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Vitamin E for the treatment of E-antigen-positive chronic hepatitis B in paediatric patients: results of a randomized phase 2 controlled study

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Abstract

Background & Aims: The treatment of chronic hepatitis B infection (CHB) in children is still an area of great uncertainty. Vitamin E is an immunostimulating/antioxidant compound proven to be safe and effective for the treatment of adult CHB. The aim of this phase 2 controlled study was to evaluate the safety and efficacy of vitamin E for the treatment of paediatric HBeAg-positive CHB. *Methods:* Forty-six children were randomized in a 1:1 ratio to receive vitamin E at a dose of 15 mg/kg/day (in galenic preparation) or no treatment for 12 months and were monitored for the subsequent 12 months. Clinical, biochemical, haematological and serovirological evaluations were carried out every 3 months. *Results:* No significant side effects were associated with the vitamin E treatment. At the end of the study, anti-HBe seroconversion was obtained in 7 of 23 (30.4%) of vitamin E-treated versus 1 of 23 (4.3%) of the control patients ($P = 0.05$), while a virological response (≥2 log decrease in HBV-DNA from baseline) was observed in 9 of 23 (39.1%) vs. 2 of 23 (8.7%) respectively ($P = 0.035$). *Conclusions:* Vitamin E administration for the treatment of paediatric CHB at the tested dosage has no significant side effects and may induce anti-HBe seroconversion. Vitamin E could represent a tool for the treatment of paediatric CHB.

Keywords
chronic hepatitis B – paediatric infection – treatment – vitamin E

Hepatitis B virus chronic infection (CHB) is a major health problem, affecting approximately 350 million people in the world and may cause a persistent necro-inflammatory liver disease leading to cirrhosis and hepatocellular carcinoma (1). Although HBV vaccination programmes have produced a significant decrease in viral transmission in wealthy and industrialized countries, a high HBV prevalence is still widespread in the geographical areas with low socio-economic development (2).

In highly endemic regions, children acquire viral infection either by means of vertical or horizontal transmission during the perinatal or preschool period, respectively (2, 3), and approximately 90% of them will develop a chronic disease (2).

Much like adult infection, CHB in children is a highly dynamic process that is defined by several serovirological profiles, reflecting different host immune reactivities against the virus (3, 4). Typically, an infection acquired perinatally enters in an immunotolerant phase

Abbreviations
ALT, alanine aminotransferase; CHB, hepatitis B virus chronic infection; CVR, complete virological response; HBeAb, HBe antibody; HBeAg, HBe antigen; HBV, hepatitis B virus; IR, immunoreactive; IT, immunotolerant; PCR, Polymerase chain reaction; ULN, upper limit of normal values; UNT, untreated; VE, vitamin E; VR, virological response.

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Key points

- Safe and effective anti-hepatitis B therapies are available in adults, but the treatment of paediatric population remains controversial.
- Given the central role of immune response in controlling the hepatitis B infection, the use of immunostimulating compounds could be a strategy for therapy.
- Vitamin E exerts both antioxidant and immunostimulatory activities.
- Vitamin E in HBeAg(+) children is safe and may induce anti-HBe seroconversion and could represent a tool for the treatment of paediatric infection.

characterized by HBeAg positivity, normal or mildly elevated serum aminotransferase activity, active HBV replication and minimal liver disease (1, 3, 4). The so-called immunoreactive phase is characterized by HBeAg positivity, a lower level of HBV replication, increased transaminase levels and more rapid fibrosis progression (1). The spontaneous or treatment-induced HBeAg loss associated with HBe antibody (HBeAb) development is considered a hallmark of the transition from active to inactive disease, as it has been shown to be frequently associated with a significant reduction in HBV replication, improvement in liver necro-inflammation and a more favourable prognosis (1).

The treatment of CHB in children is an area of great uncertainty as clear-cut guidelines are lacking and the treatment indications are based on the consensus of expert panels (5). The mere application of indications used in adult infection is considered inappropriate because children are generally thought at a low risk of developing morbidity and mortality. Nonetheless, children with CHB are exposed to the virus for a long period of time, and the data on the natural history of paediatric infection are missing. The general recommendation is that treatment indications should be very carefully evaluated, and therefore, a conservative strategy is warranted (6). Moreover, in developing countries the latter is often the only viable approach due to economic burdens. Thus, in the real-life clinical setting a large part of patients remain untreated until adulthood and/or disease progression.

As a defective antiviral immune response is the hallmark of CHB, agents able to restore immune function are thought to improve the control of infection (7).

There is evidence that vitamin E (VE) is capable of exerting both antioxidant and immunostimulatory activities because it acts as a free radical 'scavenger', protects cellular structures from oxidative damage and, most importantly, enhances cell-mediated immunity (8–11). In this view, we previously performed a randomized trial in adult CHB utilizing α-tocopherol, one of the components of the VE family, with encouraging results (12). The experience in the paediatric field has been evaluated in two trials, but the heterogeneity of these studies does not provide a firm conclusion on the therapeutic potential of VE (13, 14).

Based on this premise, our study was aimed at assessing the efficacy and safety of high doses of VE administration in children with HBeAg(+) CHB.

Methods

Study population

From January 2006 through January 2008, paediatric patients with CHB were enrolled in three Italian Centers (2 Units at the Azienda Ospedaliero-Universitaria Policlinico Sant'Orsola-Malpighi, Bologna, and 1 Unit at the Ospedale Bambino Gesù, Rome).

Originally, the study was proposed to six Italian Paediatric Centres but in January 2005, a meta-analysis reported that a supplemental intake of VE at a daily dosage exceeding 150 IU may enhance all-cause mortality and should be avoided (15). Although the results of that study are questionable and several authors' replies have been published against these conclusions (16–18), four Paediatric Centres withdrew from the study.

Inclusion criteria were the following: age between 2 and 17 years, HBeAg(+)CHB, serum HBV-DNA >20 000 IU/ml at the screening and compensated liver disease. A previous ineffective course with interferon-based treatment was not an exclusion criterion.

Patients with decompensated liver disease; co-infection with hepatitis C, hepatitis D or human immunodeficiency viruses; causes of liver disease other than HBV; or with a clinical condition that, according to investigator's opinion, could interfere with adherence to the protocol, were excluded.

The study was conducted according to international ethical and scientific principles of Good Clinical Practice and the Declaration of Helsinki and was approved by Comitato Etico Azienda Ospedaliera di Bologna (Bologna) and Comitato Etico per la Sperimentazione del Farmaco Ospedale Pediatrico Bambino Gesù (Rome).

Adequate information was provided to the parents or legal guardians of the patients at the beginning of the study, and their written informed consent was obtained.

Study design

This was a phase II, spontaneous, prospective, multicentre, randomized and controlled study (EUDRACT code: 2004-002014-36) aiming to evaluate the safety and efficacy of VE versus no treatment in HBeAg(+) CHB children.

To identify the type and dosage of VE with the best safety and efficacy profile and the lowest potential risk of toxicity and side effects, before developing the protocol we conducted a preliminary systematic review of the literature to identify the *in vivo and in vitro* studies

assessing the effects of different variants and doses of VE supplementation on the clinically relevant measures of immunity both in adults and children with CHB, including our previous study (12). Following the critical appraisal of the available data, alpha-tocopherol succinate was elected as a study drug.

The patients were randomized in a 1:1 ratio to receive either VE or no treatment for 12 months. VE (DL-α-tocopherol hydrogen succinate) was administered at a dose of 15 /kg orally once a day. VE and untreated (UNT) patients were monitored for an additional 12 months (overall study period: 24 months). The randomization list was generated by a computer program and was centralized at a coordinating centre.

Vitamin E was kindly provided by Merck-KGaA (Darmstadt, Germany) free of charge to the coordinating centre and was prepared as a galenic medicine in the Pharmaceutic Department of each centre according to a predefined, common protocol. Briefly, the drug was reconstituted in the form of a syrup and stored at a concentration of 75 mg/ml in dark glass bottles. According to the body weight, the daily dose of VE (expressed as millilitres of syrup) was established for each child by the supervising physician. The patients were provided with the adequate number of bottles to ensure 30 days of treatment to guarantee a frequent resupply, to maintain the chemical stability of the drug and to evaluate the adherence to treatment. The parents and/or legal guardians were properly trained regarding the storage condition of the preparation and on the administration schedule with the support of written information. The daily dose of VE was modulated, if necessary, at each control visit according to the new assessment of body weight.

HBV status definitions

Patients were defined as immunotolerant (IT) if alanine aminotransferase (ALT) were <1.5-fold the upper limit of normal values (ULN) and HBV-DNA >20 000 IU/ml or immunoreactive (IR) if ALT were ≥1.5-fold the ULN and HBV-DNA >20 000 IU/ml. According to the laboratory ranges of local sites, the ALT normal values were ≤41 U/L for males and ≤31 U/L for females.

Efficacy assessment

Hepatitis B virus serology (HBsAg, HBsAb, HBeAg and HBeAb) as well as biochemical (liver transaminases, albumin, INR, blood urea nitrogen, creatinine) and haematological parameters (haemoglobin, leucocytes and platelet counts) were performed at each centre.

The virological tests were centralized at a coordinating centre (Microbiology and Virology Unit, University of Bologna). HBV-DNA was quantified by real-time PCR (Abbott Diagnostics, [Rome, Italy], LoD 15 IU/ml, until 2011; then Cobas AmpliPrep/Cobas TaqMan, Roche Diagnostics, [Monza, Italy], LoD 20 IU/ml).

The serology, biochemical and virology assessments were performed at screening, baseline and every 3 months for the entire study period (24 months).

Safety assessment

A complete clinical assessment, including a physical examination, was performed at each study visit. Any adverse event that had occurred since the previous visit was annotated in a written questionnaire with explanatory notes. The data were collected from the beginning of the study during the entire 24-month period. The severity of adverse events was classified by investigators as mild, moderate or severe in accordance with the criteria adapted from the Division of AIDS, National Institute of Allergy and Infectious Diseases.

An increase of ALT levels above 5-fold the ULN was defined as a flare.

The patients' compliance to therapy was also assessed during each visit by means of: (i) a drug assumption daily written report completed by the patient or his/her parent/legal guardian; (ii) an accurate control by the supervising physician of the item returned by the patients.

Study endpoints

The primary endpoints were as follows:

1 to evaluate the safety and tolerability profile of VE administration in children with HBeAg(+) CHB;
2 to evaluate the efficacy of VE in inducing HBeAg loss and/or anti-HBe seroconversion during the study period (treatment and follow-up).

The secondary endpoints were as follows:

1 to evaluate the efficacy of VE in determining a virological response (VR) defined as ≥2 $\log_{10}$ sustained decrease in serum HBV-DNA compared to baseline during the study period (treatment and/or follow-up);
2 to evaluate the efficacy of VE in inducing a complete virological response (CVR) defined as sustained HBeAg loss/anti-HBe seroconversion together with serum HBV-DNA reduction <2000 IU/ml during the study period (treatment and follow-up).

Sample size calculation

Our previous results concerning VE treatment in adult patients with chronic HBV infection were used to calculate the sample size of the study (12). To perform an intention-to-treat analysis, we calculated that a sample size of at least 44 patients (22 in the VE group and 22 in the UNT group) would provide at least 80% power to detect an absolute difference of 42% (50% vs. 8%) in the rate of HBeAg loss/anti-HBe seroconversion on the basis of a two-sided significance level of 0.05.

We sampled 23 patients for each group for taking into consideration potential dropout patients.

Statistical analysis

Demographic data, baseline characteristics and serovirological response rates between the treatment groups were analysed using chi-square test or Fisher's exact test for categorical data and Wilcoxon rank-sum test for continuous data. The longitudinal and intergroup kinetics of HBV-DNA were compared with Wilcoxon matched-pairs test and Mann–Whitney test respectively.

Estimated long-term events (HBeAg loss/HBeAb development, VR and CVR) in both groups were assessed by the Kaplan–Meier method, and the curves were compared using the log-rank test.

Univariate and multivariable Cox's regression models were performed to identify factors associated with anti-HBe seroconversion and virological response.

A P value <0.05 was considered to indicate statistical significance, and all of the tests were two-sided. All analyses were carried out with StataSE/14.1 (StataCorpLP, College Station, TX, USA).

Results

Patients

Forty-six children and adolescents were enrolled in the study: 23 were randomized to receive VE and 23 received no treatment. The baseline characteristics are reported in Table 1. At inclusion, the VE and UNT groups were comparable for age, sex, source of infection, previous IFN-α therapy, ALT and HBV-DNA levels.

Three children, one in the VE group and two in the UNT group, dropped out immediately after randomization, and therefore, 43 started the study. Three more patients, two in the VE and one in the UNT group, were lost during the treatment and the follow-up respectively. Thus, 40 subjects completed the study.

Thirty-five subjects were classified as IT (18 in VE and 17 in UNT group), while 11 were classified as IR (five in VE and six in UNT group).

Safety

The safety profile of VE was generally good. Only mild and short-term side effects, including gastrointestinal symptoms such as nausea, vomiting and diarrhoea, were observed in both groups (Table 2). No adverse events were reported to be correlated with VE. No abnormalities were detected in the white blood cell and platelet counts, haemoglobin levels, serum electrolytes or in the renal or liver function tests. No serious adverse events were observed. The VE treatment was not discontinued in any of the patients. ALT flares occurred in four patients (two in VE and two in the UNT group; Table 2) and were not associated with worsening of liver function.

Efficacy of treatment

HBeAg loss and anti-HBe seroconversion

Overall, at the end of the study, the HBeAg loss with anti-HBe seroconversion was achieved in 7 of 23 in the VE group and 1 of 23 in the UNT group ($P = 0.047$). In five of eight patients, the e-antigen loss occurred several months before the HBeAb appearance, while in the remaining the events were simultaneous.

Table 1. Demographic and baseline characteristics of patients

	VE group ($n = 23$)	UNT group ($n = 23$)	P
Age (years), median (range)	13 (4–17)	11 (2–15)	0.12
Sex (male/female)	15/8	19/4	0.31
Race			
White	15	9	0.21
Black	2	3	
Asian	6	11	
Route of transmission			
Vertical	13	9	0.38
Unknown	10	14	
BMI (kg/m^2)	18 (14–26)	18 (15–24)	0.6
HBV-DNA ($\log_{10}$, IU/ml)			
$<10^5$	1	0	0.99
$\geq 10^5 \div <10^8$	6	6	
$\geq 10^8$	16	17	
ALT (U/L), median (range)	42 (8–382)	37 (9–117)	0.65
ALT			
<1.5 ULN (%)	18 (78)	17 (74)	0.73
≥1.5 ULN (%)	5 (22)	6 (26)	

BMI, Body Mass Index; ALT, alanine aminotransferase; ULN, upper limit of the normal range.

Table 2. Clinical adverse events and laboratory abnormalities

	VE group ($n = 23$)	UNT group ($n = 23$)
Most common adverse events: *N* (%)		
Headache	2 (8.7)	3 (13.0)
Nausea	4 (17.3)	2 (8.7)
Fatigue	2 (8.7)	2 (8.7)
Vomiting	3 (13.0)	2 (8.7)
Upper abdominal pain	2 (8.7)	3 (13.0)
Diarrhoea	4 (17.3)	2 (8.7)
Upper respiratory tract infection	3 (13.0)	4 (17.3)
Flulike symptoms	2 (8.7)	2 (8.7)
Serious adverse events		
ALT flare	2 (8.7)	2 (8.7)
Adverse events leading to study discontinuation	0	0
Laboratory abnormalities		
Grade 3 ALT (5÷10 × ULN)	1 (4.3)	0
Grade 4 ALT (>10 × ULN)	1 (4.3)	2 (8.7)

In the VE group, anti-HBe seroconversion occurred more frequently during the 12 months of treatment ($n = 6$), while only one patient seroconverted during the 12 months of follow-up. The cumulative incidence of anti-HBe seroconversion is shown in Fig. 1a.

All except 1 of the patients who seroconverted were IT. No patient in either group experienced HBeAg seroreversion once HBeAb development was obtained.

Looking at the baseline demographic, biochemical and virological parameters, Cox regression analysis revealed that HBV-DNA and VE treatment (the last at limit of statistical significance were independent predictors for anti-HBe seroconversion (Table 3). Even if not significant, also the race tended to be associated with anti-HBe seroconversion as it was observed in seven White and only in one non-White patient. Interestingly, among the factors known to impact on spontaneous anti-HBe seroconversion in children (19), neither age, ALT affected the seroconversion rate (Table 3).

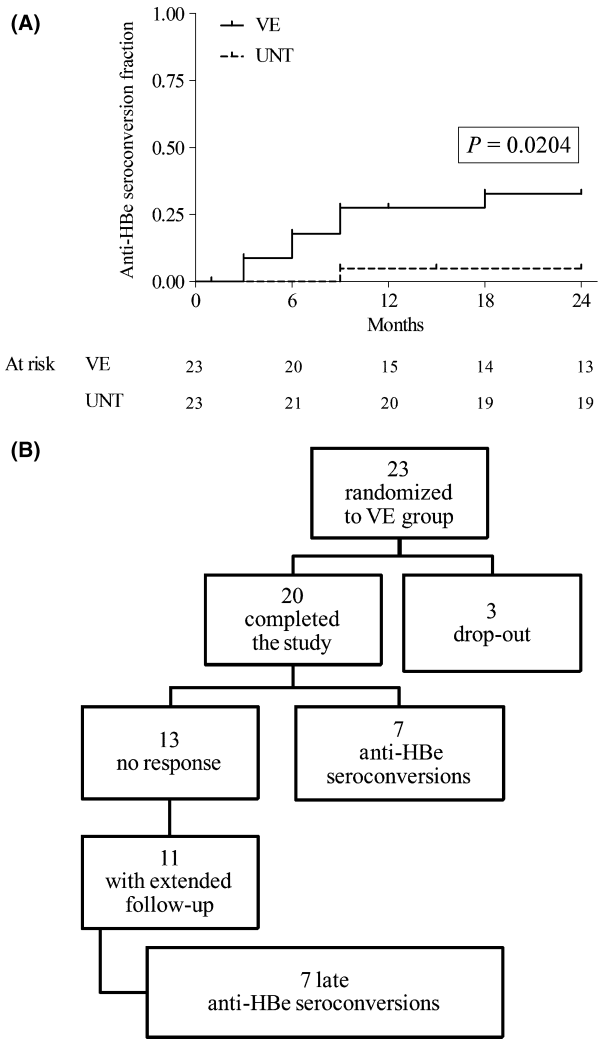

Fig. 1. (a) Cumulative incidence of anti-HBe seroconversion n the VE (solid line) and UNT groups (dashed line). (b) Flow chart of patients treated with VE during the study and the extended follow-up.

In the multivariate analysis, performed on variables with $P < 0.1$, only VE treatment and viremia remained statistically significant in predicting anti-HBe seroconversion (Table 3).

In addition, as some of the patients continued to be routinely followed at the participating centres, we had the chance to monitor the serovirological evolution in 11 subjects treated with VE for an additional 24 months after the end of the study (Fig. 1b). In this subgroup, a late anti-HBe seroconversion was observed in 7 of 11 subjects at 30 ($n = 3$), 33 ($n = 3$) and 42 ($n = 1$) months, respectively, after the beginning of VE treatment.

No patient experienced HBsAg loss or HBsAg seroconversion during the study period.

HBV-DNA kinetics

The baseline serum HBV-DNA did not differ between the VE and UNT groups (Table 1).

The virological response (VR) defined as ≥ 2 $\log_{10}$ and sustaining a decrease in the serum HBV-DNA compared to baseline was achieved more frequently in the VE group than in the UNT group (9/23 vs. 2/23, $P = 0.035$). The cumulative incidence of VR is shown in Fig. 2a. VR occurred preferentially in the anti-HBe seroconverters.

The kinetics of HBV-DNA in the VE group showed a slow and progressive decline, although of variable entity. By contrast, the HBV-DNA levels of the UNT group remained stable over time (Fig. 2b). Nevertheless, the difference between the two groups was not statistically significant.

The univariate analysis showed that treatment with VE ($P = 0.029$), the patient ethnicity ($P = 0.024$) and the ALT ($P = 0.025$) were associated with the achievement of VR, while only the White race maintained a prediction capability of virological response at the multivariate analysis (Table 4).

A complete virological response, defined as anti-HBe seroconversion, together with the achievement and maintenance of a serum HBV-DNA reduction <2000 IU/ml, was obtained in three patients of the VE group and in none of the UNT group patients (P = NS, Fig. 3). Concerning the remaining patients achieving anti-HBe seroconversion, they did not reach the inactive status, as their HBV-DNA level at last time point was >2000 IU/ml ranging from 5700 to 22 500 IU/ml.

Alanine aminotransferase behaviours

The study population was composed mainly from IT patients who started the study with ALT in the normal

Table 3. Factors predictive of anti-HBe seroconversion: univariate and multivariate analysis

	Univariate analysis		Multivariate analysis	
Variable	HR (95% CI)	*P*	HR (95% CI)	*P*
Years	1.15 (0.94−1.41)	0.180		
Female	1.93 (0.46−8.11)	0.369		
VE treatment	7.68 (0.94−62.51)	*0.057*	9.63 (1.16−78.81)	*0.036*
White race	6.90 (0.85−56.20)	0.071		
HBV-DNA $> 1 \times 10^7$ (IU/mL)	0.19 (0.05−0.78)	*0.021*	0.15 (0.04−0.62)	*0.009*
BMI (kg/m^2)	0.97 (0.76−1.25)	0.857		
ALT U/L	1.00 (0.99−1.01)	0.192		

BMI, Body Mass Index; HR, hazard ratio; CI, confidence interval; *P*, *P* value.
Italic *P* values indicate significant (and/or variables entered in multivariate analysis).

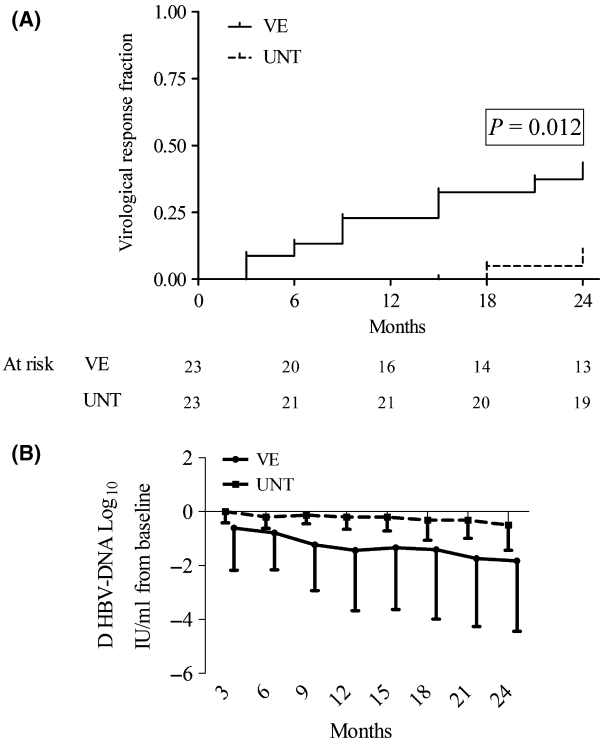

Fig. 2. (a) Cumulative incidence of the Virological Response (decrease in serum HBV-DNA from baseline ≥2 log10) in the VE (solid line) and UNT groups (dashed line). (b) Time course analysis of the HBV-DNA Log_{10} decrease from baseline (mean ± standard deviation) in the VE (solid line) and UNT group (dashed line).

range or slightly elevated (<1.5-fold the ULN in 32 of 43). The ALT levels did not substantially change during the study in the VE or the UNT group.

Among the 11 IR patients, ALT normalization was observed in those who seroconverted to HBeAb, both in the VE and UNT patients. As reported above, ALT flares occurred in four patients (two in the VE and two in the UNT group; Table 2). In the treated group, both the flares occurred in two patients experiencing anti-HBe seroconversion, concomitantly in one case (month 6th of treatment) and at month 15th in the other one, with late seroconversion after the end of protocol (month 33), and were followed by a profound decline in viremia. The unique patient becoming HBeAb positive in the untreated group did not show any ALT increase; therefore, the flares occurring in this group did not associate with anti-HBe seroconversion, at least during the study period.

Discussion

The results of our trial indicate that VE administration at a dose of 15 mg/kg/die is safe and effective in inducing anti-HBe seroconversion in a significant number of subjects.

The use of VE in children affected by HBV infection has been already evaluated by others. A recent study reported the results obtained in children receiving RRR-α tocopheryl acetate (dosage according to body weight, ranging from 200 to 600 IU) for 6 months (14). Although a higher rate of HBeAg loss was reported in the VE group (23.2%) compared to placebo (8.7%), the difference was not significant. Similar to our results, anti-HBe seroconversion occurred mostly after therapy was withdrawn. Another study performed with the same purpose demonstrated no serovirological benefit in HBeAg(+) children treated with tocopheryl acetate (100 mg/daily) for 3 months compared to no treatment (13). In respect to these previous experiences, we utilized higher doses of VE for a longer treatment and post-treatment observation time, which may underlie the differences between these results and the latter study.

An important point of interest of our study is that serovirological events occurred preferentially in immunotolerant children, which is the most typical form of HBV infection in childhood. Although long-term studies on the natural history of these patients are lacking, HBeAg(+) immunotolerant children are not indicated for any antiviral treatment and could remain at this status until adulthood. However, spontaneous HBeAg loss before 30 years of age has been reported to correlate with a decreased incidence of HBeAg(−) hepatitis as well as with a decreased

Table 4. Factors predictive of VR: univariate and multivariate analysis

	Univariate analysis		Multivariate analysis	
Variable	HR (95% CI)	*P*	HR (95% CI)	*P*
Years	1.07 (0.90–1.28)	0.423		
Female (vs. male)	1.17 (0.31–4.44)	0.810		
VE treatment	5.50 (1.18–25.52)	*0.029*	3.74 (0.79–17.65)	0.092
White race	10.67 (1.36–83.55)	*0.024*	7.94 (0.99–63.45)	0.051
HBV-DNA $\geq 1 \times 10^7$ IU/ml	0.99 (0.21–4.59)	0.991		
BMI (kg/m^2)	0.99 (0.80–1.23)	0.929		
ALT U/L	1.01 (1.00–1.01)	*0.025*		

BMI, Body Mass Index; HR, hazard ratio; CI, confidence interval; *P*, *P* value.
Italic *P* values indicate significant (and/or variables entered in multivariate analysis).

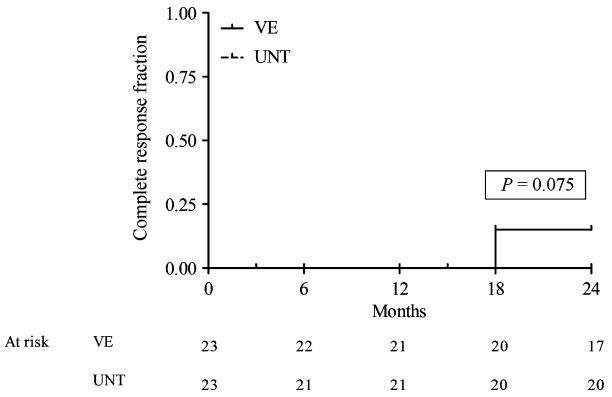

Fig. 3. Cumulative incidence of the Complete Virological Response (anti-HBe seroconversion, together with HBV-DNA <2000 IU/ml) in the VE (solid line) and UNT groups (dashed line).

risk of developing disease reactivation, cirrhosis and hepatocellular carcinoma (20). Therefore, to improve the final outcome and prognosis of these subjects, HBeAg clearance should be obtained as soon as possible. Furthermore, the concept of immunotolerance has been recently revised, suggesting the hypothesis that an immunomodulant approach could be beneficial for these patients (21).

With safety concerns, our experience confirms the already reported good tolerability profile of VE using higher doses and the syrup formulation (12–14).

The mechanisms potentially involved in VE-induced anti-HBe seroconversion are still speculative. VE has been shown to have an antioxidant effect, decrease liver damage and improve the ALT levels in some liver diseases (22, 23), and exerts a number of immunomodulatory activities, collectively resulting in an increase in the immune response (24). Furthermore, VE was recently demonstrated to modulate the synthesis of some microRNAs (miRs) in the liver as well as in immune cells (25, 26). The tight modulation of the synthesis of these miRs might increase the protective immune response against the virus due to their pleiotropic effects (27).

Interestingly, the possibility of continuing the monitoring of patients after the end of the study permitted us to observe that a gradual and progressive serovirological response within 30–36 months of enrolment can be obtained in a consistent number of patients who were classified as non-responders, consistently with our previous experience using immunomodulators for the treatment of adults with CHB (28).

An additional point deserving attention for the future is the possibility of using VE in combination with standard antiviral drugs. The rationale for combination therapy arises from evidences that prolonged viral remission triggers the mechanisms leading to the end of viral control and therefore represents the basis to act with an immunomodulatory compound that is able to boost antiviral immune reactivity (29).

We are aware that our trial has some limitations, including the small number of enrolled patients and the number of subjects who dropped out from the study. Furthermore, an important issue concerns the design of this randomized, controlled trial without a placebo. We decided not to use a placebo in the control group because the galenic preparations often contain other vitamins, which could interfere with the evaluation of the effects of VE. Moreover, the ethical considerations in conducting clinical studies are complex and the use of placebo in this particularly vulnerable population (children with infectious, potentially progressive disease) deserves careful evaluation (30). Although they must be confirmed using larger cohorts, these results might have a significant clinical impact taking into account the following considerations: (i) vertical infection is a clinical problem, particularly in some developing areas of the world with high endemicity where access to potent, effective but expensive anti-HBV drugs is limited; (ii) currently, there are no largely applicable indications for the treatment of this infection in paediatric subjects, and therefore, a large proportion of patients remain untreated. In both industrialized and developing pharmaco-economic settings, a cost-effective and low-toxicity profile drug might be a valid therapeutic support for paediatric CHB.

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