Variables determining the virologic response to adefovir treatment in patients with chronic HBV infection previously treated with lamivudine

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The objective of this study was the investigation of the variables that determine the development of virological response to adefovir treatment. In this retrospective case-control study, files of patients with chronic HBV infection which previously used adefovir throughout different periods were examined for gender, age, height, weight, Knodell and fibrosis scores in liver biopsies, serum ALT and HBV DNA levels, HBeAg status and alcohol use. Independent variables determining the virologic response to adefovir treatment were investigated. This study included 63 patients. Forty-one (65.1%) of the patients were male, and their median ages were 42 (range 19 to 65). Twenty-five (39.7%) of the study population were HBeAg positive. The cumulative virological response rates on the 6th, 12th, 18th, 24th, 30th and 36th months of adefovir therapy were found to be 31.7, 38.4, 38.4, 44, 44 and 58%, respectively. HBeAg negativity and the ratio of decrease in serum HBV DNA levels at 6th month of the treatment were the independent variables determining the response to the adefovir treatment [Odds ratio (95% confidence intervals) were 4.498 (1.194-16.939) for HBeAg negativity (p=0.026) and 1.598 (1.232-2.072) for the ratio of decrease in serum HBV DNA levels at 6th month of the treatment (p=0.0001). It was suggested that adefovir was more effective in HBeAg negative patients, and was more rational to continue the treatment in patients who had a decrease of at least 2log_{10} units in serum HBV DNA levels at 6th month of treatment.

Key words: Adefovir, virologic response, HBV.

INTRODUCTION

Chronic hepatitis B virus (HBV) infection is an important cause of morbidity and mortality worldwide. The aim of therapy in this disease is to prevent cirrhosis and hepatocellular carcinoma. This is best achieved by decreasing HBV DNA levels (Conjeevaram, 2003). Adefovir is a nucleotide analogue used for this purpose. Efficacy of adefovir is affected by both viral and host factors. Consequently different kinds of responses such as non-response, partial response and complete virologic response are achieved in clinical practice. Due to this reason, knowing the variables determining the response to adefovir is important in deciding to initiate therapy with adefovir. It has been reported in literature that pretreatment HBeAg status, serum HBV DNA and alanine aminotransferase (ALT) levels, whether a response to previous lamivudine treatment was obtained or not, and the decreases of HBV DNA levels in certain months might determine the response to adefovir (Kin et al., 2011; Jung et al., 2010; Aizawa et al., 2011;...
MATERIALS AND METHODS

In this study, the files of the cases with chronic hepatitis B infection who had received adefovir monotherapy in Chronic Hepatitis Outpatient Clinic, between January 2005-January 2012 were examined retrospectively. The patients with HBsAg-positivity for at least six months were accepted as chronic HBV infected. In this group, the patients with positive HBV DNA were accepted as eligible for adefovir treatment. Adefovir was given to the patients in dose of 10 mg/day. The follow up periods were not same in patients.

Inclusion criteria

Chronic HBV infection, pretreatment positive HBV DNA levels, at least 6 months of adefovir treatment, serum HBV DNA levels measured in each 6 months during the treatment, and liver biopsy, Liver biopsy must be assessed according Knodell Scoring System.

Exclusion criteria

Hepatitis C virus infection, HIV infection, hepatitis D virus infection, intravenous drug addiction, malignancy, pregnancy, liver transplantation, autoimmune hepatitis, hemachromatosis and the use of another antiviral drug with adefovir.

Evaluated variables

Information regarding age and gender, height, weight, alcohol use, the Knodell score and fibrosis score in liver biopsy, genotypic resistance to lamivudine, serum HBV DNA level, serum ALT level and HBeAg status before adefovir therapy were all obtained from the files.

Evaluation of liver histology

Liver biopsy samples were evaluated using the modified Knodell scoring system.

Measurement of HBV DNA level and genotypic resistance to lamivudine

The pretreatment HBV DNA levels of the cases were measured in 28 cases by hybridization (hybrid capture 2, Digene Corp., USA, detection limit 142000-170000000 copy/ml) and in 35 cases by RT-PCR (Cobas TaqMan HBV test, Roche Diagnostics, France, detection limit 30-110 000 IU/ml), HBV DNA levels of the cases were measured only by polymerase chain reaction (PCR) during treatment. To measure the genotypic resistance to adefovir and lamivudine InnoLipa HBV DR reverse hybridization II v 2 (Bayer diagnostics, USA) method was used.

Definition of the virologic response to adefovir

Virological response to adefovir therapy was accepted as HBV DNA negativity (HBV DNA <30IU/ml) determined by PCR during treatment.

Definition of relapse during adefovir treatment

Virologic relapse was defined as serum HBV DNA levels measured with PCR method; >30 IU/ml at least in two separate occasions in patients who had a virologic response during the treatment.

Evaluation of HBeAg seroconversion

During adefovir treatment, HBeAg was measured every six months, and patients were evaluated whether seroconversion occurred or not.

Statistics

The data were evaluated by using SPSS-13 (SPSS Inc., Chicago, IL) statistical software package. The end points of our study were virological response to adefovir therapy. Kaplan-Meier method (using the log rank test for comparisons) was used to find the cumulative incidence curves of the virological response to adefovir therapy. Uni- and multivariable Cox regression analyses were used in examining the variables (gender, age, alcohol use, body-mass index (BMI), presence of lamivudine resistance, HBeAg status, pretreatment serum ALT and HBV DNA levels, and the Knodell score and fibrosis score in liver biopsies) determining the response to the treatment. The patients were divided into two groups according to whether their serum HBV DNA levels at 6th months at least 1 log_{10} units, 2 log_{10} units, 3 log_{10} units and 4 log_{10} units decreased or not, and the effects of these decreases on the response rates were measured by Cox regression analysis. P < 0.05 was accepted as statistically significant. All tests were performed in two-ways.

RESULTS

Patients characteristics

Data from 63 cases with chronic HBV infection was analysed in our study. Forty-one (65.1%) of the patients were male, and their median (range) ages were 42 (19 to 65). Twenty-five (39.7%) of the study population were HBeAg-positive. All patients used lamivudine previously and in fifty-three (84.1%) of these patients, lamivudine resistance were detected. Virologic response to adefovir treatment was obtained in 28 (44.4%) patients. Data of the patients with and without virologic response are summarized in Table 1.

Virologic response to adefovir

The cumulative virological response rates on the 6th, 12th,
Table 1. Data of the responder and non-responder patients to adefovir treatment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Responded n=28 (44.4%)</th>
<th>Not responded n=35(55.6%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)*</td>
<td>49 (21-64)</td>
<td>36 (19-65)</td>
</tr>
<tr>
<td>Gender (male)**</td>
<td>18 (65.3%)</td>
<td>23 (65.7%)</td>
</tr>
<tr>
<td>Body-mass index *</td>
<td>25.6 (18.2-36.3)</td>
<td>24.6 (19.1-36)</td>
</tr>
<tr>
<td>Knodell score*</td>
<td>9 (3-12)</td>
<td>8 (2-14)</td>
</tr>
<tr>
<td>Fibrosis score*</td>
<td>1 (0-3)</td>
<td>1 (0-4)</td>
</tr>
<tr>
<td>Pretreatment HBV DNA level (x1000U/ml)*</td>
<td>612.5 (1.89-432000)</td>
<td>68658 (1.85-394000)</td>
</tr>
<tr>
<td>Pretreatment serum alanine aminotransferase level (U/L)*</td>
<td>122.5 (16-626)</td>
<td>91 (18-320)</td>
</tr>
<tr>
<td>Pretreatment HBeAg positivity **</td>
<td>3 (10.7%)</td>
<td>22 (62.9%)</td>
</tr>
<tr>
<td>Alcohol use**</td>
<td>6 (21.4%)</td>
<td>5 (14.3%)</td>
</tr>
<tr>
<td>Presence of lamivudine resistance **</td>
<td>5 (26.3%)</td>
<td>14 (40%)</td>
</tr>
<tr>
<td>Decrease in serum HBV DNA levels at sixth months of the treatment (log_{10} units)*</td>
<td>3.86 (0.19-7.01)</td>
<td>2.19 (-0.81-7.19)</td>
</tr>
</tbody>
</table>

*, Median (range); **, Number of patients.

Table 2: The results of Cox regression analyses investigating the variables determining the response to adefovir treatment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Univariable Cox analysis</th>
<th>Multivariable Cox analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio</td>
<td>95% confidence interval</td>
</tr>
<tr>
<td>Age</td>
<td>1.029</td>
<td>0.999-1.061</td>
</tr>
<tr>
<td>Gender</td>
<td>1.135</td>
<td>0.509-2.531</td>
</tr>
<tr>
<td>Body-mass index</td>
<td>1.030</td>
<td>0.949-1.118</td>
</tr>
<tr>
<td>Knodell score</td>
<td>1.112</td>
<td>0.955-1.296</td>
</tr>
<tr>
<td>Fibrosis score</td>
<td>1.118</td>
<td>0.784-1.596</td>
</tr>
<tr>
<td>Pretreatment HBV DNA level (x1000U/ml)</td>
<td>1.022</td>
<td>0.995-1.053</td>
</tr>
<tr>
<td>Serum alanine aminotransferase level</td>
<td></td>
<td>1-1</td>
</tr>
<tr>
<td>Pretreatment HBeAg negativity</td>
<td>6.676</td>
<td>1.984-22.465</td>
</tr>
<tr>
<td>Alcohol use</td>
<td>1.422</td>
<td>0.570-3.546</td>
</tr>
<tr>
<td>Presence of lamivudine resistance</td>
<td>0.472</td>
<td>0.155-1.440</td>
</tr>
<tr>
<td>Decrease in serum HBV DNA levels at sixth months of the treatment (log_{10} units)</td>
<td>1.471</td>
<td>1.218-1.777</td>
</tr>
<tr>
<td>≥1log_{10} units</td>
<td>7.339</td>
<td>0.995-54.161</td>
</tr>
<tr>
<td>≥2log_{10} units</td>
<td>3.243</td>
<td>1.117-9.419</td>
</tr>
<tr>
<td>≥3log_{10} units</td>
<td>4.539</td>
<td>1.886-10.909</td>
</tr>
<tr>
<td>≥4log_{10} units</td>
<td>4.902</td>
<td>2.172-11.062</td>
</tr>
</tbody>
</table>

18th, 24th, 30th and 36th months of adefovir therapy were found to be 31.7%, 38.4%, 38.4%, 44%, 44%, and 58%, respectively. The mean virological response time to adefovir was 29.24 months (95% confidence interval; 23.7-34.78).

Variables determining the response to adefovir treatment

The results of the uni- and multivariable Cox regression analyses of variables determining the response to adefovir treatment are summarized in Table 2. The multivariable analysis showed that the amount of decrease in serum HBV DNA level at the 6th months of treatment and HBeAg status were independent variables determining the response rate. In patients with more decreases in the serum HBV DNA level, response rate to adefovir treatment was better (Table 2). Furthermore, the virologic response also was better in HBeAg negative patients (Table 2).
In Kaplan Meier analysis, mean (95% confidence interval) response time to the treatment was 33.6 (29.69 to 37.5) and 22.12 (15.76 to 28.58) months in HBeAg positive and negative patients, respectively (p=0.0001) (Figure 1).

Mean (95% confidence interval) response time to the treatment was 20.43 (16.08 to 24.79) and 37.27 (27.61 to 46.94) months in patients whose serum HBV DNA levels at 6th months of treatment at least 2log_{10} units decreased or not, respectively (p=0.009) (Figure 2).
HBeAg seroconversion

Fifty-five patients were HBeAg positive before treatment and in 21 of them, HBeAg was measured every six month. In 5/21 (23.8%) of previously HBeAg positive, HBeAg seroconversion was observed. Because their numbers were so small, variables affecting HBeAg seroconversion were not investigated.

Virologic relapse

Virologic relapse was developed in two patients.

Renal function impairment

During adefovir treatment, serum creatinine levels were measured every three month in all patients. This level never exceeded the normal upper limit in the patients.

DISCUSSION

The cumulative virological response rates on the 6th, 12th, 18th, 24th, 30th and 36th months of adefovir therapy were found to be 31.7, 38.4, 38.4, 44, 44 and 58%, respectively. These rates were consistent with findings published in literature (Reijnders et al., 2009; Ong et al., 2011; Wang et al., 2011). Virologic response rates in some studies were higher than ours, but we thought that difference was due to the combined use of adefovir and lamivudine in other studies (Kin et al., 2011; Aizawa et al., 2011).

In our study, we observed that the decrease of serum HBV DNA at six month compared to the pretreatment, was one of the independent variables and this finding is consistent with those published in literature (Kin et al., 2011; Reijnders et al., 2009; Ong et al., 2011; Wang et al., 2011). In a study including seventy-six patients with chronic HBV infection; it was shown that the serum HBV DNA level at 24th month was a better determinant of virologic response than the 48th month's level. (Reijnders et al., 2009). In a study including 106 patients used adefovir, it was seen that during the follow up, the virologic response was continued in 87% of the patients who had a virologic response at sixth month of treatment and in 34% of the patients who had no virologic response at 6th month (p<0.05) (Ong et al., 2011). In another study on adefovir naïve 168 patients, the virologic response in patients with the serum HBV DNA level < 10^3 copies at 48th week of the treatment were better than in patients with the serum HBV DNA level > 10^5 copies (Wang et al., 2011).

In our study, pretreatment serum HBV DNA levels had no effect on the response to the adefovir treatment, which is in contrast to the reported studies in the literature (Jung et al., 2010; Aizawa et al., 2011; Reijnders et al., 2009; Wang et al., 2011; Buti et al., 2007). We explain this inconsistency with that pretreatment serum HBV DNA levels in some patients which were measured with hybrid capture 2 (a method with a low level of sensitivity) in our study and so, numbers of the patient with lower serum HBV DNA levels were small.

We observed that the pretreatment serum ALT level was not a variable determining the response to adefovir treatment which is in contrast to literature (Jung et al., 2010; Aizawa et al., 2011; Reijnders et al., 2009). We think that this might be explained with the fluctuation of serum ALT levels in patients with chronic HBV infection, and also we used only one measurement of those levels instead of the mean of the at least three separate measurements.

It has been shown that the HBV genotype is a variable that especially determines the response to interferon treatment but not a variable determining the response to adefovir treatment (Buti et al., 2007; Palumbo et al., 2007). In our study, HBV genotype was not included in the investigated variables.

It has been observed that the probability of virologic response to adefovir treatment was decreased in HBeAg-positive patients (Jung et al., 2010; Reijnders et al., 2009; Wang et al., 2011; Buti et al., 2007). Furthermore, in our study, virologic response to adefovir treatment was better in patients with HBeAg-negativity than patients with HBeAg positivity.

Gender was not reported to seemingly affect the response to adefovir treatment except one study which shows that the virologic response to adefovir was better in female patients (Kin et al., 2011; Jung et al., 2010; Aizawa et al., 2011; Reijnders et al., 2009; Ong et al., 2011; Wang et al., 2011; Buti et al., 2007). There was no effect of gender in response to the adefovir treatment in our study.

In literature, it has been reported that the response rate to adefovir treatment in patients previously treated with lamivudine was low (Zoulim et al., 2009). We observed that the response to adefovir was not decreased in the presence of lamivudine resistance. We thought that this observation was the result of a shorter duration of adefovir treatment shorter in our study than the other studies. In a study, it has been shown that adefovir treatment did not decreases the creatinine clearance in patients compared to patients without such treatment (Manolakopoulos et al., 2011). Although we did not measure the creatinine clearance, serum creatinine levels did not exceeded upper limits of normal in our patients in accordance with above-mentioned study. Adverse effect of adefovir on renal function in patients with underlying renal disease is well-known in literature. We think that the absence of renal impairment in our study might be due to the short durations of adefovir treatment and small number of patients who had no underlying renal disease.
Conclusion

These findings concludes that in patients with chronic hepatitis B infection, 1) cumulative virologic response rates on the 1\textsuperscript{st}, 2\textsuperscript{nd} and 3\textsuperscript{rd} years of adefovir treatment were 38.4, 44 and 58%, respectively; 2) HBeAg-negative patients showed a better virologic response to adefovir than HBeAg positive patients and 3) It may be more logical to continue the treatment in patients whom serum HBV DNA levels decreased at least 2log\textsubscript{10} units at sixth months of the adefovir treatment.

REFERENCES


