Predictive factors of lamivudine treatment success in a hepatitis B virus-infected pediatric cohort: A 10-year study

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BACKGROUND: Hepatitis B virus (HBV) infections are responsible for the development of chronic hepatitis in 400 million people worldwide. Currently, no consensus exists as to when treatment should be initiated for pediatric patients.

OBJECTIVES: To evaluate the risks and predictive factors of success of lamivudine treatment in children with chronic, active HBV infection.

METHODS: Forty-three children (22 male, median age 9.6 years) chronically infected with HBV and treated between 1998 and 2008 at CHU Ste-Justine (Montreal, Quebec) were included in the present chart review study. Inclusion criteria were detectable hepatitis B surface antigen and hepatitis B e antigen (HBeAg), minimum serum alanine aminotransferase (ALT) level of two times the upper limit of normal and detectable serum HBV DNA for at least three months. Patients received lamivudine for a minimum of six months (median 14 months). Genotyping was performed.

RESULTS: Lamivudine treatment was effective in 35% of cases (15 of 43) and overall virological response (during or after treatment) was achieved in 51% of patients. Three patients harboured suspected lamivudine-resistant mutations and five progressed to HBeAg-chronic HBV. Predictive factors for success of treatment were: younger age at beginning of treatment (P=0.05), elevated ALT levels throughout treatment duration (P=0.003) and loss of HBeAg during treatment (P=0.016). Asian origin did not affect treatment success or spontaneous viral control during follow-up. HBV genotype did not influence treatment success.

CONCLUSIONS: Lamivudine treatment in a carefully selected cohort of HBV patients demonstrated a good rate of success and low incidence of mutation. Younger age at the beginning of treatment and high ALT levels during treatment predicted a positive outcome.

Key Words: *Hepatitis B; Lamivudine; Pediatric; Predictive success factors*

Les facteurs prédictifs de réussite du traitement à la lamivudine chez une cohorte de patients pédiatriques infectés par le virus de l'hépatite B : une étude sur dix ans

HISTORIQUE : Les infections par le virus de l'hépatite B (VHB) sont responsables de l'apparition d'hépatite chronique chez 400 millions de personnes de par le monde. Il n'existe pas de consensus quant au moment d'amorcer le traitement chez les patients pédiatriques.

OBJECTIFS : Évaluer les risques et les facteurs prédictifs de réussite du traitement à la lamivudine chez les enfants atteints d'une infection active par le VHB chronique

MÉTHODOLOGIE : Quarante-trois enfants (22 garçons, âge médian de 9,6 ans) atteints d'une infection chronique par le VHB et traités au CHU Sainte-Justine (Montréal, Québec) entre 1998 et 2008 ont participé à la présente étude de dossiers. Les critères d'inclusion étaient un antigène de surface de l'hépatite B et un antigène e de l'hépatite B (HBeAG) décelables, un taux d'alanine-aminotransférase sérique (ALT) deux fois plus élevé que la normale supérieure et un ADN du VHB décelable dans le sérum pendant au moins trois mois (médiane de 14 mois). Les chercheurs ont procédé à un génotypage.

RÉSULTATS : Le traitement à la lamivudine était efficace dans 35 % des cas (15 cas sur 43) et on observait une réponse virologique globale (pendant ou après le traitement) chez 51 % des patients. Trois patients présentaient des mutations qu'on présumait découler d'une résistance à la lamivudine et cinq ont évolué vers une VHB chronique HBeAg-positive. Les facteurs prédictifs de réussite du traitement étaient un plus jeune âge en début de traitement (P=0,05), un taux d'ALT élevé pendant toute la durée du traitement (P=0,003) et une perte de l'HBeAg pendant le traitement (P=0,016). L'origine asiatique n'avait pas d'influence sur la réussite du traitement ou sur le contrôle viral spontané pendant le suivi. Le génotype du VHB n'avait pas d'incidence sur la réussite du traitement.

CONCLUSIONS : Le traitement à la lamivudine dans une cohorte de patients atteints du VHB soigneusement sélectionnée s'associe à un fort taux de réussite et à une faible incidence de mutation. Un âge plus jeune en début de traitement et des taux d'ALT élevés pendant le traitement étaient indicateurs d'une issue positive.

Hepatitis B virus (HBV) infections are responsible for the development of chronic hepatitis in 400 million people worldwide and 600,000 in Canada (1,2). In most cases, patients acquired the infection during childhood (1). It has been estimated that approximately 25% of chronically infected individuals, who contracted HBV in childhood, develop complications in adulthood such as cirrhosis and hepatocellular carcinoma and die of liver-related complications (3,4). In addition to the risk of health complications, chronic HBV infections in childhood carry the risk of sexual transmission as children grow older and become sexually active.

These risks of viral transmission and long-term health complications call for prevention through education and close clinical monitoring, but also for viral control with optimized therapeutic strategies. Currently, drugs licensed for the treatment of HBV chronic infection in pediatric populations are interferon-alpha (INF-alpha) and lamivudine (LAM), which have similar hepatitis B e antigen (HBeAg) seroconversion rates (5-7). INF therapy has the advantage of finite duration of treatment, lack of acquired drug resistance and higher likelihood of hepatitis B surface antigen (HBsAg) clearance over LAM. However, INF use is associated with many adverse effects

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including derangement of weight gain and growth, discomfort on administration and flu-like syndrome (5). LAM is known to induce drug-resistant mutations (7-9) and the ideal duration of the treatment remains unclear; however, it is administered orally, better-tolerated and safer (5,8).

To date, no consensus exists as to if and when treatment should be initiated in pediatric patients. HBeAg seroconversion is more sustainable and HBV recurrence occurs less frequently in childhood than in adult patients (5,10,11). Children with chronic HBV infections are often in an immune tolerance phase and do not benefit from treatment (12). Actual knowledge suggests that only children with immune active disease should undergo treatment (5,7,13,14).

An important part of our knowledge concerning the pediatric response to LAM treatment has come from one, extensively studied, single placebo-controlled cohort (7,14-16). Studies on different cohorts have validated and expanded these findings as well as their applicability to different populations (5,13,17). The aim of our study was to evaluate the risks and predictive factors of LAM treatment success in a Canadian pediatric population with chronic HBV infection who were in an immune-active phase.

METHODS

Population

The present retrospective study reviewed the charts of all children with chronic HBV infection and treated with LAM at St Justine Children's Hospital in Montreal, Quebec, between July 1998 and July 2008, who fulfilled the inclusion criteria. During this period, 43 children (22 male) received LAM treatment and fulfilled the following inclusion criteria: detectable HBsAg and HBeAg (for at least three months), minimum serum alanine aminotransferase (ALT) level of two times the upper limit of normal (ULN; normal 25 IU/mL) and detectable serum HBV DNA for at least three months. All other causes of elevated ALT levels were discarded at inclusion. The median age at beginning of treatment was 9.6 years (range 1.1 to 18.4 years). HBV infection occurred in more cases vertically (or perinatally) (86%) than were horizontally transmitted (14%). No patients were coinfected with either hepatitis C virus or HIV; one patient was infected with hepatitis D virus. Two patients received IFN-alpha before the study. The present study was approved by the institutional ethical committee of CHU Sainte-Justine and was conducted in conformity with the guidelines for human experimentation.

Study design

All patients received 3 mg/kg/day to a maximum of 100 mg of LAM orally daily. The duration of treatment was determined on a case-by-case basis (median 14 months, range five to 38 months). Treatment was discontinued if: the physician determined that treatment was successful (see definitions), a LAM-resistant mutation occurred or treatment was unsuccessful. Patients were assessed every 12 weeks after initiation of therapy for biochemical markers of liver disease and HBV serological markers: serum HBV DNA according to either a hybrid capture assay (Digene, Qiagen, USA [detection limit 1030 UI/mL]) if assessed before 2005, or HBV test (COBAS AmpliPrep/COBAS TaqMan HBV Test (Roche, USA [detection limit 20 UI/mL]) if assessed after 2005, and HBsAg and HBeAg by automated immunochemical assay (AxSYM, Abbot Laboratories, USA). Thirty-three patients were biopsied before the beginning of LAM treatment (48 h to seven days before treatment) and assessed blindly at the end of the study by a single pathologist using the METAVIR algorithm (18). There were no reports of lack of adherence to therapy and no withdrawals. Patients were followed post-treatment for a mean (± SD) of 48.5±32.2 months, eight were lost to follow-up before the end of treatment. Treatment risk, such as development of virus mutations or adverse effects, were evaluated based on clinical data (see definitions).

Definitions

HBV virological response was defined as a loss of HBeAg and detectable serum HBV DNA (7,15). Seroconversion-e implied a virological response along with normalization of ALT level and acquisition of hepatitis B e antibody (HBeAb) (5,7,15). A successful outcome was defined as sustained normalization of ALT levels and HBV virological response during the LAM treatment period and follow-up (17). LAM-resistant mutations were suspected in patients whose HBV DNA concentrations declined then subsequently increased with no decline in HBeAg and no appearance of HBeAb. Precore mutations were suspected in HBe-seroconverted patients who maintained increased levels of HBV DNA (patients progressing to HBeAg-negative chronic HBV).

Genotyping

Viral DNA was prepared from 200 µL of frozen serum using the QIAamp MinElute Virus Spin kit (Qiagen, USA) according to manufacturer's instructions or by microwave technique (19). HBV DNA was amplified by polymerase chain reaction (PCR) using HBV primers for the *S* gene (20). In cases where an amplicon was not visible on an agarose gel, a nested PCR was performed using forward primer position 302 to 319, and reverse primer position 741 to 760. Genotyping was performed on PCR products by restriction fragment length polymorphism using *TasI* and *HinfI* restriction enzymes (Fermentas, Canada) (20).

Statistical analysis

All statistical analyses were performed using SPSS version 17.0 (IBM Corporation, USA). All statistical tests (χ^2, Fisher's exact test, unpaired *t* test and ANOVA) for comparison between success and failure of treatment groups and resulting P values were two tailed. Kaplan-Meyer survival curves were used to illustrate cumulative virological response in different groups, and groups were compared using the Mandel-Cox log-rank test. $P \leq 0.05$ was considered to be statistically significant.

RESULTS

Efficacy of treatment in the studied population

Baseline demographic and clinical characteristics of chronically HBV-infected children treated with LAM are summarized in Supplemental Table 1. Approximately one-half of the children were of Asian descent (22 of 43). Median serum ALT level at the beginning of treatment (highest value within six months of the beginning) was 3.7×ULN (mean 7×ULN±8.4; range 2×ULN to 43×ULN). The highest value within the six months preceding treatment was used to assess whether the serum ALT levels were a predictive factor of response (13). The median serum HBV DNA level at baseline was 7.64 $\log_{10}$. Based on liver biopsy data, patients had a median grade of liver inflammation of 1 and median stage of liver fibrosis score of 1 at baseline. One patient received prednisone and mycophenolate mofetil during the course of treatment.

Fifty-one per cent of patients (22 of 43) achieved a virological response over the course of the study period (mean follow-up 48.5±32.2 months). Thirty-five per cent of all patients (15 of 43) achieved this response while under LAM therapy (period of treatment: median 14 months, range five to 38 months) and were thus considered to have a successful response to treatment (Table 1). The mean time to response to LAM therapy was 10.1 months (Figure 1). For the 15 patients who experienced a successful response to LAM-treatment: four achieved virological response within the first six months of treatment, six reached this response between six and 12 months of treatment and five more in the second year of treatment (Supplemental Figure 1). After 12 months of treatment, 23% of all patients (10 of 43) had already achieved virological response (Supplemental Figure 1). When patients achieved a virological response after the first year of treatment, LAM was discontinued. LAM therapy was continued for a second year in 14 patients. Of these, five (33%) experienced a successful response to treatment during this period. After stopping LAM treatment, 50% of patients who experienced a poor outcome to treatment achieved spontaneous viral control within 3.5 years (seven of 28).

TABLE 1
Overall effect of lamivudine treatment

Treatment success	
No	28 (65.1)
Yes	15 (34.9)
Overall virological response	
No	21 (48.8)
Yes	22 (51.2)
Seroconversion-e	
No	17 (39.5)
Yes	24 (55.8)
Transient	2 (4.7)
Seroconversion-s	
No	37 (86.0)
Yes	6 (14.0)
Suspected lamivudine-resistance mutation	
No	40 (93.0)
Yes	3 (7.0)
Suspected precore mutation (HBeAg-chronic HBV)	
No	38 (88.4)
Yes	5 (11.6)

Data presented as n (%). HBeAg Hepatitis B e antigen

Over a mean follow-up of more than four years, virological response obtained with LAM treatment was sustained in all patients except one. This patient experienced a virological recurrence with elevation of HBV DNA levels one year after treatment while maintaining seroconversion-e ($HBeAg^-$, anti-HBe^+). This patient was suspected to have developed a precore mutation. The two patients who had received previous HBV treatments (IFN-alpha) both experienced a sustained virological response and seroconversion-e but this response occurred during LAM treatment in only one of the two. The number of non-naive patients who previously underwent LAM treatment was too small to draw any conclusions with respect to their response to LAM treatment.

Safety and LAM-associated resistance mutations

No safety problems or serious adverse effects associated with LAM therapy occurred in the present cohort of patients. LAM was well tolerated with no reported withdrawals. LAM-associated resistance mutations (such as YMDD mutations) were suspected in three patients (11%). Furthermore, three of the children progressed to HBeAg-negative chronic HBV (suspected precore mutation) and went on to achieve virological response (two under treatment and one spontaneously) (Table 2). HBV genotype was not associated with LAM-resistant mutant occurrence (one of genotype B, one of genotype D and one of unknown genotype [P=0.742, χ^2 test]) or precore mutation (two patients infected with genotype C, two with genotype D and one unknown [P=0.101, χ^2 test]).

Predictive factors of success at beginning of treatment

Contrary to previous studies that reported a delayed virological response to LAM treatment in Asian populations (21,22), in the present study cohort, Asian descent (50% of patients) was not associated with a reduced rate of success or with unusual virological response kinetics (spontaneous or due to treatment) (Figure 2).

Age at the beginning of treatment was the only factor significantly associated with treatment outcome (Table 2). In fact, younger age at the beginning of treatment led to a more favourable response to treatment (P=0.05 [unpaired two-tailed *t* test]). A Spearman negative correlation of −0.307 (P=0.045) was found between age at the beginning of treatment and success of treatment.

Other potential predictive factors, such as ALT levels and liver inflammation, were not found to be significantly associated with treatment success (P=0.565 and P=0.425 [unpaired *t* tests], respectively).

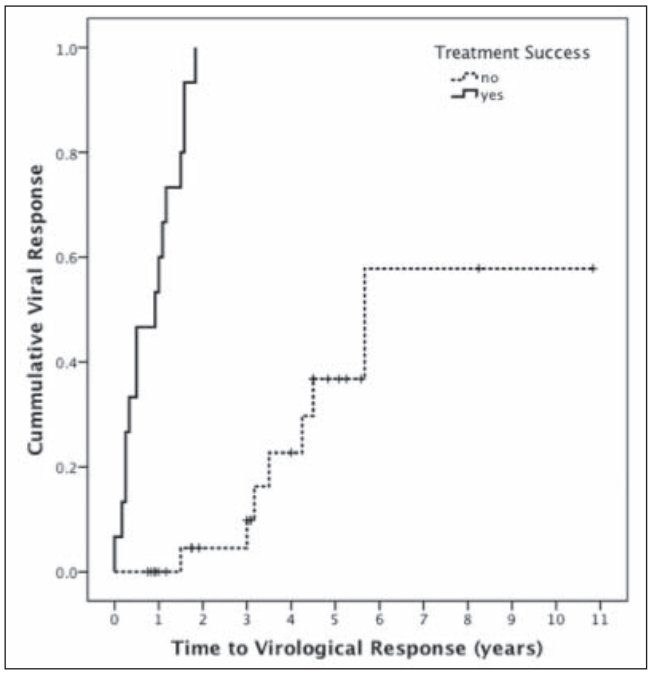

Figure 1) *Hepatitis B virus (HBV)-infected children who experienced successful treatment with lamivudine achieved virological response in a short period of time. Cumulative proportion of children achieving virological response (loss of hepatitis B e antigen and loss of detectable serum HBV DNA) with regard to success of treatment calculated by Kaplan-Meyer analysis. Censored data are depicted. (n=43, P<0.001 [Mandel-Cox log-rank test])*

However, a tendency was observed for fibrosis score before treatment, with higher scores in patients who achieved a virological response during treatment (P=0.085 [unpaired *t* test]). HBV genotype was not associated with treatment outcome.

Serological markers during treatment

A statistically significant diminution of HBV DNA load was observed during treatment in patients with treatment success and failure (P=0.0245 and P=0.0218, respectively) (Figure 3A). At three months, patients with a positive outcome lost a mean of 2.88 $\log_{10}$ of HBV DNA, whereas patients who did not achieve virological response with LAM lost a mean of 2.25 $\log_{10}$ (P=0.263, unpaired two-tailed *t* test). At the end of treatment, HBV DNA was significantly lower in patients who successfully responded to treatment (P=0.0001 [n=31]).

HBeAg loss during treatment was significantly higher in the treatment success group at three months (n=31, P=0.016 [Fisher's exact test]) and at the end of treatment (n=26, P<0.0001 [Fisher's exact test]). Nine patients experienced a loss of HBsAg (21% of all patients), of whom eight achieved virological response during treatment, representing 53% of the treatment success group. Of the nine patients who seroconverted, two experienced transient losses. Of the seven patients who had a sustained loss, two lost HBsAg during treatment and five in follow-up years (Table 2).

Although there was no marked difference between ALT levels at baseline (n=36, P=0.565), patients who successfully responded to treatment had consistently higher ALT levels throughout treatment (three months P=0.009 [n=35]; six months P=0.003 [n=29]; nine months P=0.049 [n=18]) (Figure 3B). Patients with a positive outcome showed a tendency toward lower ALT values (P=0.151 [n=25]).

DISCUSSION

Although there are few treatment options for chronic HBV infection in pediatric patients, LAM is one of the treatments appropriate for this patient population. Previous studies on pediatric response to LAM

TABLE 2
Demographic, biochemical and viral characteristics of patients with treatment failure and successful response to treatment

	Effective treatment			
	No	Yes	Total	P (statistical test)
Sex				
Male:female	16:12	6:9	22:21	0.284 (χ^2 test); 0.347 (Fisher's exact test)
Transmission, %				
Horizontal	3	3	6 (14)	0.402 (χ^2 test); 0.647 (Fisher's exact test)
Vertical (or perinatal)	25	12	37 (86)	
Suspected YMDD mutation, n (%)	3 (11)	0 (0)	3 (7)	0.189 (χ^2 test); 0.541 (Fisher's exact test)
Suspected precore mutation (%)	3 (11)	2 (13)	5 (11.6)	0.798 (χ^2 test); 1.000 (Fisher's exact test)
Liver inflammation score, mean ± SD	1.1±0.6	1.3±0.7		0.425 (*t* test)
Liver fibrosis score, mean ± SD	1.3±0.9	1.9±0.9		0.085 (*t* test)
Descent, n (%)				
East Asian	15 (54)	7 (46)	22 (51)	0.529 (χ^2 test)
African	2 (7)	1 (7)	3 (7)	
European	0 (0)	2 (13)	2 (4.7)	
Canadian	6 (21)	3 (20)	9 (21)	
Latin and South American	2 (7)	1 (7)	3 (7)	
Bangladeshi, Indian, Pakistani	1 (4)	1 (7)	2 (4.7)	
Russian	2 (7)	0 (0)	2 (4.7)	
Asian verus non-Asian, n (%)				
Asian	15 (54)	7 (47)	22 (51)	0.666 (χ^2 test); 0.755 (Fisher's exact test)
Non-Asian	13 (46)	8 (53)	21 (49)	
Hepatitis, n (%)				
A	2 (10)	0 (0)	2 (4.7)	0.742 (χ^2 test)
B	8 (38)	2 (29)	10 (23.3)	
C	3 (14)	1 (14)	4 (9.3)	
D*	8 (38)	4 (57)	12 (27.9)	
Serum ALT (× upper limit of normal), mean ± SD	6.5±6.2	8.2±11.9		0.565 (*t* test)
Serum HBV DNA, $\log_{10}$, IU/mL, mean ± SD				
Before treatment	7.66±0.84	7.11±2.17		0.263 (ANOVA)
3 months in treatment	5.17±1.51	4.31±2.23		0.214 (ANOVA)
6 months in treatment	5.02±1.03	4.57±1.80		0.389 (ANOVA)
9 months in treatment	4.69±1.06	4.87±0.30		0.714 (ANOVA)
At end of treatment	6.05±1.59	3.01±2.26		0.000I (ANOVA)
HBeAg positivity, n (%)				
Before treatment	19 (95)	8 (80)		0.197 (χ^2 test); 0.251 (Fisher's exact test)
End of treatment	14 (77)	0 (0)		<0.0001 (χ^2 test) (Fisher's exact test)
HBsAg loss, n (%)	1 (transient) (3)	8 (1 transient) (53)		<0.0001 (χ^2 test); 0.0003 (Fisher's exact test)
Age, years, mean ± SD	10.7±5.1	7.5±4.9		0.050 (*t* test)
Length of treatment, mean ± SD	17.3±7.9	16±5.8		0.786 (*t* test)
Follow-up, months, mean ± SD	48.6±32.1	48.3±33.4		0.971 (*t* test)
Time necessary to respond to treatment, months				
Mean		10.1		
Median		11.0		
SD		7.2		

**Restriction fragment length polymorphism analysis cannot distinguish between hepatitis virus (HBV) D3 and HBV E; therefore, all patients in this category have been classified as D. HBeAg Hepatitis B e antigen; HBsAg Hepatitis B surface antigen*

treatment have resulted in recommendations of who to treat (7,14-16). Consequently, we selected our population for the present study following recommendations outlined by Sokal et al (7), thereby focusing on patients in an immune active phase with ALT levels of at least 2×ULN. Clearance of HBV infection is rarely achievable with currently available drugs, hence, our goal for therapy was based on surrogate markers previously associated with favourable long-term prognosis (4): seroconversion of HBeAg to HBeAb, reduction of HBV DNA to undetectable level and normalization of ALT levels.

The present study reports a treatment success rate of 24% after 12 months of treatment, and 35% at the end of treatment (regardless of treatment duration). These results are similar to the treatment success rate of 23% after 12 months (15) and 30% after 24 months (7) reported in previous studies. The rate of seroconversion-e achieved in our cohort in the second year of treatment reached 36%. Undergoing a second year of treatment was, therefore, beneficial for one-third of our patients; however, LAM-resistant mutations developed only in patients who underwent a prolonged period of treatment (21% of patients who were treated for two years). In analyzing the results of HBV treatment protocols, spontaneous e-seroconversion is considered to occur in approximately 11% of HBV-infected children every year (21).

Development of clinically resistant mutations (eg, YMDD mutations) with LAM treatment is the major drawback of this drug. Previous studies involving pediatric populations have reported a YMDD mutation rate of 19% to 24% after 12 months of treatment, and a mutation rate as high as 64% following a 36-month treatment

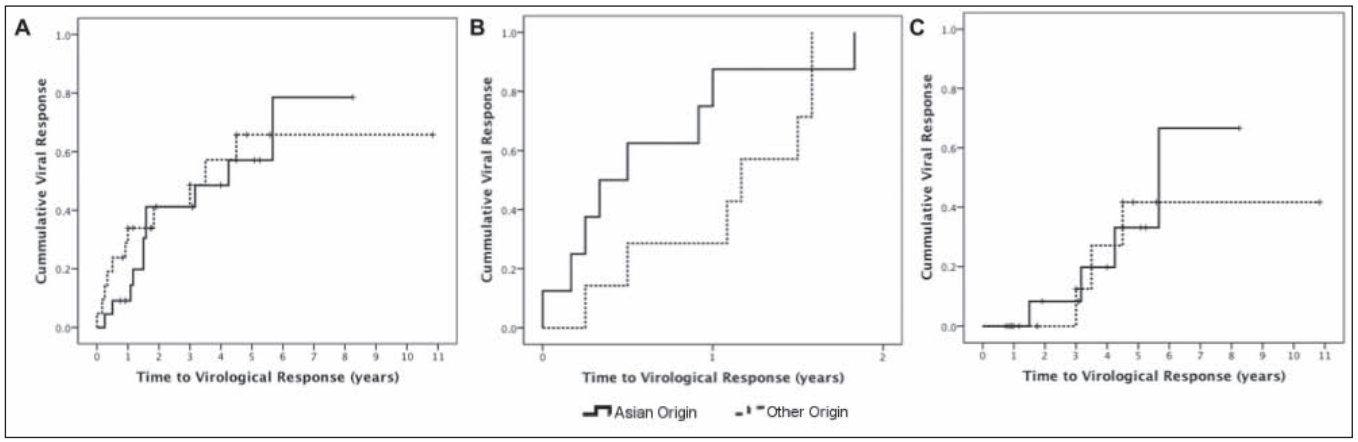

Figure 2) *Asian descent does not influence time to virological response in lamivudine-treated hepatitis B virus (HBV)-infected patients. Cumulative proportion of children achieving viral control (loss of hepatitis B e antigen and loss of detectable serum HBV DNA) calculated by Kaplan-Meyer analysis. In all children (n=43, P=0.633)* (**A**)*, in children with successful response to treatment* (**B**) *and in children with treatment failure* (**C**) *(n=43, P=0.542 [Mandel-Cox log-rank test] for treatment success adjustment)*

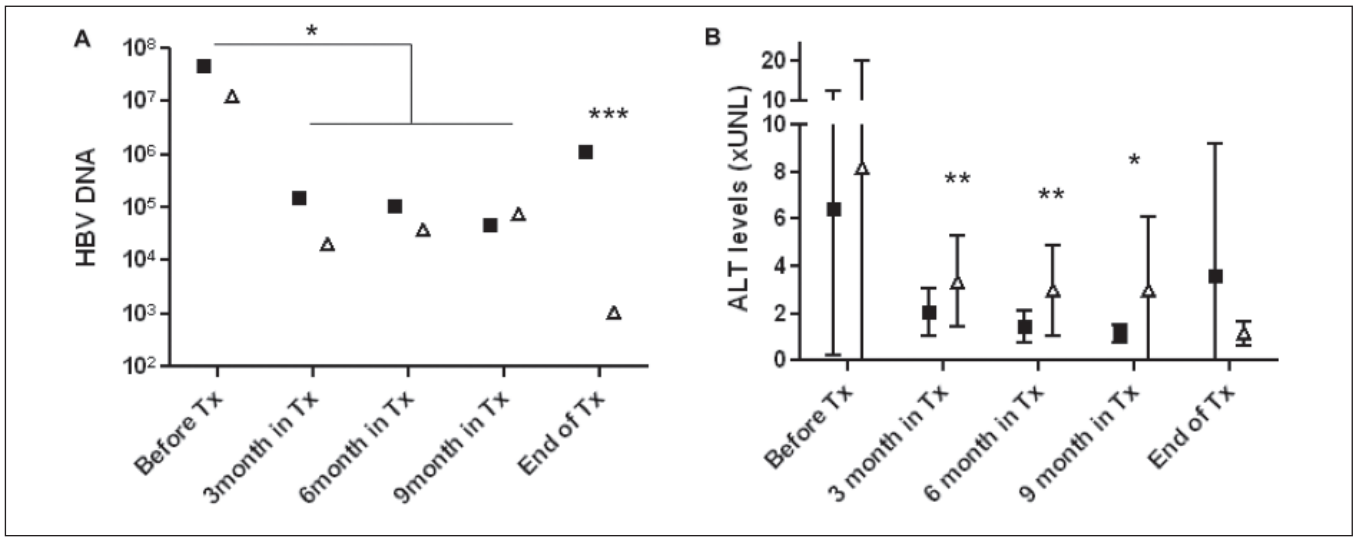

Figure 3) *Hepatitis B virus (HBV) DNA and alanine aminotransferase (ALT) levels in patients with regard to treatment success.* **A** *HBV DNA levels were similar in all patients at the beginning of the treatment (Tx) (P=0.263, n=37), diminished during the treatment (P=0.0245 and P=0.0218 in failure and success to treatment, respectively) and was significantly lower in patients who successfully responded to treatment (P=0.0001, n=31).* **B** *ALT levels were not significantly different in patients who succeeded at the beginning (P=0.565, n=36) and end of treatment (P=0.151, n=25); however, these patients will have significantly higher ALT levels throughout treatment (P=0.009, n=35; P=0.003, n=29; P=0.049, n=18). ANOVA with Tukey post-tests;* ■ *Treatment failure;* Δ *Treatment success. ULN Upper limit of normal*

regimen (7,13). Interestingly, our patient population had a low percentage of suspected LAM-resistant mutations (11%). Although this may be partially due to the excellent adherence of patients to treatment and the mean age of our patient population (23), other confounding factors may also be responsible for this low mutation rate. The emergence of YMDD mutation has also been associated with descent (less frequently in subjects of Asian descent), pronounced liver pathology at baseline, alcohol consumption, high viral load at inclusion (>5×10^6 IU/mL) and previous drug treatment for HBV infections (23). Our patient population's lack of high alcohol consumption, previous drug treatment (except in two patients), pronounced viral pathology and a high proportion of patients of Asian descent could account for the low percentage of LAM-resistant mutations. As expected, patients with suspected LAM-resistant mutations in our cohort did not achieve virological response. These data are consistent with Liaw et al (24) who found no benefit in continued LAM therapy after the emergence of YMDD mutations. As for precore mutations (progression to HBeAg-negative chronic HBV), there was no significant association with treatment success and progression to this stage did not impair response to treatment. This is consistent with previous studies involving adult (8,25) and pediatric (26) patients.

Recently, a very high rate of recurrence in patients treated with nucleoside analogues, especially LAM, has been described in chronically HBV-infected adults (27). In our pediatric cohort, there were no issues regarding virological or serological recurrence except for one patient suspected to harbour a precore mutation. However, chronically infected children who experience HBeAg seroconversion have a more sustainable response and decreased chances of HBV recurrence (reappearance of detectable HBV DNA levels while maintaining seroconversion-e) compared with adult patients (5,10,11). Moreover, other studies in pediatric populations have shown satisfactory sustainability in virological response subsequent to LAM treatment (15,16). Furthermore, the low HBV recurrence rate in our cohort may have been due to the high rate of HBsAg seroconversion in patients with treatment success (53%), which is regarded as a surrogate marker for excellent long-term outcome (4). The high degree of HBsAg loss in our study, compared with previous studies using LAM (27), may be explained by the mean age of our population (28).

Patients of Asian descent showed no differences from other populations with respect to likelihood of treatment success or virological response when patients have been selected according to ALT levels. These results are consistent with several recent studies on treatment response (6,14). In contrast, Asian descent seems to have an impact on HBeAg seroconversion rate in natural history of the disease. Previous studies have described more frequent/rapid spontaneous seroconversion-e and virological response in non-Asian patients (21,29).

The young age of the patients was a significant predictive factor for treatment success in our cohort. A Spearman negative correlation of −0.307 (P=0.045) was found between age groups at the beginning of treatment and treatment success. This correlation may explain approximately 10% of the variance in a population of 43 patients. Consistent with our findings, Sokal et al (7) observed a slightly higher virological response rate in patients between two and six years of age. This trend was also noted in chronically HBV-infected children treated with INF (6,28,30) and led to the conclusion that beginning treatment at a younger age may prove to be beneficial in the long term. In contrast, Hom et al (14) found that age had no effect on response to LAM treatment. This discrepancy could be explained by patient inclusion criteria and the duration of treatment. Our cohort had higher ALT levels before treatment (at least two times normal values versus 1.3 times) – a known predictor of treatment success.

Other putative predictive factors of favourable outcome, such as elevated ALT levels at baseline and liver fibrosis stage (7,13,15), were not statistically significant in our study. A factor that could explain this observation is the selection of our population. We selected patients with elevated ALT levels at baseline as recommended in recent reports (7,15). With a mean value of 7×ULN, our selected population had a favourable chance of treatment success (17), perhaps masking ALT's role as a predictive factor.

Serological and virological markers during treatment were found to be different in patients with a positive outcome to treatment. ALT levels were systematically and significantly higher in patients responding to treatment than in patients who did not respond. LAM treatment is known to result in marked elevation of ALT levels during treatment (31). This phenomenon could reflect active immunological activity in the liver leading to viral control (5).

A reduction in HBV DNA levels throughout treatment was observed in both groups of patients with no statistically significant association with treatment success. This was also observed in a pediatric study involving patients resistant to INF who were given LAM (32). However, a tendency toward lower HBV DNA levels was observed in our successfully treated patients. Similar observations were also made by Hagmann et al (13) in a small pediatric cohort. These lower values are comparable with those observed in adult patients, in which a diminution of 3.6 $\log_{10}$ of HBV DNA levels at four weeks of treatment was highly predictive of treatment success at five years (8). Another study found that reaching a HBV DNA level below 10,000 copies/mL at eight weeks of treatment was predictive of a favourable outcome (33). Our study and others (13) suggest that guidelines proposed for adult patients could be applicable to chronically infected children treated with LAM.

The present study was limited by its retrospective nature and the small number of children included. Furthermore, the heterogeneity of treatment duration limits the conclusions that can be drawn with the present cohort. Interpretation of the data must be made in light of other independent studies to draw a comprehensive portrait of the LAM treatment response in chronically HBV-infected children.

There is no consensus as to whether to treat chronically HBV-infected children; treatment does not seem to augment the absolute number of patients with seroconversion-e once they reach adulthood but only accelerates the process (21,29). Our conclusion is that if the decision to treat is made, LAM should be administered in well-selected patients. The recommendation to treat patients with elevated ALT levels and during the immune active phase of the disease is appropriate and our results suggest that there is a greater chance of treatment success in younger patients. Further research is needed to identify biological markers predictive of treatment outcome during the course of treatment to prevent unnecessary exposure of children to drugs. The pediatric population would benefit from studies of new therapeutics such as pegylated-IFN, new generations of nucleos(t)ide analogues and combination therapies.

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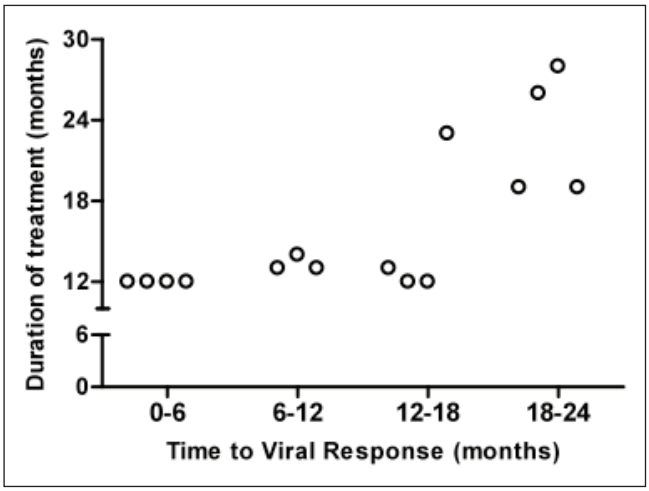
SUPPLEMENTAL TABLE 1
Baseline characteristics of children treated with lamivudine (n=43)

Age at beginning of treatment, years	
Median	9.6
Mean ± SD	9.6±5.2
Range	1.1–18.4
Length of treatment, months	
Median	14.0
Mean ± SD	17.1±7.2
Range	5–38
Liver inflammation score	
Median	1.0
Mean ± SD	1.2±0.6
Range	0.0–3.5
Liver fibrosis score	
Median	1.0
Mean ± SD	1.5±0.9
Range	0.0–3.5
Serum ALT (x ULN)	
Median	3.7
Mean ± SD	7.0±8.4
Range	1.4–42.8
Serum HBV, $\log_{10}$, IU/mL	
Median	7.6412
Mean ± SD	7.4614±1.4688
Range	0.0–9.30
Sex, male:female, n:n (%:%)	22:21 (51.2:48.8)
Ethnicity, n (%)	
Asian	22 (51.2)
Not Asian	21 (48.8)
African	3 (7)
European	2 (4.7)
Canadian	9 (20.9)
Latin and South American	3 (7)
Bangladeshi, Indian, Pakastani	2 (4.7)
Russian	2 (4.7)
Hepatitis, n (%)	
A	2 (4.7)
B	10 (23.3)
C	4 (9.3)
D*	12 (27.9)
Unknown	15 (34.9)
Transmission, n (%)	
Horizontal	6 (14)
Vertical (or perinatally)	37 (86)

**Restriction fragment length polymorphism analysis cannot distinguish between hepatitis virus (HBV) D3 and HBV E; therefore, all patients in this category have been classified as D*



Supplemental Figure 1) *Time to viral response in hepatitis B virus (HBV)-infected patients with successful response to lamivudine according to duration of treatment. Five of 15 patients (33%) who successfully responded to treatment achieved a viral control in their second year of treatment (loss of hepatitis B e antigen and loss of detectable serum HBV DNA)*

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