Background: Our aim was to determine the short-term natural course of viraemia and the response to lamivudine treatment in HBeAg-negative chronic hepatitis B patients with a persistently low hepatitis B virus (HBV)-DNA level.

Methods: A total of 55 patients were included. Group 1 consisted of 37 patients with low-level viraemia and high serum alanine aminotransferase (ALT) levels and further randomized to two groups: group 1a (n=19) patients received 1 year of lamivudine therapy and group 1b (n=18) patients were untreated controls. Group 2 consisted of 18 inactive carriers who were followed as controls of untreated low viraemic chronic hepatitis B patients. HBV DNA was longitudinally determined by real-time polymerase chain reaction assay.

Results: A female predominance in group 2 was observed while males were predominant in group 1. Mean age and baseline HBV-DNA levels did not differ between group 1 and 2 patients while group 1 patients had a higher histological score (P<0.01). Of group 1a patients, 44% had complete ALT normalization at end of treatment, whereas 21% untreated group 1b patients had normal ALT at the end of the follow-up. No change in histological activity was observed in group 1a patients at the end of treatment. HBV-DNA levels did not significantly change from baseline to end-of-treatment/observation period in patient groups. The viraemia course was not different across the groups.

Conclusions: Low viraemic HBeAg-negative patients with high ALT present with minimal/mild histological activity. Inactive carriers cannot be differentiated from low viraemic patients with high ALT based on HBV DNA determination. Although lamivudine treatment can be effective in some cases, observation rather than a prompt treatment attempt seems to be more logical because of mild histological changes and low response rate to treatment in these patients.

Introduction

Spontaneous hepatitis B e antigen (HBeAg) to hepatitis B e antibody (anti-HBe) seroconversion is usually associated with sustained remission of liver disease during the natural course of chronic hepatitis B virus (HBV) infection. This inactive carrier state (ICS) is characterized by undetectable HBV DNA by commercial hybridization assays and the absence of significant liver injury [1]. However, the vast majority of HBeAg-negative/anti-HBe-positive ICs have detectable HBV-DNA in serum when measured by very sensitive polymerase chain reaction (PCR)-based assays [2]. In contrast to this benign clinical presentation, a subset of patients develops active liver injury and HBV viraemia despite HBeAg seroconversion. HBV DNA is detectable by conventional hybridization assays in most of these patients [3].

In the recent NIH workshop on the management of hepatitis B, an arbitrarily chosen HBV-DNA cut-off level of 10^5 copies/ml measured by PCR-based assays was recommended to differentiate chronic hepatitis B from the ICS [1]. Studies on the treatment of HBeAg-negative patients have used the detection limits of hybridization assays, which are slightly above 10^5 copies/ml. However, 13% of HBeAg-negative patients with continuing inflammatory activity in the liver have been reported to have HBV-DNA levels of less than 10^5 copies/ml [4]. Given the fluctuating course of the disease, the HBV-DNA level can drop below the detection limit of hybridization assays at several time points during the natural course of HBeAg-negative chronic hepatitis B [5]. According to a recent study, the HBV-DNA level may drop below
10^5 copies/ml in one third of HBeAg-negative chronic hepatitis B patients during longitudinal observations [6]. However, there appears to be a group of HBeAg-negative patients with elevated alanine aminotransferases (ALTs) who have HBV-DNA levels persistently below the detection limit of non-proliferative assays, where seemingly no confounding factors for ALT elevation, such as alcohol and drug intake, exist and the body mass index (BMI) is normal. Clinical course and response to currently available drugs in these patients are still unknown.

Currently, lamivudine has a major role in the treatment of chronic HBV infection. It inhibits the reverse transcriptase activity of HBV and effectively suppresses viral replication without any significant side effects [1,7]. It is effective in both HBeAg-positive and HBeAg-negative patients but prolonged therapy is necessary to gain benefits from the treatment [8–15]. In most published series, HBeAg-negative patients who had HBV-DNA levels below the detection limit of non-PCR-based assays were not included [11,12,14,16,17]. The efficacy of lamivudine treatment in these low viraemic patients is currently unknown.

In this prospective-controlled study, our aim was to determine the clinical characteristics, natural short-term course of viraemia and response to lamivudine treatment in non-cirrhotic, low viraemic, HBeAg-negative patients with high ALTs who had undetectable HBV DNA by a hybridization assay. To this end, lamivudine-treated low viraemic patients were compared with untreated low viraemic patients. We also included ICs for comparison of the natural course of viraemia in untreated low viraemic patients with active liver disease versus ICs. Special attention has been paid to exclude any confounding factors such as a high BMI, alcohol intake and drug-related toxicity in this group of patients with low viraemia and high ALT.

**Materials and methods**

**Patients**

This prospective controlled trial included a total of 55 consecutive patients. These patients were selected by screening 390 HBeAg-negative/anti-HBe-positive HBsAg carriers who were regularly seen in our outpatient clinic between January 1999 and June 2000. Among them, 37 patients were identified (group 1) with elevated ALT levels (>1 × upper limit of normal) and an HBV-DNA level below the detection limit of a hybrid capture assay at monthly/bimonthly assessments during 1 year of follow-up prior to entry into the present study. Alcohol intake was absent or less than 20 g per week and BMI was below 30 kg/m² in all of these patients. Presence of non-alcoholic steatohepatitis (NASH) and liver steatosis, if significant, was an exclusion criteria. Inflammatory activity and fibrosis scores were assessed according to Knodell's scoring system [18] while Brunt et al.'s classification [19] was used if liver steatosis was present. NASH was ruled out by a biopsy not suggestive of NASH in 35 patients while the remaining two patients without liver biopsy did not show an echo pattern suggestive of steatosis on ultrasound. None of the patients had any other identifiable causes of transaminase elevation such as drug toxicity, autoimmune and metabolic liver disease or delta virus infection. None had received prior anti-HBV treatment. These patients were consecutively enrolled into a controlled trial to assess the efficacy of lamivudine therapy. Group 1a (n=18) consisted of the treatment group (lamivudine 100 mg daily for 1 year) and group 1b patients (n=19) were untreated controls with at least 1 year of follow-up after entry into the study. We also included 18 ICs (group 2) to compare the natural course of HBV viraemia in untreated low viraemic group 1b patients with ICs. These ICs had persistently normal liver enzymes and undetectable HBV-DNA levels by the hybrid capture assay at monthly assessments for at least 1 year of follow-up. Selection of inactive carriers was mainly restricted to the availability of stored sera for longitudinal evaluation of viraemia.

Informed consent was obtained from the patients and the study was approved by the local ethics committee. After entry, all patients were followed at monthly/bimonthly intervals during the 12-month treatment (group 1a) or observation (group 1b) period. After completion of the 12-month treatment period, group 1a patients were followed-up for a further 6 months. Biochemical response was determined at the end of the 12-month therapy/observation period and at 6 months of follow-up in treated subjects. Biochemical response was defined as complete ALT normalization. A decrease in Knodell's score of ≥2 was accepted as a histological response. Pretreatment liver biopsy was carried out immediately before commencement of therapy or within the previous 6 months. The second liver biopsy was done at the end of the treatment in group 1a patients.

**Serological tests**

HBsAg, antibody against HBsAg (anti-HBs), HBeAg, anti-HBe, IgM and IgG antibodies against hepatitis B core antigen (anti-HBcAg), antibody against hepatitis C virus (anti-HCV), IgM and IgG antibodies against cytomegalovirus (anti-CMV) and HIV antibody (anti-HIV) were determined by the micro particle enzyme immunoassay. Antibodies against hepatitis delta virus (anti-HDV) (Abbott Laboratories, North Chicago, IL, USA) and Epstein–Barr virus (anti-EBV) (Virotech, Rüsselheim, Germany) were also determined by specific enzyme immunoassays.
Quantification of HBV DNA
HBV-DNA levels were measured by commercial hybrid capture assay (detection limit: 5 pg/ml; Digene Corp, Gaithersburg, MD, USA). A real-time PCR-based HBV-DNA quantification (detection limit: 100 copies/ml) was performed in serum samples obtained at baseline, at month 6 of therapy, at the end of therapy and at the 6-month follow-up in lamivudine-treated patients as previously described [20]. In untreated patients (group 1b and group 2), the same assay was used in patients’ sera obtained at baseline, month 6 and month 12 of the observation period.

Lamivudine-resistant mutant analysis
Genotypic resistance was tested on the serum samples of the treatment group irrespective of presence or absence of clinical breakthrough at end of treatment by line probe assay, according to the instructions of the manufacturer (Inno-Lipa HBV DR; Innogenetics NV, Ghent, Belgium).

Statistics
Student’s t-test, Mann–Whitney U test, Wilcoxon signed ranks test, χ² and Fisher’s Exact tests, and logistic regression analysis were used where appropriate. P<0.05 was considered as statistically significant.

Results
Baseline characteristics
Table 1 summarizes the clinical characteristics of all patient groups at baseline. In contrast to group 1, a female predominance in group 2 was observed. Baseline ALT, HBV-DNA levels and histological scores were not different in male versus female group 1 patients. Mean age was not different between group 1 and 2 patients (35.4 ±9.8 vs 37.6 ±8.7, respectively). No statistically significant difference in median HBV-DNA levels measured by real-time PCR was observed between group 1 and 2 at baseline (1.9x10³ copies/ml vs 2.5x10³ copies/ml, respectively).

Lamivudine-treated (group 1a) and untreated (group 1b) patients were not different in terms of age, baseline median ALT and HBV-DNA levels. None of the patients, except one of the group 1b patients (3.6x10⁵ copies/ml) and one group 2 patient (2.5x10⁵ copies/ml), had an HBV-DNA level above 10⁵ copies/ml. Three group 1a (16.7%), five group 1b (26.3%) and four group 2 patients (22.2%) were PCR-negative at baseline.

Thirty-five group 1 patients (94.6%) and 15 group 2 patients (83.3%) underwent liver biopsy. None of them had any histological evidence of NASH. Mild steatosis was observed in two group 1 patients (10 and 20%). The two group 1 patients without liver biopsy did not show an echo pattern suggestive of steatosis on ultrasound. Group 1 patients had higher median histology activity index (HAI, Knodell score) than group 2 patients (4.0 vs 2.0, P<0.01) while Knodell score was similar in group 1a and 1b patients (Table 1). Fibrosis, although in mild degree (Knodell fibrosis score 1), was observed in 10 group 1 patients (29%, six in group 1a and four in group 1b) versus none of the group 2 patients.

Response to lamivudine treatment
Lamivudine treatment decreased the median serum ALT levels by the end of treatment compared with baseline in group 1a patients (63.5 vs 41.0 IU/l, P<0.05) (Table 2). This trend was still observed at the 6-month follow-up visit (42.0 IU/l vs baseline, P<0.05). However, in untreated group 1b patients, baseline and the end of follow-up ALT levels were the same (48.0 vs 48.0 IU/l). At end of treatment, 44.4% (8/18) of patients in group 1a had complete ALT normalization (Figure 1), whereas only 21.1% (4/19)

Table 1. Baseline characteristics of patient groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Age, mean ±SD</th>
<th>Gender, male/female</th>
<th>Mean BMI, kg/m²</th>
<th>Median ALT level, IU/l (range)</th>
<th>Median HBV-DNA level, copies/ml (range)</th>
<th>Median HAI (range)</th>
<th>Presence of fibrosis, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>35.4 ±9.8</td>
<td>30/7</td>
<td>27.2</td>
<td>53 (35.0–186.0)</td>
<td>1.9x10³ (1x10⁵ – 3.6x10⁵)</td>
<td>4.0 (1.0–16.0)</td>
<td>10/35 (29%)</td>
</tr>
<tr>
<td>Group 1a</td>
<td>31.9 ±11.1</td>
<td>17/1</td>
<td>25.6</td>
<td>63.5 (38.0–186.0)</td>
<td>1.2x10³ (1x10⁵ – 3.6x10⁵)</td>
<td>4.5 (1.0–16.0)</td>
<td>6/18 (33%)</td>
</tr>
<tr>
<td>Group 1b</td>
<td>39.0 ±7.2</td>
<td>13/6</td>
<td>28.4</td>
<td>48.0 (35.0–168.0)</td>
<td>4.2x10³ (1x10⁵ – 3.6x10⁵)</td>
<td>4.0 (1.0–8.0)</td>
<td>4/17 (24%)</td>
</tr>
<tr>
<td>Group 2</td>
<td>37.6 ±8.7</td>
<td>3/15*</td>
<td>25.4</td>
<td>17.0 (11.0–30.0)</td>
<td>2.5x10³ (1x10⁵ – 5.2x10³)</td>
<td>2.0 (1.0–4.0)</td>
<td>0/15 (0%)</td>
</tr>
</tbody>
</table>

Upper limit of ALT was 37 and 31 IU/l for males and females, respectively. Detection limit of PCR assay was 100 copies/ml. HAI was assessed according to Knodell’s scoring system. *Group 2 versus group 1, P<0.05. † Group 2 versus 1 P<0.001. BMI, body mass index; ALT, alanine aminotransferase; HAI, histology activity index; PCR, polymerase chain reaction.
of untreated group 1b patients had complete ALT normalization at the end of 12 months of follow-up ($P=0.12$).

Liver histology at baseline in group 1 patients displayed mild chronic hepatitis. A second liver biopsy was available in 16 out of 18 (88.9%) group 1a patients. Baseline and end-of-treatment HAI scores were similar in group 1a patients (4.5 vs 2.5) (Table 2). However, a decrease of the HAI score by $\geq 2$ points was observed in six out of 10 (60.0%) group 1a patients in whom HAI was $\geq 3$ at baseline.

**Course of viraemia**

The viraemia course did not differ across the groups. From baseline to the end of the 12-month observation/treatment periods, HBV-DNA levels did not significantly change in group 1a, 1b and group 2 patients (Table 2).

**Development of lamivudine-resistant mutants**

Among the lamivudine-treated patients, 11 out of 18 (61%) subjects had mutation at either position 180 or 204 of the polymerase gene (Table 3). All but one patient had a mixed population of mutant and wild-type strains. Despite this high frequency of mutant detection, none of the lamivudine-treated patients developed clinical breakthrough.

**Discussion**

A persistent low-level viraemia ($<10^{5}$ copies/ml) that cannot be recognized by commercial hybridization assays is frequently observed in HBeAg-negative cirrhotic patients in clinical practice. However, besides significant drops in the HBV-DNA level because of the fluctuating course of viraemia, a persistently low viraemic course is a rare event in non-cirrhotic HBeAg-

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**Table 2. Clinical response to lamivudine treatment**

<table>
<thead>
<tr>
<th></th>
<th>ALT level, median (range)</th>
<th>HBV-DNA level, median (range)</th>
<th>HAI, median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>EOT-O</td>
<td>Follow-up</td>
</tr>
<tr>
<td>Group 1a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=18)</td>
<td>63.5*</td>
<td>41.0</td>
<td>42.0</td>
</tr>
<tr>
<td></td>
<td>(38.0–186.0)</td>
<td>(16.0–118.0)</td>
<td>(24.0–99.0)</td>
</tr>
<tr>
<td>Group 1b</td>
<td>48.0</td>
<td>48.0</td>
<td>N/A</td>
</tr>
<tr>
<td>(n=19)</td>
<td>(31.0–168.0)</td>
<td>(24.0–67.0)</td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>17.0</td>
<td>16.0</td>
<td>N/A</td>
</tr>
<tr>
<td>(n=18)</td>
<td>(31.0–30.0)</td>
<td>(13.0–45.0)</td>
<td></td>
</tr>
</tbody>
</table>

Upper limit of ALT was 37 and 31 IU/L for males and females, respectively. Detection limit of PCR assay was 100 copies/ml. HAI was assessed according to Knodell’s scoring system. *Baseline versus EOT and follow-up, $P<0.05$. Follow-up: 6 months after cessation of treatment. ALT, alanine aminotransferase; EOT-O, end of treatment or observation; N/A, not applicable.
negative patients with active disease. Thus, our study population included a unique and relatively rarely observed group of patients infected with HBV.

Special attention has been paid to exclude patients with confounding factors in these low viraemic subjects. In this context, all patients in this group were carefully investigated for well-known aetiological factors associated with transaminase elevation, including idiopathic NASH. Of the 37 patients with persistent low-level viraemia, liver biopsy was available in 35, and of them, only two patients had non-significant steatosis (<30%), which was not associated with any of the histological hallmarks of NASH. In the two cases where liver biopsy was not performed, ultrasound examination of the liver did not reveal increased echogenicity suggestive of liver steatosis. Absence of evidence for a different aetiology and presence of histological findings of mild chronic hepatitis strongly suggest that liver injury in these low viraemic patients is probably related to HBV. We cannot, of course, rule out that our low viraemic patients may have bursts of detectable HBV DNA during longer duration of follow-up, although we did not find detectable HBV DNA by hybridization assays performed at monthly/bimonthly intervals during 1 year of follow-up before entry into the study. Furthermore, none of the untreated low viraemic patients had an HBV-DNA level of ≥10^6 copies/ml measured by real-time PCR on three occasions and all had undetectable HBV DNA by hybridization assay on 6–12 occasions during the 1-year study period. Thus, during a 2-year period, these patients remained with low-level viraemia. All these findings are consistent with the existence of a subgroup of hepatitis B patients with low-level viraemia. Longer duration follow-up studies are needed to understand the natural course of viremia and liver disease in these individuals.

The finding of the presence of low histological activity and low fibrosis scores in these patients suggests that this may be a relatively mild form of HBeAg-negative chronic hepatitis B. However, the mean age of our study population was only 35 years and these low viraemic patients may have a risk of further progression of liver disease over time. In addition, low viraemic patients with high ALTs obviously differ from ICs since they not only had elevated ALT levels but also had higher histological scores. A male predominance in this group may be another clue supporting the idea that these patients are different from the group of ICs in which a female predominance exists. The finding of overlapping and similar HBV-DNA levels in both low viraemic patients with active disease and ICs, strongly suggests that these two groups of patients may not be differentiated by means of viral load determinations. However, the previously suggested cut-off HBV-DNA level of 10^5 for differentiation of inactive healthy carriers from chronic hepatitis B disease [21] may differentiate a milder form of chronic hepatitis B from a more progressive disease. This suggestion needs to be confirmed by further longitudinal studies on a greater number of patients.

The low replication rate of the virus in this relatively rarely observed group of patients is unlikely to be due to a strong immune response against virus-infected hepatocytes because most had only slightly elevated ALT levels and low histological activity. Reduced hepatocyte expression of viral proteins because of an inherited low replication rate of the infecting virus could be a potential mechanism for low replication rate and a poor immune response.

Complete ALT normalization was more frequently observed in lamivudine-treated versus untreated patients, although it did not reach statistical significance. However, only 44% of low viraemic patients reached normal ALT levels at the end of treatment. This response rate is lower compared with that of the 60–70% response rate observed in classical lamivudine-treated chronic hepatitis B patients. One possible explanation for the relatively low efficacy of lamivudine in these patients may be low HBV polymerase activity due to an infection with a slowly replicating predominant viral strain. The antiviral effect of lamivudine depends, in part, on the level of DNA polymerase activity because one of its mechanisms of action on HBV replication is competition of its active metabolite, lamivudine 5′-triphosphate, with both the viral DNA- and RNA-dependent DNA polymerase, during viral DNA synthesis [22]. Another explanation of the low efficacy of lamivudine may be linked to the immunological component of the antiviral response to lamivudine. A decrease/loss of infected cell mass and covalently closed circular DNA in infected cells can be achieved by an antiviral immune response. Lamivudine can break the hyporesponsiveness to viral proteins and enhance the specific antiviral immune responses [23]. Patients who have difficulty in mounting this immune response with lamivudine may experience little or no change in viral load and clinical course of the disease.

YMDD mutant strains were detected in 61% of lamivudine-treated patients at the end of the 12-month

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**Table 3. The observed drug-resistant mutants at the end of the lamivudine treatment**

<table>
<thead>
<tr>
<th>Group 1a (n=18)</th>
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</thead>
<tbody>
<tr>
<td>PCR-negative</td>
<td>3 (17%)</td>
</tr>
<tr>
<td>Wild-type</td>
<td>4 (22%)</td>
</tr>
<tr>
<td>Mutant</td>
<td>8 (61%)</td>
</tr>
<tr>
<td>180M+204V</td>
<td>3 (17%)</td>
</tr>
<tr>
<td>204I</td>
<td>8 (44%)</td>
</tr>
</tbody>
</table>
treatment period. Almost all patients had a mixed population of mutant and wild-type strains. The high sensitivity of the line probe assay enables the detection of as low as 10% of a specific variant in a mixed viral population before development of clinical breakthrough [24]. Detection of mutant viruses at such a high frequency supports the previous observations that mutant strains may also be present as a mixed population before lamivudine treatment [25,26]. Although none of the patients with genotypic resistance developed clinical breakthrough, development of phenotypic resistance in HBsAg-negative patients is almost universal in YMDD mutant infection on continuing lamivudine treatment [13].

In summary, a subgroup of HBsAg-negative patients is presented with low-level viraemia and continuing minimal/mild histological activity during the course of their disease. These patients represent a different group of patients from inactive healthy carriers, although they appear to be similar and not likely to be differentiated based on their HBV-DNA levels. The potential risk for progression of their liver disease by longer duration of follow-up remains to be determined. An individual balance between host immune response and the virus possibly determines the outcome and response to antiviral treatment in these patients. Although lamivudine treatment can be effective in some cases, observation rather than a prompt treatment attempt seems to be more logical because of mild histological changes and the low response rate to treatment in these patients.

Acknowledgements

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References


