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Acute exacerbation during interferon alfa treatment of chronic hepatitis B: frequency and relation to serum β-2 microglobulin levels

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Background. We aimed to determine the frequency of alanine aminotransferase (ALT) elevation during interferon-α treatment, the so-called "flare", its relation to serum beta-2 microglobulin levels, and its impact on the outcome of treatment in chronic hepatitis B. ***Methods.*** The files of 53 treatment-naive patients with chronic hepatitis B (17 hepatitis B e antigen (HBeAg) +ve, 36 HBeAg −ve) who had been treated with 10 MU interferon-α 2b three times per week for 24 weeks were reviewed. We analyzed the fluctuations in serum ALT, β_2-microglobulin, and HBV-DNA levels before, during, and after flare. ***Results.*** We detected flare in 4/17 (24%) of the HBeAg +ve and 7/34 (21%) of the HBeAg −ve patients. ALT level peaked between weeks 2 and 16 (mean, week 8). After flare, HBV-DNA disappeared in 5/7 (71%) HBeAg −ve vs 3/4 (75%) HBeAg +ve patients (all seroconverted to anti-HBe). The overall sustained response rate was 41%: 55% in the patients with flare, and 38% in those without ($P > 0.05$). Basal serum β_2-microglobulin levels were significantly higher in responders vs nonresponders (2.19 ± 0.32 vs 1.78 ± 0.34 mg/l, mean ± SD; $P < 0.005$). In addition, during treatment, serum β_2-microglobulin levels increased significantly only in responders, and the degree of increase was significantly higher in responders with flare vs responders without flare (3 ± 0.33 vs 2.34 ± 0.35 mg/l; $P < 0.001$). ***Conclusions.*** This study, with a limited sample size, showed that, in chronic hepatitis B, there is a trend for a higher response in patients with exacerbation of hepatitis B with interferon-α treatment. However, the difference does not reach statistical significance to be of predictive value. On the other hand, serum β_2-microglobulin levels before and during treatment may be useful in predicting the outcome.

Key words: hepatitis B, chronic, therapy, beta 2-microglobulin

Introduction

During the treatment of chronic hepatitis B infection (CHB) with interferon alfa (IFN), an increase in serum alanine aminotransferase (ALT) level, the so-called flare, is not uncommon.[1–3] Despite exacerbation of hepatitis is relevant in such a circumstance as it is characterized by heightened immunity, it is detected in fewer than one-third of the subjects treated. It is generally asymptomatic and can be detected with close biochemical monitoring. ALT flare during IFN treatment needs to be differentiated from superimposed hepatitis A, D, and C infections.[4] ALT flare is more common in hepatitis B e antigen-positive subjects (HBeAg +ves), and subsequent seroconversion to anti-HBe is usual.[1–3,5,6] However, this sequence of events is seldom associated with the clearance of hepatitis B surface antigen (HBsAg) and the development of anti-HBs.[1,3,5] The data on the frequency and significance of ALT flare during IFN treatment of HBeAg −ve subjects is insufficient. IFN increases the expression of HLA-I antigens on hepatocytes, which attracts T-lymphocytes, with subsequent cytolytic and noncytolytic viral inactivation. Because beta-2 microglobulin (β_2-MG) is part of the HLA-I antigen family and is present in the circulation, its serum level reflects the degree of HLA-I expression on the cell surface.[7–9] This study aimed to detect the frequency of hepatitis exacerbation, not only in HBeAg +ve but also in anti-HBe +ve subjects (an underinvestigated subpopulation) in

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the course of IFN treatment, and the relationship of this exacerbation to serum β_2-MG levels and treatment outcome.

Patients and methods

The files of 53 patients with CHB (39 male, 14 female; median age, 36 years; range, 14–65 years) who had been treated with 10 MU IFN 2b three times per week (tiw) for 24 weeks and followed-up for at least 24 weeks, untreated, were reviewed. Seventeen patients (32%) were HBeAg +ve and 36 (68%) were anti-HBe +ve. All patients had persistently elevated (more than two times the upper limit of normal for at least 6 months) ALT and detectable HBV-DNA by liquid hybridization before treatment. Histological diagnosis of chronic hepatitis was available. Patients with findings of decompensated liver disease, such as ascites, hepatic encephalopathy, or esophageal varices, as well as patients positive for anti-HDV, anti-HCV, and HIV were excluded. However, patients with histologically proven cirrhosis were included. All patients were antiviral treatment-naive. Patients were followed with clinical, biochemical, and hematological examinations at weekly intervals for the first month and every month thereafter. HBV-DNA measurements were done for each patient, before, at the end of the treatment, and at several occasions during the follow-up. An additional sample was taken during flare, if it occurred.

An end-of-treatment response (ETR) to therapy was defined as the loss of HBV-DNA (and HBeAg, if it had been present) with complete normalization of ALT at the end of the treatment, and sustained response (SR), was defined by normal ALT and undetectable HBV-DNA and HBeAg during the post-treatment follow-up. Relapse was defined as the reappearance of serum HBV-DNA and HBeAg and an increase in ALT level. During treatment, an increase in serum ALT levels equal to or greater than twice the basal value was defined as flare. We analyzed the fluctuations in serum ALT and HBV-DNA before, during, and after the flare. Serum β_2-MG levels were measured from the stored sera of 31 patients without flare at baseline and at week 8 of treatment. For the patients with flare, the second sample tested was the one obtained at the time of flare.

Serological markers of HBV (HBsAg, anti-HBs, anti-HBc, HBeAg, anti-HBe), HDV, and HCV were tested with a commercial enzyme-linked immunosorbent assay (ELISA; Abbott EIA; Abbott, Wiesbaden, Germany). β_2-MG was determined using a microparticle enzyme immunoassay (Abbott IMx system; Abbott, Wiesbaden, Germany). HBV-DNA was measured quantitatively by liquid hybridization (Digene, Hybrid capture; Murex Diagnostics, Brussels, Belgium).

Liver biopsy specimens were evaluated according to the histological activity index of Knodell et al.[10] The sum of the necroinflammation scores (0–18) was expressed as the activity score, and staging was done according to the fibrosis score (0–3). Stage 4 was termed cirrhosis. All patients had given their written informed consent previously and the study had been approved by the Ethics Committee of our hospital.

Statistical analysis was performed with Pearson's or Spearman's correlation (two-tailed) and Mann-Whitney *U*-tests. Categorical variables were compared by using Fischer's exact test, and *P* values below 0.05 were considered significant.

Results

Fifty-three patients, treated with 10 MU IFN 2b for 24 weeks, were analyzed. Two HBeAg −ves were excluded because they did not complete the treatment due to intolerance. While 17 of the remaining 51 were HBeAg +ve (33%), the other 34 (67%) were HBeAg −ve. Twelve of the 17 (71%) HBeAg +ves, and 19 of the 34 (56%) HBeAg −ves responded. Relapse occurred in 10 patients. Two relapses occurred within 6 months after the end of the treatment, and the others relapsed between 6 and 12 months of follow-up. Twenty-one patients (41%) were sustained responders. In 3 of the 21 (1 of them with flare) HBsAg also became undetectable, and anti-HBs developed subsequently in 1 of them. The mean untreated follow-up period was 23 months (range, 6–37 months).

We observed ALT flare in 11 of 51 (22%) patients: 4/17 HBeAg +ve (24%) and 7/34 HBeAg −ve (21%) patients. There was no correlation between ALT flare and HBeAg status. All but 1 patient, who experienced fatigue, were asymptomatic during flare. ALT level peaked between weeks 2 and 16 (mean, week 8) (Fig. 1). While serum HBV-DNA level increased (+88.86 ± 39.2%) in 3 patients, it decreased in 5 (−88.53 ± 17.14%) and became undetectable in 3 during flare (Fig. 2). After the resolution of flare, HBV-DNA was no longer detectable in 5/7 (71%) anti-HBeAg +ve vs 3/4 (75%) HBeAg +ve patients; all seroconverted to anti-HBe. The overall sustained response rate was 41%; 55% in patients with flare and 38% in those without (Fig. 3). There was no correlation between the emergence of flare and the sustained response rate ($P > 0.05$). The pretreatment serum ALT and HBV-DNA levels and fibrosis score were not predictive of either ALT flare or sustained response. Some characteristics of the patients studied and results are summarized in Table 1.

Basal serum β_2-MG levels were significantly higher in responders vs nonresponders (2.19 ± 0.32 vs 1.78 ±

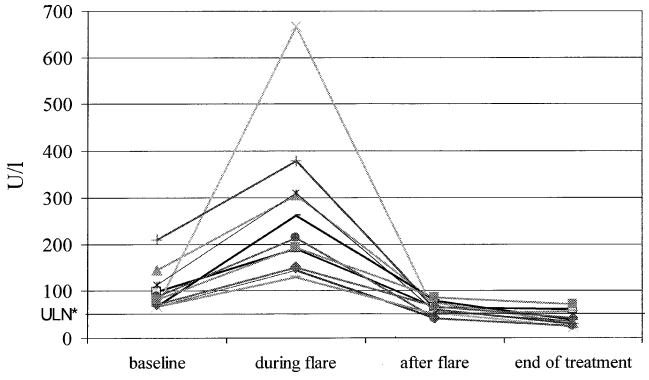

Fig. 1. Course of serum alanine aminotransferase (ALT) levels in patients with in-treatment flare. *ULN*, Upper limit of normal

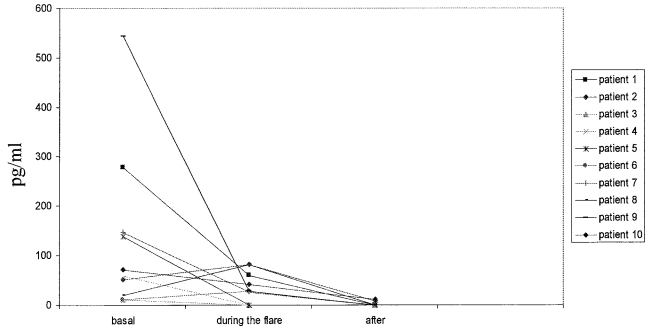

Fig. 2. Serum hepatitis B virus (HBV)-DNA levels before, during, and after flare. Numbers 8, 9, and 10 are hepatitis B virus envelope antigen-positive (HBeAg +ve) patients; patient 11 was not included because of very high HBV-DNA value (4500 pg/ml)

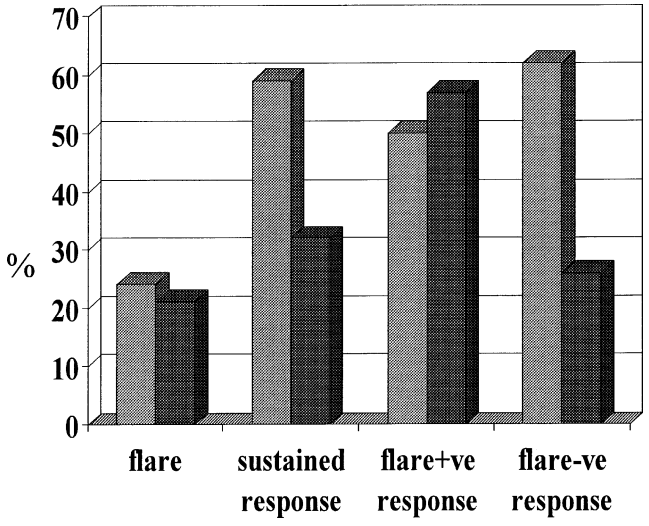

Fig. 3. ALT flare during interferon alfa (IFN) treatment and sustained response in chronic hepatitis B. *Light gray bars*, HBeAg-positive; *dark gray bars*, anti-HBe-positive. Differences between values are not significant

0.34 mg/l; $P < 0.005$). In addition, during therapy, serum β_2-MG concentrations increased significantly only in responders, and the degree of increase was significantly higher in responders with flare vs responders without flare (3 ± 0.33 vs 2.34 ± 0.35 mg/l; $P < 0.001$) (Table 2). There was no correlation between the basal serum β-2 MG levels of the patients and their activity scores on liver biopsy. All patients were seronegative for HDV, HCV, and HIV markers during flare. Most patients tolerated IFN treatment with minor side effects. However IFN was discontinued in two patients because of intolerance.

Discussion

We observed ALT flare in 11 of 51 (22%) chronic hepatitis B patients followed-up with monthly blood tests during IFN treatment, and the frequency of flare in HBeAg −ves was not different from HBeAg +ves. Spontaneous or IFN treatment-related hepatitis exacerbation (flare) with possible HBe seroconversion in HBeAg +ve chronic hepatitis B infection was reported previously.[1–6,11,12] Flare is believed to be associated with heightened immunomediated antiviral response, and the result is usually precipitously decreased serum HBV-DNA levels.[1–3,13] This is marked by increased hepatocyte destruction as the result of increased hepatocyte HLA class I antigen expression followed by an attack of cytotoxic T cells and is characterized by a sometimes striking increase in serum transaminase levels.[14–16] Previous studies showed that serum β_2-MG levels may be elevated by IFN treatment and the degree of the increase was associated with the magnitude of HLA class I antigen expression on hepatocytes.[7–9] In this study, serum β_2-MG levels were higher in responders, before as well as during treatment, in comparison with nonresponders. The clearance of HBV-DNA in responders without elevation of serum ALT levels may occur via a noncytolytic mechanism. It is possible that cytolytic and noncytolytic mechanisms are usually complementary for HBV-DNA clearance. This study showed that flare occurred in approximately 20% of both HBeAg+ and HBeAg −ve patients during IFN treatment. In contrast to previous reports,[1–3] no significant relationship was found between the flare and the response rate in this study. However, there was a trend for a higher sustained response rate in the patients with flare. The relatively small sample size in this study prevents us from reaching a firm conclusion about the predictive value of flare in reference to sustained response. It was previously observed that elevation of serum ALT levels above ten times the upper limit of normal during IFN treatment appearred to predict a successful outcome.[1–3] In our study, in only one patient,

Table 1. Some characteristics of the patients, and summary of the results

Total treated $n = 51$	Patients with flare $n = 11$	Patients without flare $n = 40$	P value
Male/female	10/1	29/11	NS
Age (years)[a]	30 (14–57)	35 (17–62)	NS
Basal ALT level (U/l)[b]	103 ± 44.98 (68–209)	180.7 ± 138.8 (69–559)	NS
Basal HBV-DNA level (pg/ml)[b]	629.8 ± 1425.1 (11–4404)	396.7 ± 906.3 (5.6–4116)	NS
Basal β_2 microglobulin level (mg/l)[b]	2.15 ± 0.34 (1.6–2.7)	2.06 ± 0.39 (1.4–2.8)	NS
Activity[b,c]	9.7 ± 3.4 (6–16)	10.9 ± 3.8 (4–17)	NS
Fibrosis[b,d]	2.2 ± 1.2 (0–3)	1.7 ± 0.99 (0–3)	NS
With cirrhosis	2	2	NS
End-of-treatment response	8 (73%)	23 (58%)	NS
Relapse	2 (25%)	8 (35%)	NS
Sustained response	6 (55%)	15 (38%)	NS

NS, Not significant
[a] Median (range)
[b] Mean ± SD (range)
[c] Sum of necroinflammatory scores on histology activity index
[d] Fibrosis scores on histology activity index (Knodell et al.[10])

Table 2. Changes in serum β_2-microglobulin level in chronic hepatitis B with interferon alfa treatment

Category	Number of patients	Baseline value (mg/l)[a]	Second value (mg/l)[a,b]	P value
Flare + ve and response	8	2.21 ± 0.29 (1.9–2.7)	3 ± 0.33 (2.6–3.6)	<0.001
Flare + ve and nonresponse	3	2.0 ± 0.53 (1.6–2.6)	2.2 ± 0.53 (1.8–2.8)	NS
Flare − ve and response	14	2.18 ± 0.34 (1.6–2.6)	2.34 ± 0.35 (1.7–2.9)	<0.005
Flare − ve and nonresponse	16	1.69 ± 0.22 (1.4–1.9)	1.8 ± 0.26 (1.4–2.1)	NS
All responses	22	2.19 ± 0.32 (1.6–2.8)	2.56 ± 0.46 (1.7–3.6)	<0.005
All nonresponses	19	1.78 ± 0.34 (1.4–2.6)	1.92 ± 0.38 (1.4–2.8)	NS

** $P < 0.005$
NS, Not significant
[a] Mean ± SD (range)
[b] At week 8 in patients without flare; at the time of flare in patients with flare

serum ALT exceeded ten times the basal ALT level and he was, indeed, a sustained responder. However, such an aggressive flare seems to be an unusual event in the course of IFN treatment. A recent study examining the efficacy of a pre-S2-containing hepatitis B vaccine in the treatment of CHB showed flare of over ten times the upper limit of normal occurring with vaccine administration and this was highly predictive of sustained response as well.[17] No relationship was found between baseline serum ALT and HBV-DNA levels or age and occurrence of flare.

We demonstrated that 6 months of IFN treatment with the dose of 10MUtiw in both HBeAg +ve and HBeAg −ve patients resulted in sustained suppression of HBV DNA, in 59% and 32% of these groups, respectively. Previous studies reported that IFN induced remission of disease in 28%–30% of HBeAg +ves[18,19] and 18%–33% of HBeAg −ves.[20,22] The better results obtained in our cohort may be related to the 6 months of treatment instead of 4, and the inclusion of patients with at least two times the upper limit of normal ALT values. Prolonged[18,21] or high-dose[22] IFN treatment was shown to enhance the response rate in CHB.

It was previously noted that increases in ALT levels were generally preceded by increased HBV-DNA, HBeAg, and anti-HBc IgM levels, and were followed by subsequent cessation of viral replication and HBeAg seroconversion.[12,13,16] In contrast, during the reactivation episodes, serum HBsAg concentrations usually remain unchanged, and the proliferative response of T lymphocytes to HBV envelope antigens remains weak and undetectable.[5] However, in this study, we detected a preceding HBV-DNA rise in only 3 of 11 patients (1 of whom was HBeAg +ve), and peak values were not too striking (<3 times basal HBV-DNA value). HBV-DNA decreased in 5 patients and became undetectable in 3 patients during flare. Maruyama et al.[13] showed that the highest serum HBV-DNA levels occurred 1–4 weeks before the peak ALT. However, our study was retrospective, and we might not have detected a preceding increase of HBV-DNA level in patients with flare, due to poor sampling. Guidotti et al.[23] showed that the

HBV-DNA content of serum and liver may decrease with little or no biochemical or histological evidence of hepatocyte injury in acute hepatitis B infection (so-called noncytolytic clearance). The results of their study demonstrated that clearance of more than 90% of the viral DNA, actually, does not require destruction of infected cells in acute hepatitis B. Virus-specific cytotoxic T-lymphocyte (CTL) response results in the secretion of cytokines (e.g., IFNγ, tumor necrosis factor [TNF], interleukin-2 [IL-2], and IL-12) during HBV infection, and viral clearance by means of a noncytolytic mechanism may be more efficient than the destruction of infected cells.[14,23,24] The presence of a correlation between sustained response and serum β_2-MG, and the lack of correlation between sustained response and flare show that increased expression of viral particles to the elements of cell-mediated immunity does not necessarily result in cell lysis, and noncytolytic antiviral actions are effective as well.

One of our patients (age 17 years; male) who was HBeAg +ve initially responded to treatment with loss of HBeAg and normalization of serum ALT, without flare in the course of treatment. His serum HBV-DNA was undetectable at the end of treatment as well. However, at week 4 of follow-up, his serum ALT rose to 363 U/l, although he was asymptomatic, and there was a spontaneous drop to 163 U/l at week 6, with the normal range shown at week 8. At the end of the untreated follow-up he was a sustained responder. His serum HBV-DNA, as well as HBeAg, remained undetectable in this period. The meaning of post-treatment ALT flare in a patient with ETR not associated with detectable viral reactivation in serum, with eventual SR, remains to be explained.

In conclusion, this study showed that, in CHB, exacerbation of ongoing infection with IFN treatment occurred in approximately one-fifth of both HBeAg +ve and HBeAg −ve patients. Although there was a trend for higher response in the patients with flare, in this small-sample study, we failed to find a significant relationship between the occurrence of flare during treatment and the response rate. However, high serum β_2-MG levels before and during treatment predict better outcome. It seems likely that the response to IFN treatment in patients with CHB is mediated by both cytolytic and noncytolytic mechanisms, and the biological behavior of HBeAg +ve and −ve patients is similar.

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