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Full Length Research Paper

Effects of pegylated interferon-related neutropenia and thrombocytopenia on treatment of chronic HBV infection

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This study was carried out to investigate the effect of neutropenia and thrombocytopenia on virologic response in cases with chronic hepatitis B virus (HBV) infection receiving treatment with pegylated interferon. Patients with chronic HBV infection treated with pegylated interferon were analyzed retrospectively. Patients with or without end-of-treatment and sustained virologic response were compared in terms of basic parameters and pre-treatment, and during treatment leukocyte, neutrophil and platelet counts. Sixty-two (62) cases with chronic HBV infection (47 male; mean age was 41 ± 10.91 years) were included in the study. The ratio of male gender, the ratio of previous standard interferon users and the percentage of cases with a platelet count of <100000/mm^3 at the end of the 3rd month of treatment was higher in the group with end-of-treatment virologic response compared to non-responder (male gender was 33 (84.6%) and 14 (60.9%), p = 0.035; patients with previous standard interferon use were 13 (33.3%) and 2 (8.7%), p = 0.034; and patients with thrombocytopenia were 11 (28.2%) and 1 (4.3%), p = 0.024, respectively). Pre-treatment serum HBV DNA levels were lower in cases with end-of-treatment response compared to non-responders (p < 0.005). One patient (2.6%) with end-of-treatment response and 7 (30.4%) non-responders were hepatitis B virus "e" antigen (HBeAg)-positive (p = 0.003). The only independent predictor of end-of-treatment virologic response was previous standard interferon use (odds ratio 8.157, p = 0.048). There was no difference between groups with and without sustained virologic response in terms of evaluated variables. Cytopenia development during treatment did not affect pegylated interferon treatment response in our study. End-of-treatment response to treatment with pegylated interferon is enhanced in previous standard interferon users compared to treatment-naive patients.

Key words: Hepatitis B virus, thrombocytopenia, neutropenia, interferons.

INTRODUCTION

One of the current treatments of chronic hepatitis B virus (HBV) infection consists of treatment with pegylated interferon (Marcellin et al., 2005; Akarca, 2010). Although numerous adverse effects have been reported with interferon, hematologic toxicity represents particular clinical significance (Fried, 2002). In the event of interferon-induced bone marrow suppression, reduction of dosage is recommended; however, lower doses also may reduce effectiveness of treatment (Fried, 2002). Predictors of response to treatment of chronic HBV infection with interferon have been well defined in

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literature, and it has recently been suggested that cytopenia during treatment may be one of these variables (Marcellin et al., 2005; Thomas et al., 1991; Suwantarat et al., 2010; Turbide et al., 2008; Linday et al., 2008; Alvarez-Uria et al., 2010). Although studies investigating the impact of cytopenia on response to treatment in cases with chronic hepatitis C virus (HCV) infection report contradictory results, the majority of these studies indicate a greater decline in neutrophil and platelet counts during the treatment in responders. The cytopenia effect of interferon and its antiviral effects are considered to be interrelated with interferon-induced changes in gene expression and therefore the smaller antiviral efficacy in cases with a less pronounced cytopenia. There exists no study evaluating the effect of the development of interferon-related cytopenia on response to treatment in cases with chronic HBV infection in the literature. The aim of this study was to investigate the impact of neutropenia and thrombocytopenia during treatment on end-of-treatment virologic response and sustained virologic response in cases with chronic hepatitis B infection treated with pegylated interferon.

MATERIALS AND METHODS

The medical records of patients receiving a 12-month of treatment with pegylated interferon for chronic HBV infection in our Chronic Hepatitis Outpatient Clinic between January, 2001 and January, 2010 were retrospectively evaluated.

Inclusion criteria

All of the following criteria had to be true for inclusion in the study:

1) Hepatitis B virus surface antigen (HBsAg) positivity for at least 6 months.
2) Pre-treatment HBV DNA positivity.
3) Twelve (12) months of monotherapy with pegylated interferon treatment.
4) The presence of end-of-treatment HBV DNA measurement.
5) The presence of serum leukocyte, neutrophil and platelet counts. pre-treatment, at the end of the 3rd month of the treatment, and end-of-treatment.

Exclusion criteria

The presence of any of the following was reason for exclusion from the study:

1) Active HCV infection, HIV infection, or hepatitis D virus (HDV) infection
2) Intravenous drug abuse
3) Malignancy
4) Pregnancy
5) Liver transplantation
6) Autoimmune hepatitis
7) Hemochromatosis
8) Lack of or incompletion of 12 months of treatment with pegylated interferon
9) Lack of serum leukocyte, neutrophil and platelet counts pre-treatment, at the end of the 3rd month of treatment, and end-of-treatment
10) Lack of end-of-treatment serum HBV DNA level measurements

Variables evaluated in the study

The variables are data pertaining to age at liver biopsy, gender, height, weight, alcohol use, Knodell score and fibrosis score at liver biopsy; previous treatment with standard interferon, total dose of previous standard interferon and the period between treatments with standard interferon and pegylated interferon; serum HBV DNA and alanine aminotransferase (ALT) levels; hepatitis B virus “e” antigen (HBeAg) positivity; total dose of pegylated interferon administered, pre-treatment and end of 3rd and 12th months of treatment: blood leukocyte, neutrophil and platelet counts were recorded from patient files. Cases included in the study were allocated into two groups according to end-of-treatment and sustained virologic response, and the groups were compared in terms of variables being evaluated.

Evaluation of liver histology

Liver biopsy specimens were evaluated according to the modified Knodell scoring system.

Alcohol use

All patients declaring alcohol use were considered positive for alcohol consumption regardless of the amount or duration of use.

Measurement of HBV DNA

Pre-treatment serum HBV DNA levels were measured by bDNA (branched DNA) signal amplification (Versant HBV DNA 3.0 Assay, Bayer Corp. Diagnostics, USA, detection range 2000-100000000 copies/ml) and reverse transcription polymerase chain reaction **(**RT-PCR) (1-Cobas TaqMan HBV test, Roche Diagnostics, France, detection range 30-110000000 IU/ml; 2-BioRad iCycler iQ system, Quiagen DNA isolation kit, Germany, detection range 20 IU/ml).

Definition of virologic response

End-of-treatment virologic response to treatment with pegylated interferon was defined as end-of-treatment HBV DNA negativity by PCR. Sustained virologic response was defined as HBV DNA negativity by PCR 1 year after the completion of treatment with pegylated interferon.

Determination of the total dose of pegylated interferon

The total intended dose of pegylated interferon over a 12-month period was acknowledged as 1.2 units, if the patient completed the course without dose reduction. The total interferon dose of cases was calculated based on that.

Calculation of the dose of previous standard interferon

The total dose of previous treatment with standard interferon was calculated in million units, regardless of the type of standard interferon used.

Evaluation of changes in serum leukocyte, neutrophil and platelet count during treatment

The decreases in blood leukocyte, neutrophil and platelet counts at 3rd month and 12th month of treatment were calculated. The number of patients with a blood leukocyte count <3000/mm^3, neutrophil count < 2500/mm^3 and platelet count <100000/mm^3 at 3rd and 12th months of treatment were computed.

Statistics

The SPSS-17 (SPSS Inc., Chicago, IL) statistics program was used in the evaluation of data. Continuous variables without normal distribution were expressed as median and range; continuous variables with normal distribution as mean ± standard deviation, and categorical variables as number and percentage of cases. In the comparison of patient groups with and without virologic response, the Student t-test was used for continuous variables with normal distribution, the Mann Whitney U-test for continuous variables without normal distribution, and the χ^2 test for categorical variables. The logistic regression analysis was used in the multivariate comparison of the two patient groups. The two-way analysis was used in all statistical tests. A statistical significance level of $p < 0.05$ was accepted.

An ethics committee approval was not obtained because of the retrospective nature of the study.

RESULTS

We evaluated 377 chronic hepatitis cases that were previously treated with pegylated interferon. Only 62 of the patients met the study criteria, 47 of them were male and 15 were female and the average age was 41 ± 10.91. Peginterferon alfa-2a (180 µg subcutaneously, once weekly) was used in 42 cases (67.7%) and peginterferon alfa-2b (1.5 µg/kg subcutaneously, once weekly) was used in 20 cases (32.3%). 15 (24.2%) of the patients had lower doses than planned. Eight patients were HBeAg-positive. The general characteristics of patients are shown in Table 1.

The post-treatment HBeAg status had been evaluated in only 5 of the 8 HBeAg-positive patients, and 3 (60%) of these 5 had achieved HBeAg seroconversion. Only 1 of the 3 patients with HBeAg seroconversion showed end-of-treatment virologic response. End-of-treatment virologic response was achieved in 39 (62.9%) patients, while 23 (37.1%) were non-responders. Comparative data between the two patient groups with and without end-of-treatment virologic response are shown in Tables 2, 3 and 4. 33 (84.6%) of the 39 patients with virologic response to treatment, and 14 (60.9%) of the 23 non-responders were male ($p = 0.035$). Mean pre-treatment serum HBV DNA levels were 176000 IU/ml (ranges between: 230 to 110000000) in end-of-treatment responders, and 20300000 IU/ml (ranges between: 82000 to 110000000) in non-responders ($p < 0.005$).

Only 1 patient (2.6%) with end-of-treatment viral response was HBeAg-positive, while 7 non-responders (30.4%) demonstrated HBeAg positivity ($p = 0.003$). Thirteen (13) responders (33.3%) and 2 non-responders (8.7%) had received previous treatment with standard interferon ($p = 0.034$). There was no difference between the responder and non-responder groups in terms of pre-treatment leukocyte, neutrophil and platelet counts or a reduction in these counts at 3rd and 12th months of treatment in comparison with pre-treatment levels (Table 3). At the end of the 3rd month of treatment, platelet count of <100000/mm^3 was detected in 11 (28.2%) responders and 1 (4.3%) non-responder ($p = 0.024$).

The results of the logistic regression analysis of independent predictors of end-of-treatment virologic response are shown in Table 5. Multivariate analyses revealed only the independent variable with an impact on end-of-treatment virologic response to be previous treatment with standard interferon (Odds ratio 8.158, $p = 0.048$).

HBV DNA measurements had been performed in 57 of the total 62 patients included in the study at the end of 12th month after completion of treatment. Of these 57 patients, 14 (24.6%) had achieved sustained virologic response, while 43 (75.4%) were non-responders. Mean pre-treatment HBV DNA level was 83.5 (1.84 to 110000) (×1000 IU/ml) in patients with sustained virologic response, and 1390 (32.5 to 100000) (×1000 IU/ml) in non-responders ($p = 0.08$). There was no difference between the two groups in terms of basic variables or pre-treatment blood leukocyte, platelet and neutrophil counts and changes in these counts at the 3rd and 12th months of treatment (Table 6) (Values relating to blood cells are not included in Table 6).

DISCUSSION

Variables such as being of Asian descent, infection during childhood, male gender, immunosuppression, HBeAg negativity, low serum ALT levels, infection with genotype D HBV, high serum HBV DNA levels and mild inflammation at liver biopsy have been indicated as predictors of poor response to interferon treatment in patients with chronic HBV infection (Marcellin et al., 2005; Thomas et al., 1991; Buster et al., 2009; Wu et al., 2009; Hansen et al., 2010; Wong and Chan, 2009; Zhong et al., 2008). In our study, although end-of-treatment response was poorer in female patients with high pre-treatment serum HBV DNA levels, HBeAg positivity and no previous treatment with standard interferon, previous standard interferon use was determined as the only independent predictor of response to treatment. The discrepancies among studies investigating variables predictive of response to treatment in chronic HBV infection are due to deficiencies in our understanding of the mechanism of action of interferon in the treatment of chronic HBV infection. Besides virus-related factors such as HBV genotype, host-related factors such as differences in interferon-related gene expression are considered to have a significant impact on response to treatment (Chen et al., 2005; Taylor et al., 2007). Gene

Table 1. General characteristics of cases included in the study.

Variable	Cases receiving pegylated interferon for chronic HBV infection (n = 62)
Male gender*	47 (75.8)
Age (years)**	41 ± 10.91
Body mass index**	26.36 ± 4.39
Histopathologic activity index ***	9 (5 - 13)
Fibrosis score***	1 (0 - 4)
Pre-treatment HBV DNA level (×1000IU/ml)***	896 (0.23 - 110000)
Pre-treatment serum alanine aminotransferase level (U/L)***	70 (15 - 461)
HBeAg positivity*	8 (12.9)
Pegylated interferon alfa-2a/pegylated interferon alfa-2b*	42 (67.7) / 20(32.3)
Alcohol use*	14 (22.6)
Previous standard interferon use*	15 (24.2)
Total dose of previous standard interferon use (million units)***	271.44 (162 - 360)
Period between the end of previous standard interferon treatment and start of pegylated interferon treatment (months)***	47.93 (12 - 93)
Total pegylated interferon dose***	1.2 (0.775 - 1.2)
Pre-treatment blood leukocyte count (/mm^3)**	7471 ± 1799
Pre-treatment blood neutrophil count (/mm^3)**	4038 ± 1192
Pre-treatment blood platelet count (/mm^3)**	212806 ± 48658

*: count (%)
**: mean ± standard deviation
***: median (range)

Table 2. Comparative data regarding patient groups with and without end-of-treatment virologic response to pegylated interferon.

Variable	Cases with end-of-treatment virologic response; n=39 (62.9%)	Cases without end-of-treatment virologic response; n=23 (37.1%)	p
Male gender*	33 (84.6)	14 (60.9)	0.035
Age (years)**	41.1 ± 10.44	40.82 ± 11.91	0.924
Body mass index***	26.64 (18.52 - 32.87)	25.81 (18.69 - 47.96)	0.294
Histopathologic activity index***	9 (5 - 13)	7 (5 - 13)	0.221
Fibrosis score***	1 (0 - 4)	1 (0 - 3)	0.939
Pegylated interferon alfa-2a/pegylated interferon alfa-2b*	26 (66.7) / 13 (33.3)	16 (69.6) / 7 (30.4)	0,814
Pre-treatment HBV DNA level (x1000IU/ml)***	176 (0.23 - 110000)	20300 (82 - 110000)	< 0.005
Pre-treatment serum alanine aminotransferase level (U/L)***	75 (15 - 461)	67 (33 - 370)	0.531
HBeAg positivity*	1 (2.6)	7 (30.4)	0.003
Alcohol use*	12 (30.8)	2 (8.7)	0.061
Previous standard interferon use*	13 (33.3)	2 (8.7)	0.034
Total dose of previous standard interferon (million units)***	324 (162 - 360)	349 (338.4 - 360)	0.121

Table 2. Contd.

Period between two interferon treatments (months)***	44 (12 - 93)	52 (24 - 81)	0.932
Total pegylated interferon dose***	1.2 (0.854 - 1.2)	1.2 (0.775 - 1.2)	0.135

*: count (%).
**: mean ± standard deviation.
***: median (range).

Table 3. Comparative data pertaining to case groups with and without end-of-treatment virologic response to pegylated interferon in terms of pre-treatment leukocyte, neutrophil and platelet counts and changes during treatment.

Variable		Cases with end-of-treatment virologic response; n = 39 (62.9%)	Cases without end-of-treatment virologic response; n = 23 (37.1%)	p
Blood leukocyte count (/mm^3)	Pre-treatment*	7598 ± 1936	7256 ± 1557	0.475
	3rd month*	4388 ± 1550	4643 ± 1440	0.524
	12th month**	4100 (2100 - 11900)	4000 (2600 - 6200)	0.516
Blood neutrophil count (/mm^3)	Pre-treatment*	4053 ± 1310	4013 ± 987	0.902
	3rd month*	2096 ± 799	2278 ± 771	0.384
	12th month**	2100 (1100 - 3700)	2000 (1200 - 6099)	0.610
Blood thrombocyte count (×1000/mm^3)	Pre-treatment**	199 (123 - 383)	227 (132 - 309)	0.055
	3rd month*	134 ± 46	147 ± 36	0.228
	12th month**	128 (63 - 267)	139 (68 - 210)	0.489
Decrease in leukocyte versus pre-treatment count (/mm^3)	3rd month*	3209 ± 1560	2613 ± 1491	0.145
	12th month*	3337 ± 1769	2887 ± 1300	0.293
% decrease in leukocyte count versus pre-treatment	3rd month**	42.59 [(-9.43) - 71.43]	39.47 [(-26.92) - 60.76]	0.149
	12th month**	47.22 [(-1.47) - 68.37]	41.3 [(-0.02) - 58.38])	0.229
Decrease in neutrophil versus pre-treatment count (/mm^3)	3rd month**	1800 [(-1100) - 4900]	2000 [(-2100) – 3100]	0.948
	12th month**	2000 [(-400) - 6000]	1900 [(-3999) – 3400]	0.873
% decrease in neutrophil count versus pre-treatment	3rd month**	47.36 [(-35.48) - 81.82]	48.57 [(-116.67) - 70.45]	0.771
	12th month*	43.47 ± 22.22	33.48 ± 54.06	0.311
Decrease in platelet count versus pre-treatment count (×1000/mm^3)	3rd month**	75 [(-88) - 259]	78 [(-9) – 170]	0.821
	12th month**	73 (10 - 176)	83 [(-3) – 142]	0.091
% decrease in platelet count versus pre-treatment	3rd month**	34.08 [(-41.71) - 67.62]	36 [(-6.82) - 58.49]	0.471
	12th month**	32.8 (4.74 - 72.06)	37.77 [(-2.21) - 52.2]	0.250

*: mean ± standard deviation.
**: median (range).

expression studies have demonstrated apparent distinctions between patients with chronic HCV infection with and without sustained response to treatment in terms of genes with increased or decreased expression with interferon (Chen et al., 2005; Taylor et al., 2007). We attribute discrepancies between various studies to the

Table 4. Comparative data pertaining to case groups with and without end-of-treatment virologic response to pegylated interferon in terms of changes in leukocyte, neutrophil and platelet counts during treatment.

Variable	Cases with end-of-treatment virologic response; n = 39 (62.9%)	Cases without end-of treatment virologic response; n = 23 (37.1%)	p
Cases with blood leukocyte <3.000/mm^3 at 3rd month of treatment, n (%)	6 (15.4)	3 (13)	1.000
Cases with blood leukocyte <3.000/mm^3 at 12th month of treatment, n (%)	7 (17.9)	3 (13)	0.731
Cases with blood neutrophil <1.500/mm^3 at 3rd month of treatment, n (%)	11 (28.2)	4 (17.4)	0.378
Cases with blood neutrophil <1.500/mm^3 at 12th month of treatment, n (%)	12 (30.8)	3 (13)	0.138
Cases with blood platelet <100.000/mm^3 at 3rd month of treatment, n (%)	11 (28.2)	1 (4.3)	0.024
Cases with blood platelet <100.000/mm^3 at 12th month of treatment, n (%)	9 (23.1)	3 (13)	0.508

Table 5. Results of logistic regression analysis evaluating independent predictors of end-of-treatment virologic response.

Variable	Beta value	Odds ratio	p-value
Gender	1.315	3.724	0.119
Pre-treatment serum HBV DNA level	0	1	0.128
HBeAg status	-0.639	0.528	0.673
Previous standard interferon use	2.099	8.157	0.048
Platelet count <100.000/mm^3 at 3rd month of treatment	-1.649	0.192	0.161

failure of including interferon-responsive genes among variables that have an impact on response to treatment. The systemic effects of interferon such as cytopenia, depression and loss of weight, and its antiviral affects are considered to be interrelated with interferon-induced changes in gene expression (5 to 8). We aimed to incorporate genetic factors, the most important host-related factor, as a variable influencing response to treatment by including cytopenia, a systemic effect of interferon, among the variables investigated. We encountered four previous studies investigating the effect of interferon-related cytopenia on response to treatment in chronic HCV infection, but none conducted in the chronic HBV infection setting (Suwantarat et al., 2010; Turbide et al., 2008; Linday et al., 2008; Alvarez-Uria et al., 2010). Our study is the first in this regard. A study on 111 patients with chronic HCV infection receiving 48 weeks treatment with standard interferon plus ribavirin demonstrated a greater decline in blood leukocyte count during the 2nd week of treatment and in blood neutrophil count during the 2nd and 4th weeks of treatment in the patient group who did not achieve sustained virologic response (Turbide et al., 2008). There was no difference between patient groups in terms of platelet count during treatment. It has been suggested that in patients with chronic HCV infection, neutrophils comprise the location of conservation and replication for HCV, that patients with a high HCV RNA load within neutrophils demonstrate a greater decline in neutrophil count with interferon treatment, and that the high HCV RNA load causes poorer response to treatment. As opposed to the early phase (2nd and 4th weeks) of treatment in this study, we evaluated neutrophil and leukocyte counts in later phases (3rd and 12th months) of treatment. Even if statistically insignificant, the drop in neutrophil counts at the 3rd month of treatment was less pronounced in patients with sustained virologic response compared to non-responders.

Table 6. Comparative data pertaining to case groups with and without sustained virologic response to pegylated interferon.

Variable	Cases with sustained virologic response; n = 14 (24.6%)	Cases without sustained virologic response; n = 43 (75.4%)	p
Male gender*	12 (85.7)	31 (72.1)	0.478
Age (years)**	46 (34-59)	40 (31 - 61)	0.846
Body mass index**	25.95 (22.95 - 28.69)	26.45 (21.97 - 30.48)	0.934
Histopathologic activity index **	9.5 (7 - 13)	9 (5 - 12)	0.880
Fibrosis score**	2 (1 - 4)	1 (0 - 3)	0.680
Pre-treatment HBV DNA level (×1000IU/ml)**	83.5 (1.84 - 110000)	1390 (32.5 - 100000)	0.080
Pre-treatment serum alanine aminotransferase level (U/L)**	56 (15 - 71)	75 (48 - 337)	0.993
HBeAg positivity*	0 (0)	7 (16.3)	0.176
Alcohol use*	4 (28.6)	8 (18.6)	0.463
Pegylated interferon alfa-2a/pegylated interferon alfa-2b*	8 (57.1) / 6 (42.9)	30 (69.8) / 13 (30.2)	0.384
Previous standard interferon use*	6 (42.9)	9 (20.9)	0.106
Total dose of previous standard interferon (million units)**	252 (162 - 360)	338.4 (162 - 360)	0.510
Period between two interferon treatments (months)**	39 (12 - 93)	44 (12 - 84)	0.636
Total dose of pegylated interferon **	1.2 (1.1 - 1.2)	1.15 (0.854 - 1.2)	0.200

*: count (%).
**: median (range).

It has been suggested that in patients with chronic HCV infection undergoing treatment with pegylated interferon and ribavirin, a decline in blood leukocyte, neutrophil and platelet counts indicate better response to treatment (Suwantarat et al., 2010; Linday et al., 2008; Alvarez-Uria et al., 2010). Although we did not find any difference in neutrophil and leukocyte counts during treatment, a serum platelet count of <100000/mm^3 in 3 months of treatment was more common in the patient group with end-of-treatment virologic response compared with non-responders, albeit without significance in the multivariate analysis. We attribute this to the small number of patients included in our study.

We found previous standard interferon use to be correlated with an increased end-of-treatment virologic response to pegylated interferon. Previous studies have reported 18.4 to 36.9% end-of-treatment or post-treatment virologic response with a second course of standard interferon following relapse or non-response with previous standard interferon treatment (Manesis et al., 2001; Ruiz-Moreno, 1995; Liu et al., 2007). In a study conducted on non-responders to the first course of treatment with standard interferon that were treated with pegylated interferon and monitored for 26 weeks following treatment, virologic response was 35% at the end of the follow-up period (Flink et al., 2006). Similarly, we found end-of-treatment virologic response was 33.3% with pegylated interferon treatment in patients who received previous therapy with standard interferon. We considered the better treatment response was with pegylated interferon treatment in the presence of previous use of standard interferon to the continuing effects of interferon. The previously reported ongoing beneficial effects of interferon in chronic HBV infection during the follow-up period have been justified with the following theories:

1) The diminishing antiviral T-cell response to wild type HBV by mutant T-cell epitopes in the precore/core region and the amendment of T-cell response to the virus due to the reduction of these mutants by interferon (Marinos et al., 1996).
2) The impairment of T-cell response due to high serum HBV DNA levels and the reduction in HBV DNA levels by interferon resulting in enhanced T-cell activity (Chisari et al., 1995; Bertoletti et al., 1994).
3) The stimulation of virus-specific response by the immunomodulatory effect of interferon (Papatheodoridis et al., 2001; van Zonneveld et al., 2004).

Regarding the post-treatment effects of interferon;

despite a lack of difference between interferon and placebo or lamivudine usage in terms of end-of-treatment virologic response, pegylated interferon gave better results in the long-term follow-up after treatment (Carreno et al., 1999; Marcellin et al., 2009; Lın et al., 1999). Furthermore, in support of the long-term effects of interferon treatment, biochemical response and HBeAg seroconversion were improved at 24 weeks after completion of interferon treatment compared to end-of-treatment results (Caruntu et al., 2009). However, the long-term effects of interferon are controversial due to studies reporting no difference with or without interferon treatment in terms of virologic response during long-term follow-up (Truong et al., 2005; Hsu et al., 2008).

Previous studies have indicated that relapses generally occur during the first year following treatment with pegylated interferon, and thus, we selected the end of the first year post-treatment for the evaluation of sustained virologic response (Brunetto et al., 2003). We found pre-treatment HBV DNA levels to be lower, though not with statistical significance, in responders compared with non-responders at the end of the first year following treatment. We attribute the lack of any difference between responders and non-responders at the end of the first year post-treatment in terms of variables with an established impact on response to treatment to the insufficient number of patients evaluated.

The results of this study indicate that a declining platelet or neutrophil count during treatment with pegylated interferon has no impact on response to treatment in cases with chronic HBV infection, and end-of-treatment response to treatment with pegylated interferon is enhanced in previous standard interferon users compared to treatment-naive patients.

REFERENCES

Akarca US (2010). Treatment of chronic hepatitis B. Türkiye Klinikleri J Gastroenterohepatol-Special Topics 3 (1):53-63.

Alvarez-Uria G, Day JN, Nasir AJ, Russell SK, Vilar FJ (2010). Reduction in neutrophil count during hepatitis C treatment: Drug toxicity or predictor of good response? Dig. Dis. Sci. 55(7):2058-2062.

Bertoletti A, Costanzo A, Chisari FV, Levrero M, Artini M, Sette A, Penna A, Giuberti T, Fiaccadori F, Ferrari C (1994). Cytotoxic T lymphocyte response to a wild type hepatitis B virus epitope in patients chronically infected by variant viruses carrying substitutions within the epitope. J. Exp. Med. 180(3):933-943.

Brunetto MR, Oliveri F, Colombatto P, Coco B, Ciccorossi P, Bonino F (2003). Treatment of HBeAg-negative chronic hepatitis B with interferon or pegylated interferon. J. Hepatol. 39(1):164-167.

Buster E, Hansen BE, Lau G, Pıratvısuth T, Zeuzem S, Steyerberg EW, Janssen H (2009). Factors that predict response of patients with hepatitis B e antigen-positive chronic hepatitis B to peginterferon-alfa. Gastroenterol. 137(6):2002-2009.

Carreno V, Marcellin P, Hadziyannis S, Salmeron J, Diago M, Kıtıs GE, Vafiadis I, Schalm SW, Zahm F, Manzarbeitia F, Jiménez FJ, Quiroga JA (1999). Retreatment of chronic hepatitis B e antigen-positive patients with recombinant interferon alfa-2a. Hepatol. 30(1):277-282.

Caruntu FA, Streinu-Cercel A, Gheorghe LS, Grigorescu M, Sporea I, Stanciu C, Andronescu D, Voinea F, Diculescu M, Oproiu A, Voiosu R (2009). Efficacy and safety of peginterferon alfa-2a (40KD) in HBeAg-positive chronic hepatitis B patients. J. Gastrointestin Liver Dis. 18(4):425-431.

Chen L, Borozan I, Feld J, Sun J, Tannis LL, Coltescu C, Heathcote J, Edwards AM, McGilvray ID (2005). Hepatic gene expression discriminates responders and nonresponders in treatment of chronic hepatitis C viral infection. Gastroenterol. 128(5):1437-1444.

Chisari FV, Ferrari C (1995). Hepatitis B virus immunopathogenesis. Annual Rev. Immunol. 13:29-60.

Flink HJ, Hansen BE, Heathcote EJ, Feinman SV, Simsek H, Karayalcin S, Mach T, Leemans WF, de Man RA, Verhey E, Schalm SW, Janssen HL; HBV 99-01 Study Group (2006). Succesful treatment with peginterferon alfa-2b of HBeAg-positive HBV non-responders to standard interferon or lamiudine. Am. J. Gastroenterol. 101(11):2523-9.

Fried MW (2002). Side effects of therapy of hepatitis C and their management. Hepatol. 36(5):237-244.

Hansen BE, Buster EH, Steyerberg EW, Lesaffre E, Janssen HL (2010). Prediction of the response to peg-interferon-alfa in patients with HBeAg positive chronic hepatitis B using decline of HBV DNA during treatment. J. Med. Virol. 82(7):1135-1142.

Hsu HY, Tsai HY, Wu TC, Chiang CL, Ni YH, Chen PJ, Chang MH (2008). Interferon-alpha treatment in children and young adults with chronic hepatitis B: a long-term follow-up study in Taiwan. Liver Int. 28(9):1288-1297.

Lın SM, Sheen IS, Chien RN, Chu CM, Liaw YF (1999). Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. Hepatol. 29(3):971-975.

Linday KL, Morishima C, Wright EC, Dienstag JL, Shiffman ML, Everson GT, Lok AS, Bonkovsky HL, Lee WM, Morgan TR, Ghany MG (2008). Blunted cytopenia and weight loss: New correlates of virologic null response to re-treatment of chronic hepatitis C. Clin. Gastroenterol. Hepatol. 6(2):234-241.

Liu DL, Luo KX, Feng XR, Fuo QX, Hou JL (2007). Study of the relapse of patients with chronic hepatitis B undergoing first and repeated recombinant interferon-alpha therapy during long-term follow-up. Zhonghua Yi Xue Za Zhi, 87(26):1840-1843.

Manesis EK, Hadziyannis SJ (2001). Interferon alpha treatment and retreatment of hepatitis B Eantigen-negative chronic hepatitis B. Gastroenterol. 121(1):101-109.

Marcellin P, Asselah T, Boyer N (2005). Treatment of chronic hepatitis B. In: Thomas HC, Lemon S, Zuckerman AJ (eds). Viral Hepatitis. Blackwell Publishing Ltd., Massachusetts, A.B.D. pp.323-336.

Marcellin P, Bonino F, Lau GK, Farci P, Yurdaydın C, Pıratvisuth T, Jin R, Gurel S, Lu ZM, Wu J, Popescu M, Hadziyannis S; Peginterferon alfa-2a in HBeAg-negative Chronic Hepatitis B Study Group (2009). Sustained response of hepatitis B e antigen-negative patients 3 years after treatment with peginterferon alfa-2a. Gastroenterol. 136(7):2169-2179.

Marinos G, Naoumov NV, Williams R (1996). Impact of complete inhibition of viral replication on the cellular immune response in chronic hepatitis B virus infection. Hepatol. 24(5):991-995.

Papatheodoridis GV, Manesis E, Hadziyannis SJ (2001). The long-term outcome of interferon-alpha treated and untreated patients with HBeAg-negative chronic hepatitis B. J. Hepatol. 34(2):306-313.

Ruiz-Moreno M (1995). Interferon treatment in children with chronic hepatitis B. J Hepatol. 22(1):49-51.

Suwantarat N, Tice AD, Khawcharoenporn T, Chow DC (2010). Weight loss, leukopenia and thrombocytopenia associated with sustained virologic response to hepatitis C treatment. Int. J. Med. Sci. 7(1):36-42.

Taylor MW, Tsukahara T, Brodsky L, Schaley J, Sanda J, Stephens MJ, McClintick JN, Edenberg HJ, Li L, Tavis JE, Howell C, Belle SH (2007). Changes in gene expression during pegylated interferon and ribavirin therapy of chronic hepatitis C virus distinguish responders from nonresponders to antiviral therapy. J. Virol. 81(7):3391-3401.

Thomas HC, Karayiannis P, Brook G (1991). Treatment of hepatitis Bvirus infection with interferon. Factors predicting response to interferon. J. Hepatol. 13(1):4-7.

Truong BX, Seo Y, Kato M, Hamano K, Ninomiya T, Katayama M, Kato H, Yano Y, Hayashi Y, Kasuga M (2005). Long-term follow-up of Japanese patients with chronic hepatitis B treated with interferon-

alpha. Int. J. Mol. Med. 16(2):279-284.
Turbide C, Soulellis CA, Deschenes M, Hilzenrat N (2008). Does a rapid decline in the hematological and biochemical parameters induced by interferon and ribavirin combination therapy for the hepatitis C virus predict a sustained viral response. Can. J. Gastroenterol 22(2):149-152.
van Zonneveld M, Honkoop P, Hansen BE, Niesters HG, Darwish MS, deMan RA, Schalm SW, Janssen HL (2004). Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. Hepatol. 39(3):804-810.
Wong GL, Chan HL (2009). Predictors of treatment response in chronic hepatitis B. Drugs 69(16):2167-2177.
Wu XJ, Wang Y, Chen J, Wang GQ (2009). Baseline predictors of virological response for chronic hepatitis B patients. World J. Gastroenterol. 15(34):4311-4315.
Zhong RX, Lai CY, Liang D, Long H (2008). Responses of peginterferon-alpha 2a antiviral therapy in chronic hepatitis B patients. Zhonghua Gan Zang Bing Za Zhi 16(4):279-282.