ASSESSMENT OF PATIENTS WITH THERAPY INDUCED HBEAG SEROCONVERSION: A REAL-LIFE EXPERIENCE

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ABSTRACT

Objective: *We aimed to evaluate clinical and laboratory features of chronic hepatitis B virus (HBV) patients before and after the therapy-induced Hepatitis B envelope antigen (HBeAg) seroconversion.*

Material and methods: *The data including patients' serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), HBV deoxyribonucleic acid (DNA) levels, fibrosis scores and histological activity index (HAI) scores at baseline and after HBeAg seroconversion, treatment regimen used till HBeAg seroconversion and the elapsed time for HBeAg seroconversion after the beginning of therapy were recorded. Statistical analyses were performed by SSPS 20.0.*

Results: *Overall, 67 (87%) men and 10 (13%) women were included in the study. The elapsed time for HBeAg seroconversion after the beginning of therapy was significantly shorter (p=0.011) in patients used IFNs (n=65, 411.75±509.55 days) compared with patients used only NAs (n=12, 708.92±524.50 days). Baseline serum ALT levels (140.02±95.58 IU/ml vs. 101.15±75.80 IU/ml, p=0.055) and AST levels (73.30±37.63 IU/ml vs. 54.38±25.90 IU/ml, p=0.045) were higher and serum HBV DNA levels were lower (6.63±2.17 log10 IU/ml vs. 7.23±1.28 log10 IU/ml, p=0.459) in patients (n=64, 83.1%) with the duration of equal or less than 1000 days for HBeAg seroconversion after the beginning of therapy. In this regard, we also evaluated a novel scoring system including first treatment regimen, baseline serum ALT, AST and HBV DNA levels to predict that whether or not the elapsed time for HBeAg seroconversion after the beginning of therapy may be less than 1000 days. Area under the ROC curve (AUROC) for the novel score was 0.719 (p=0.013).*

Conclusion: *Score models consisting of first treatment regimen, baseline serum ALT, AST, and HBV DNA levels to predict the elapsed time for HBeAg seroconversion may be constituted.*

Keywords: *Chronic hepatitis B, HBeAg seroconversion, treatment regimen, score models.*

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Introduction

Chronic hepatitis B virus (HBV) infection is a major health problem, leading to complications such as chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC), with an estimated 240 million chronically infected individuals worldwide[(1)]. To date, interferons (IFNs) including conventional interferon-α (IFN-α) and pegylated IFN-α (pegIFNα 2a and 2b) and nucleos(t)ide analogues (NAs) such as lamivudine, telbivudine, adefovir, tenofovir and entecavir are the approved agents for HBV therapy[(1, 2)]. Hepatitis B surface antigen (HBsAg) seroconversion is considered an ideal outcome of treatment, however, it occurs infrequently, namely less than 5%[(3-5)]. Currently, inhibiting HBV deoxyribonucleic acid (DNA) replication, particularly with long-term use of new generation NAs, is well accepted and the most frequently used approach for HBV treatment, since undetectable HBV DNA coincides with decreased liver injury and minor risk for development of cirrhosis or HCC,

and so better prognosis[1, 2]. Hepatitis B envelope antigen (HBeAg) loss or particularly seroconversion is defined as a critical event in the natural course of chronic HBV infection and an important end-point during the treatment, since it is associated with naturally decreased HBV DNA levels in sera and covalently closed circular DNA (cccDNA) in the hepatocyte nucleus, sustained or long-lasting suppression of viral replication and so reduced risk for progression of liver disease[5-17].

HBeAg seroconversion rates in the literature varies from 29-40% with IFNs[8, 11-13], 6-27.27% with NAs depending on the duration of therapy and antiviral agent[5, 7, 9, 11-13], 27-36% with a combination therapy of pegIFNα and lamivudine[11-13], and lastly, 33-76.9% with a combination therapy of pegIFNα and new generation NAs such as entecavir or tenofovir[5, 14-17]. Furthermore, serum alanine aminotransferase (ALT) levels and serum HBV DNA levels at baseline or serum HBsAg, HBeAg, antibodies to hepatitis B core antigen (anti-HBc) titres at baseline, decline trends in serum HBsAg and HBeAg titres or in serum HBV DNA levels, HBV genotype and body mass index (BMI) have been described as predictors for virologic response or therapy-induced HBeAg seroconversion in different trials with patients receiving NAs or IFN-α[7-9]. In this regard, we aimed to evaluate clinical and laboratory features of chronic HBV patients before and after the therapy-induced HBeAg seroconversion and, if there is, the association or the correlation between the elapsed time for HBeAg seroconversion after the beginning of therapy and some variables such as patients' age and gender, antiviral agent, serum aspartate aminotransferase (AST), ALT and HBV DNA levels at baseline.

Materials and methods

Patients and data

For this study, ethics committee approval was obtained from Gülhane Medical School Non-invasive Clinical Research Ethics Committee. Chronic HBV patients being treated at Gülhane Medical School Department of Infectious Diseases were evaluated retrospectively. Overall, 201 patients were evaluated, and of these, therapy induced HBeAg seroconversion was detected in 94 (46.7%) patients. However, 17 patients were excluded from the study, since they did not have pretreatment and/or control liver biopsy results. Ultimately, 77 patients were included in the study. The data including patients' gender and age on the HBeAg seroconversion date, serum AST, ALT, HBV DNA levels, fibrosis scores and histological activity index (HAI) scores at baseline and after HBeAg seroconversion, treatment regimen used till HBeAg seroconversion and the elapsed time for HBeAg seroconversion after the beginning of therapy were recorded. Both conventional IFN-α and pegIFNα were defined and recorded as IFNs.

HBeAg seroconversion was defined as the negativity of HBeAg using the chemiluminescent microparticle immunoassay (CMIA) test and the positivity of antibodies to HBeAg (anti-HBe) using the same test. HBV DNA levels were detected by Taqman Real-Time PCR assay, Fluorion HBV QNP 2.0 (Istanbul, Turkey). Fibrosis score and HAI score were evaluated via means of modified histological activity index. For evaluating the difference in variables, patients were divided into two groups according to their elapsed time for HBeAg seroconversion after the beginning of therapy such as equal and less than 1000 days or higher than 1000 days.

Score

To predict whether or not the elapsed time for HBeAg seroconversion after the beginning of therapy may be less than 1000 days, a novel scoring system were calculated for all patients using baseline serum ALT, AST and HBV DNA levels and first treatment regimen based on the following Table 1 and formula:

Score = Point for baseline serum ALT level + Point for baseline serum AST level + Point for baseline serum HBV DNA level + Point for the first treatment regimen.

Point	Baseline serum ALT level (IU/ml)	Baseline serum AST level (IU/ml)	Baseline serum HBV DNA level (log10 IU/ml)	First treatment regimen
1	<80	<40	>7	NA
2	80-160	40-80	4-7	interferon
3	>160	>80	<4	interferon+NA

Table 1: A novel scoring system to predict the elapsed time for HBeAg seroconversion.
NA: Nucleos(t)ide analogue. ALT: Alanine aminotransferase. AST: Aspartate aminotransferase . HBV DNA: Hepatitis B virus deoxyribonucleic acid.

Statistical analyses

Statistical analyses were performed by SSPS 15.0 (SSPS Inc., Chicago, ILL., USA). The variables were investigated using visual (histograms, probability plots) and analytic methods (Kolmogorov-Smirnov/Shapiro-Wilk's test) to determine whether or not they are normally distributed. All continuous

variables were summarised as mean±standart deviation or median [interquartile range (Iq)]. The Mann-Whitney U test or Student's t-test were applied to compare continuous variables, Paired Student's t-test or the Wilcoxon test were used to compare the measurements at two time points (baseline and after HBeAg seroconversion) and lastly correlations between the variables were examined using Pearson's test or Spearman's correlation test, depending on the normality of the data distribution.

And also, the differences in the variables were analyzed using analysis of variance (ANOVA) or the Kruskal–Wallis tests. The Chi-square test was used to compare the proportions in different groups. p values <0.05 were considered to be statistically significant for all analysis.

The capacity of score in predicting that whether or not the elapsed time for HBeAg seroconversion after the beginning of therapy may be less than 1000 days were analyzed using ROC (Receiver Operating Characteristics) curve analysis. When a significant cut-off value was observed, the sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) were presented.

Results

Overall, 67 (87%) men and 10 (13%) women with therapy induced HBeAg seroconversion were included in the study. The age variable was not distributed normally and mean age was 43.16±8.04 years and median age (Iq) was 42 (10) with an age range of 29-70 years. There was no difference (p=0.633) in age variable between men (42.72±7.01 years) and women (46.10±12.86 years). Also, there was no difference in serum ALT levels (132.25±92.17 IU/ml) (p=0.892), serum AST levels (p=0.832), serum HBV DNA levels (p=0.699), fibrosis scores (p=0.682) and HAI scores (p=0.096) at baseline between men and women (respectively; 132.25±92.17 IU/ml vs. 141.50±105.11 IU/ml, 69.45±35.67 IU/ml vs. 74.50±43.36 IU/ml, 6.67±2.18 log10 IU/ml vs. 7.13±1.54 log10 IU/ml, 1.96±1.33 vs. 2.20±1.47 and 7.22±3.10 vs. 8.80±2.57). Age and laboratory variables of patients are described in Table 2. There were significant difference in serum ALT levels (p<0.001), serum AST levels (p<0.001), serum HBV DNA levels (p<0.001), fibrosis scores (p<0.001) and HAI scores (p<0.001) between pretreatment period and post-HBeAg seroconversion period. In the post-seroconversion period, 60 patients (77.9%) were negative for serum HBV DNA, while 17 patients (22.1%) had serum HBV DNA positivity. In patients with positivity of HBV DNA in the post-seroconversion period, there was significant difference (p=0.010) in HBV DNA levels between pretreatment period (7.22±1.86 log10 IU/ml with a range of 3.62-9.93 log10 IU/ml) and post-seroconversion period (5.22±1.71 log10 IU/ml with a range of 2.91-7.98 log10 IU/ml).

Variables	Mean±Standart Deviation (Minimum-Maximum)	Median (Interquartile Range)
Age (years)	43.16±8.04 (29-70)	21 (2)
The elapsed time for HBeAg seroconversion (days)	458.06±519.84 (26-3036)	270 (381)
Laboratory Characteristics at Baseline		
AST (U/l)	70.10±36.48 (25-213)	61 (39)
ALT (U/l)	133.45±93.25 (43-487)	101 (97)
HBV DNA (log10 IU/ml)	6.73±2.11 (0 IU/ml -10)	6.89 (2.60)
Fibrosis Score	1.99±1.34 (0-5)	2 (2)
HAI score	7.43±3.07 (3-13)	7 (5)
Laboratory Characteristics After HBeAg Seroconversion		
AST (U/l)	28.56±13.48 (15-105)	25 (10)
ALT (U/l)	31.05±16.92 (12-125)	27 (16)
HBV DNA (log10 IU/ml)	1.15±2.32 (0 IU/ml -7.98)	0 IU/ml (0 IU/ml)
Fibrosis Score	1.21±1.23 (0-5)	1 (1)
HAI score	3.68±2.64 (0-14)	3 (3)

Table 2: Age and laboratory variables of subjects.
HBeAg: Hepatitis B envelope antigen. AST: Aspartate aminotransferase. ALT: Alanine aminotransferase. HBV DNA: Hepatitis B virus deoxyribonucleic acid. HAI: Histological activity index.

Treatment regimens used till HBeAg seroconversion were described in Table 3. In patients included in the study, a combination therapy of IFN and lamivudine were seen as the most commonly (48.1%) used treatment regimen till HBeAg seroconversion.

Also, patients used sequential lamivudine therapy following this combination therapy till HBeAg seroconversion were the second frequent group (27.3%). Furthermore, in patients with HBV DNA negativity in the post-seroconversion period, the use of a combination of IFNs and NAs was significantly higher (p=0.048) than those with HBV DNA positivity in the post-seroconversion period (83.3% vs. 58.8%).

Treatment regimens	n (%)
Lamuvudine/Tenofovir	1 (1.3)
Interferon/Lamuvudine	1 (1.3)
Interferon	3 (3.9)
Interferon/Interferon+Lamuvudine	1 (1.3)
Interferon+Lamuvudine	37 (48.1)
Interferon+Lamuvudine/Lamuvudine	21 (27.3)
Interferon+Lamuvudine/Lamuvudine/Lamuvudine+Adefovir	1 (1.3)
Interferon+Lamuvudine/Lamuvudine/Lamuvudine+Entecavir/Entecavir	1 (1.3)
Lamuvudine	7 (9.1)
Lamuvudine/Lamuvudine+Adefovir	2 (2.6)
Lamuvudine+Adefovir	1 (1.3)
Entecavir	1 (1.3)
Total	77 (100)

Table 3: Treatment regimens used till HBeAg seroconversion.

The elapsed time for HBeAg seroconversion after the beginning of therapy was significantly shorter (p=0.011) in patients used IFNs (n=65, 411.75±509.55 days) compared with patients used only NAs (n=12, 708.92±524.50 days). All the same, whereas the elapsed time for HBeAg seroconversion were shorter in patients who used IFNs combined with lamivudine at the beginning (n=60, 402.17±506.385 days) than patients used only IFNs at the beginning (n=5, 526.80±595.10 days), there was no significant difference (p=0.375) between groups.

There was no correlation between the elapsed time for HBeAg seroconversion after the begining of therapy and patients' age (r=0.137, p=0.236), baseline serum ALT levels (r=-0.118, p=0.308), baseline serum AST levels (r=-0.130, p=0.258), baseline serum HBV DNA levels (r=0.125, p=0.278), baseline fibrosis scores (r=0.034, p=0.766) or baseline HAI scores (r=-0.170, p=0.139). Morever, the elapsed time for HBeAg seroconversion was not different (p=0.904) between men (461.09±528.84 days) and women (437.80±480.11 days).

Patients were divided into two groups according to their elapsed time for HBeAg seroconversion after the beginning of therapy. In the first group, there were patients (n=64, 83.1%) with the duration of equal or less than 1000 days for HBeAg seroconversion after the beginning of therapy, and in the second group (n=13, 16.9%), patients' elapsed time for HBeAg seroconversion after the beginning of therapy were higher than 1000 days. In the first group, baseline serum ALT levels were higher (140.02±95.58 IU/ml vs. 101.15±75.80 IU/ml) and serum HBV DNA levels were lower (6.63±2.17 log10 IU/ml vs. 7.23±1.28 log10 IU/ml), but there was no statistically significant difference between two groups (respectively p=0.055, p=0.459). Also, there was no difference in baseline fibrosis scores (1.95±1.21 vs. 2.15±1.90, p=0.876) and HAI scores (7.58±3.12 vs. 6.69±2.84, p=0.351) between two groups. However, baseline serum AST levels were statistically higher (p=0.045) in the first group (73.30±37.63 IU/ml vs. 54.38±25.90 IU/ml). Also, the use of IFN or IFN plus lamivudine were higher (respectively, 87.5% vs. 69.2% and 81.3% vs. 61.5%) in the first group, but there was no statistically difference (p=0.098 and p=0.236).

In this regard, we also evaluated a novel scoring system including first treatment regimen, baseline serum ALT, AST and HBV DNA levels to predict that whether or not the elapsed time for HBeAg seroconversion after the beginning of therapy may be less than 1000 days. Area under ROC curve (AUROC) for the novel score was 0.719 (p=0.013) (Figure 1). We considered that the optimal cut-off value for the score was 8 with a sensitivity of 76.6%, a specificity of 69.2%, a PPV of 92.5% and a NPV of 37.5%.

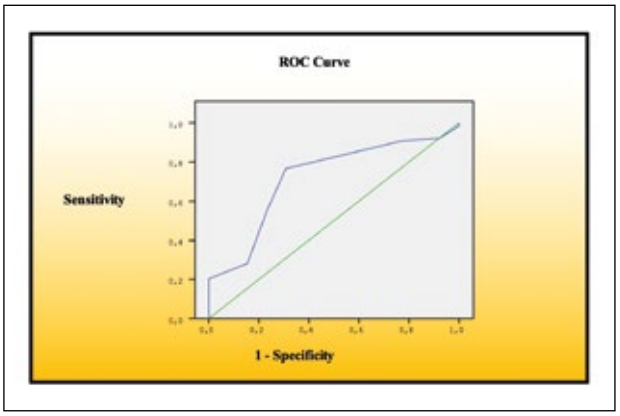

Figure 1: ROC curve analysis for the score to predict the elapsed time for HBeAg seroconversion.

Discussion

HBeAg loss and/or seroconversion is an important event during the treatment, since it is associated with decreased HBV DNA levels in sera and reduced cccDNA in the hepatocyte nucleus, long-lasting suppression of viral replication and so reduced risk for progression of liver disease[5-17]. Therefore, we evaluated clinical and laboratory features of chronic HBV patients with therapy induced HBeAg seroconversion and association

between the elapsed time for HBeAg seroconversion after the beginning of therapy and some variables.

In our cohort, the rate of the therapy induced HBeAg seroconversion was 46.7% (94/201), a little higher compared with former trials consisting of patients receiving IFN with or without NAS. This condition might have particularly resulted from the difference in the duration of therapy or used NA between the trials relevant to this matter in the literature[5-9, 14, 17, 18]. Also, the existence of a sequential therapy history n most of our patients may be another factor affecting HBeAg seroconversion rate in our study. HBV genotype and BMI have also been considered to be associated with HBeAg seroconversion[7-9]. Hence, the difference in HBV genotype and BMI in patients may be the other cause for the diversity of prevalence.

HBeAg loss and/or seroconversion has been showing to be associated with reduced HBV DNA levels in sera and regression of liver disease[5-17]. Similarly, in our patients, there were significant decrease in serum ALT, AST and HBV DNA levels, fibrosis scores or HAI scores in the post-seroconversion period. After HBeAg seroconversion, most of our patients were negative for serum HBV DNA, and also, in the other patients with HBV DNA positivity in the post-seroconversion period, there was a definite decline in HBV DNA levels.

The combination therapy of IFN and lamivudine, the most commonly (48.1%) used treatment regimen till HBeAg seroconversion in our study, were seen more frequently in patients with the elapsed time for HBeAg seroconversion less than 1000 days (81.3% vs. 61.5%) and in patients with HBV DNA negativity in the post-seroconversion period (83.3% vs. 58.8%). Furthermore, the elapsed time for HBeAg seroconversion were shorter, even if not different significantly, in patients used IFNs combine with lamivudine at the beginning (n=60, 402.17±506.385 days) than patients used only IFNs at the beginning (n=5, 526.80±595.10 days). Consequently, it can be said that a combination therapy of IFN and lamivudine seems to be more effective on achieving HBeAg seroconversion in a shorter time with definite suppression of viral replication as a result of our study. But, the combination of IFN and lamivudine is reported to be no more effective than IFN monotherapy in earlier randomized trials[11-13], while the combination therapy have been showing to have better efficacy in terms of HBeAg loss or seroconversion and HBV DNA undetectable rate compared with NAs monotherapy including lamivudine, entecavir or adefovir[4]. At the same time, it is considered that these randomized trials showing no difference between combination therapy and IFN monotherapy were limited, since both IFNs and lamivudine had been given for the same time finite duration such that lamivudine was discontinued before the desire end-point such as HBeAg seroconversion[14]. In parallel with this opinion, patients used sequential lamivudine therapy following this combination therapy till HBeAg seroconversion were the second frequent group (27.3%) in our study. Defined another drawback of these randomized trials[11-13] evaluating the combination of IFN plus lamivudine is the modest antiviral potency and low genetic barrier of lamivudine. Moreover, up to 77% HBeAg seroconversion rates have been indicated with a combination therapy of pegIFNα and new generation NAs such as entecavir or tenofovir in trials conducted to overcome the problem originating from lamivudine[5, 14-17].

In our study, baseline serum ALT and AST levels were higher and serum HBV DNA levels were lower in patients with the duration of equal or less than 1000 days for HBeAg seroconversion after the beginning of therapy, even if there was only statistically significant difference in serum AST levels. As mentioned above, serum ALT levels and serum HBV DNA levels at baseline or decline trends in serum HBV DNA levels have been described as predictors for therapy induced HBeAg seroconversion or virologic response[7-9]. There are studies showing a higher incidence of HBeAg seroconversion in patients with baseline serum ALT levels >4 × upper limit of normal (UPN) and defining baseline serum ALT levels (≥200 IU/ml or >4×UPN) as an important predictor of HBeAg seroconversion[7, 8]. Also, while the predictive value of baseline serum HBV DNA levels were indicated to be not high, HBV DNA level at week 12 after the beginning of therapy or undetectable HBV DNA within 24 weeks after the beginning of therapy was demonstrated to be associated with higher rates of HBeAg seroconversion[7-9, 19, 20].

Furthermore, in a recent study, authors constructed a model including baseline serum ALT level with a cut-off of 200 IU/ml, baseline serum HBV DNA level with a cut-off of 9 log10 IU/ml, BMI and undetectable HBV DNA within 24 week for predicting whether the probability for HBeAg seroconversion was low, intermediate or high and they considered that the model may have potential[7].

In this regard, we wanted to evaluate a scoring system including first treatment regimen, baseline serum ALT, AST, and HBV DNA levels to predict that whether or not the elapsed time for HBeAg seroconversion after the beginning of therapy may be less than 1000 days. We also used serum AST levels at baseline and first treatment regimen in the model, since they were significantly different between groups according to the elapsed time for HBeAg seroconversion. Ultimately, we considered that the optimal cut-off value for the score was 8 with a sensitivity of 76.6%, a specificity of 69.2%, a PPV of 92.5% and a NPV of 37.5%. In our cohort, the prevalence of patients with the duration of equal or less than 1000 days for HBeAg seroconversion after the beginning of therapy was very high (83.1% vs 16.9%). PPV and NPV depend on prevelance. With increasing prevalence NPV decreases, while PPV increases, or in contrast, with decreasing prevalence NPV increases, while PPV decreases. So, the marked difference between PPV and NPV might have resulted from high prevalence.

There are several limitations in this study. First of all, the retrospective design is the most important one of these limitations. In addition, the number of study patients was limited. Accordingly, it can be said that the number of patients receiving new generation NAs such as entecavir or tenofovir was a limitation for our study. That the study was a single-center study may be another drawback and a cause of the limited number of study patients. Lastly, since retrospective design causes data loss, we did not evaluate the other possible predictors for therapy induced HBeAg seroconversion such as serum HBsAg, HBeAg, anti-HBc titres at baseline, decline trends in serum HBsAg and HBeAg titres or in serum HBV DNA levels.

Conclusion

HBeAg seroconversion is a critical event associated with long-lasting suppression of viral replication and reduced risk for progression of liver disease. Accordingly, there were significant decrease in serum ALT, AST, and HBV DNA levels, fibrosis scores or HAI scores in the post-seroconversion period in our patients. Baseline serum ALT and HBV DNA levels were priorly reported to be important to predict the probability for HBeAg seroconversion, also as a result of our study, score models consisting of first treatment regimen, baseline serum ALT, AST and HBV DNA levels to predict the elapsed time for HBeAg seroconversion may be constituted. Furthermore, in proper patients, sequential therapy following a combination therapy of IFN and NAs, favourably entecavir or tenofovir since their higher antiviral potency and genetic barrier to resistance compared with lamivudine, seems to be more effective on achieving HBeAg seroconversion in a shorter time with definite suppression of viral replication. However, in order to highlight the role and importance of baseline serum ALT, AST and HBV DNA levels for predicting the elapsed time for HBeAg seroconversion and sequential therapy following a combination therapy of IFN and new generation NAs to achieve HBeAg seroconversion in a shorter time, randomized large-scale studies are required.

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