are being continued on strict follow-up every 3 months for timely identification of BR and possible re-treatment.
emphasizes on qHBsAg levels at the time of stopping therapy and recently Asia Pacific Association for Study of Liver Diseases has suggested less than 100 IU/mL as a safe endpoint for stopping long term antivirals.23 qHBsAg levels were not significantly higher in the patients who relapsed in the present study either on the univariate or in the multivariate model. HBCrAg and HBV-RNA are among the newer serological markers predicting relapse either singly or in combination.24 We evaluated the role of liver tissue ccc-DNA which although higher in relapse was not statistically different. This could be due to the smaller sample size and would need evaluation in larger multicentric studies. Recently, Lai et al51 investigated the effect of antiviral withdrawal in patients with undetectable ccc-DNA in the hepatocytes in adults. All 13 patients with undetectable ccc-DNA randomized to stop therapy had VR. Although either of HBeAg-positive status or a quantitative HBeAg could be used to predict relapse, it may be more prudent to use qualitative HBeAg as many centers may not have the facility for doing a quantitative assay.

The main strength of our study is the pilot effort in determining the relapse rate and its risk factors in a carefully curated cohort of children with CHB. Even though our study period coincided in part with the COVID-19 pandemic which could have limited the enrollment, we managed to ensure timely follow-up of each enrolled patient using online consultation wherever necessary. Our study is limited by a small sample size being a single-center study. Although limited by a short follow-up of 12 months, we continue to follow-up these patients for timely identification and treatment of new relapses. We also could not identify the predictors of BR due to small number of patients (n = 5) developing BR. The predominance of boys in the study is a limiting factor and unfortunately reflects the health-seeking behavior of the population in this part of the world. The predominance of genotype D in our cohort is representative of the population. To conclude, virological and biochemical relapse rates were 38.7% and 161.1%, respectively within 12 months of stopping antivirals in HBsAg-positive children with most relapses occurring within a month of stopping therapy. HBeAg-positive status at the time of stopping therapy and the longer time required for HBV-DNA to become undetectable while on therapy are important predictors of relapse after stopping antivirals.

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