

Pegylated Interferon α Therapy in Chronic Delta Hepatitis: A One-Center Experience

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Background: The only established therapy for chronic viral delta hepatitis, the most severe form of viral hepatitis is treatment with pegylated-interferon α (Peg IFN α).

Objectives: In this study, we aimed to determine the efficacy of pegylated-interferon α 2a (Peg-IFN α 2a) and 2b (Peg IFN α 2b) in the treatment of patients infected with chronic delta hepatitis virus.

Patients and Methods: The sample size was based on available patients potentially to be recruited. Data of 63 patients receiving either Peg IFN alpha 2a or Peg IFN alpha 2b were retrospectively assessed in the present cohort study performed in Turkey. Of 56 patients completed the study, 41 received Peg IFN α 2a and 15 received Peg IFN α 2b for 12 months. Patients were evaluated for biochemical and virological responses at the end of given treatment and six months after the treatment.

Results: Stage of fibrosis was found high in both groups (85.4% vs. 86.7%), while cirrhosis was higher in the group of Peg IFN α 2b (53.3% vs. 34.1%). At the end of treatment, either hepatitis delta virus RNA (HDV RNA) alone or both HDV RNA and hepatitis b virus DNA (HBV DNA) had negative results in 32% of patients. Although HDV RNA negativity was sustained in 30.3% of patients, negativity of both HDV RNA and HBV DNA was decreased to 19.6% six months after completion of the treatment. HBV DNA became positive in one third of patients with response at six months after completion of the treatment (10.7% of all patients). HDV RNA negativity at month six was found as a predictor of positive response. No significant difference was found between Peg IFN α 2a and Peg IFN α 2b for virological response rate.

Conclusions: Treatment with Peg IFN α achieved a sustained negativity of HDV RNA in about one third of patients. Duration of Peg IFN α therapy might be prolonged to at least 24 months or more to prevent the occurrence of Hepatitis B virus (HBV) relapse encountered six months after completion of the treatment.

Keywords: Hepatitis D; Interferon; Therapeutics

1. Background

Hepatitis Delta Virus (HDV) infection is widely distributed worldwide. It is believed that about 15 million people are infected with HDV (1). Infection with hepatitis delta virus (HDV) is a major health problem, at least in some regions of the world. HDV is a defective RNA virus. It requires helper function of hepatitis B virus (HBV) for transmission and replication (2). Clinically, HDV is usually defined as superinfection in individuals with viral hepatitis B. HDV infections defined in Europe and our country are usually associated with severe liver damage (3-5). Levels of HDV viremia change over the course of HDV infection, being significantly higher in patients with early chronic hepatitis than cirrhosis. Chronic HDV infection leads to more severe liver disease than chronic HBV monoinfection with an accelerated course of fibrosis progression, an increased risk of hepatocellular carcinoma and early decompensation in the setting of established cirrhosis (6). Prevalence of HDV varies in different geographical

regions (7). Prevalence of Hepatitis Delta Virus infection has been decreased in western regions of Turkey, while it is still high in eastern and southern regions (8). Treatment need among chronic viral hepatitis is highest for chronic HDV due to its most rapid progressive course of disease among hepatotropic virus infections. However, treatment of chronic HDV infection is difficult as it does not have an enzymatic function as a target, such as polymerases and proteases of HBV and hepatitis C virus (9). In the past, treatments failed with thymosin, ribavirin and acyclovir, famciclovir and lamivudine (10-14). The therapy available today is not different from limited interferon treatment attempted more than 20 years ago (15).

2. Objectives

The aim of this study was to review retrospectively the efficacy of Pegylated-interferon α 2a (Peg IFN α 2a) alone

and 2b (Peg IFN α 2b) alone in the treatment of patients infected with chronic delta hepatitis virus. HDV usually has suppressive effects on HBV-DNA levels. Thus, monitoring HBV DNA levels during antiviral therapy is of vital importance. Thus, we aimed to determine effects of therapeutic regimen with Peg IFN α 2a and Pen IFN α 2b on the levels of HBV RNA and HDV RNA. We also aimed to evaluate normalization of alanine aminotransferase (ALT) level and HBV DNA and HDV RNA negativity after six months of follow-up.

3. Patients and Methods

3.1. Study Population

Adult patients (> 18 years old) with CDH admitted to Gastroenterology outpatient clinic of Firat University Hospital, Elazig, Turkey between 2004 and 2012 were analyzed retrospectively. Firat University Hospital is a general referral government hospital with more than 800 hundred beds, thirty different medical and surgical sections. Patients who had compensated liver disease, positive results for HBsAg and total anti-HDV antibodies for at least three months and positive results for HDV RNA on polymerase-chain reaction assay with ALT levels not $\geq 10 \times$ ULN were included in the study. Furthermore, data on liver biopsy obtained within the past 12 months demonstrating liver disease consistent with chronic hepatitis were also sought.

3.2. Exclusion Criteria

1. Presence of any other etiology chronic liver disease: positive (+) HAV IgM Ab, HCV-RNA or HCV Ab, serum ceruloplasmin and $\alpha 1$ antitrypsin concentrations consistent with increased risk of metabolic liver disease. 2. Seropositivity for HIV antibody (anti HIV). 3. Patients with a history of hemorrhage from esophageal varices or evidence of decompensated liver disease (Childs B-C). 4. Presence of a white blood cell count lower than 3000 mm^3 or platelet count lower than $5000/\text{mm}^3$ in complete blood count. 5. Patients who received therapy for hepatitis D in past six months were excluded.

Efficacy of two different Peg IFN regimens given to 63 treatment naive CHD patients for 12 months was compared and no control group was included in the study. This study was a retrospective cohort study. No approval from the institutional ethics committee was required because of the retrospective analysis of patients records. The study protocol conformed to the ethical guidelines of the 1975 declaration of Helsinki. The patients were evaluated at baseline, at the end of therapy and six months after completion of therapy for alanine aminotransferase (ALT), serum level of direct and indirect bilirubin, albumin and whole blood count and once every six months for HBsAg, Anti-Delta, HBV DNA and HDV RNA levels. Adverse effects of drugs were recorded from the medical re-

ports of patients. The reason for withdrawal of patients from treatment was investigated. In patients with liver disease consistent with chronic hepatitis in liver biopsy performed over the last 12 months, necroinflammation and fibrosis were assessed with the Knodell histology activity index (HAI) scoring system and the Ishak modification of this system. The pathologist was blinded to the patients' clinical outcomes and biopsy sequence. Serological markers were studied using ELISA assay and levels of HBV DNA (Roche molecular systems, Branchburg, USA) and HDV RNA (Roche molecular systems, Branchburg, USA) were studied using PCR method.

3.3. Endpoints

Eradication of HDV RNA detected through PCR as the main target of antiviral therapy in Delta hepatitis. Response at the end of treatment was defined as normalization of ALT level and HDV RNA negativity at the end of treatment. Sustained biochemical and virological response was defined as normalization of ALT level and HDV RNA negativity at the end of six months of follow-up.

3.4. Statistical Analysis

The sample size was based on available patients that could be recruited. Baseline characteristics and measures of clinical and demographic predictors were summarized using mean, median, standard error, standard deviation (SD) and minimum and maximum values. The results were expressed for hazard ratios with 95% confidence intervals (CI). All analyses were performed using SPSS 22.0 program (SPSS® Statistics Grad Pack 22). The chi-square, Mann-Whitney U and Kruskal Wallis tests were used in the evaluation of different variables when appropriate. Nonparametric Friedman test was used for repeated analysis. $P < 0.05$ was considered statistically significant.

4. Results

Sixty-three patients received treatment with Pegylated-interferon for positive chronic viral B + Delta hepatitis. Seven patients discontinued the study because of adverse effects of medications. Fifty-six patients completed the study. Of these patients, 39 were male and 17 female. Forty-one patients received Peg IFN α 2a (Pegasys 180 μg , ROCHE) and 15 patients received Peg IFN α 2b (PegIntron 1.5 $\mu\text{g}/\text{kg}$, MSD). Mean age of patients were 47.6 ± 9.5 years for patients receiving Peg IFN α 2a and 51.1 ± 12.4 years for those receiving Peg IFN α 2b. Only one patient in each group had positive result for HBe Ag and the rest were Hbe Ag negative. All patients had positive results for HDV RNA; whereas, only 61% of those receiving Peg IFN α 2a had positive results for HBV DNA. However, the rate of positive HBV DNA was even higher in those receiving Peg IFN 2b (80%). Stage of fibrosis was high in the both groups (85.4% vs. 86.7%, respectively), while presence of cirrhosis

was higher in the group receiving Peg IFN α 2b compared to the group receiving Peg IFN α 2a (53.3% vs. 34.1%). Baseline characteristics of patients are shown in Table 1.

Eradication of HDV RNA detected through PCR is the main target of antiviral therapy in delta hepatitis. We found a statistically significant difference for eradication of HDV RNA at month six, the end of therapy and six months after completion of therapy [χ^2 (3) = 50.272, (IQR: 0, 1, 1, 1) $P = 0.001$], using nonparametric Friedman test. At the end of treatment, either HDV RNA alone or both HDV RNA and HBV DNA was found negative in 32% of all patients (Standard Error: 6, 2, 95% CI: 19.6 - 44.6). Although HDV RNA negativity was sustained in 30.3% of patients, negativity of both HDV RNA and HBV DNA was decreased to 19.6% six months after completion of treatment. HBV DNA became positive in one third of patients with response at six months after the end of treatment (10.7% of all patients). HDV RNA was found negative in 18 of 56 patients (32.1%) at month six and negativity at month six during the treatment was found as a predictor of positive response ($P < 0.001$). No significant differ-

ence was found between Peg IFN α 2a and Peg IFN α 2b for virological response rate (Pearson χ^2 : 3.323; P : 0.068, Fisher exact test: 0.106). Of 18 patients who achieved sustained virological response, 16 received Peg IFN α 2a, while two received Peg IFN α 2b. Moreover, 11 patients with sustained virological response had negative results for both HBV DNA and HDV RNA, whereas HBV DNA was found positive albeit HDV RNA was negative in six patients six months after the treatment. Only one patient had virologic relapse with reappearance of HDV RNA at six months after treatment and virologic response sustained in rest of patients who had achieved HDV RNA negativity at the end of treatment. Virological response outcomes are given in Table 2. There was a significant decrease at baseline mean ALT levels for all patients and for those receiving Peg IFN α 2a at the end of treatment and six months after the end of treatment ($P < 0.05$, $P < 0.01$, respectively), but it did not reach statistical significance in the Peg IFN α 2b group. ALT normalization was present in 19 (32.2%) among all patients six months after completion of treatment.

Table 1. Baseline Characteristics ^{a, b}

Parameters	Peg IFN α 2a (n = 41)	Peg IFN α 2b (n = 15)	P Value
Age, y	47.6 \pm 9.5	51.1 \pm 12.4	0.240
Gender			0.772
Male	29	10	
Female	12	5	
Hemoglobin, g/dL	13.2 \pm 1.8	13.5 \pm 1.8	0.591
The mean platelet count, 1000/mm ³	155.8 \pm 108.7	148.8 \pm 69.8	0.810
Mean ALT, IU/L	88.2 \pm 101.6	81.6 \pm 62.9	0.704
Albumin, g/dL	4.0 \pm 0.6	3.9 \pm 0.7	0.940
HbeAg positivity	1 (2.4)	1 (6.7)	
HBV DNA positivity	25 (61)	12 (80)	0.187
HBV DNA level, COPY/mL	548 $\times 10^3 \pm 274 \times 10^3$	23 $\times 10^3 \pm 35 \times 10^3$	0.007
HDV RNA Level, IUL/ML	135 $\times 10^3 \pm 394.6$	68.5 $\times 10^3 \pm 201.21$	0.703
Cirrhosis	14 (34.1)	8 (53.3)	
Fibrosis stage > 2	35 (85.4)	13 (86.7)	

^a Abbreviations: ALT, Alanine aminotransferase; HBV; Hepatitis B virus; HDV RNA, Hepatitis D virus.

^b Data are presented as No. (%) or Mean \pm SD.

Table 2. Response Rates of Therapeutic Groups of Peg IFN 2a and Peg IFN 2b at the End of Treatment and Six Months After Completion of Treatment ^{a, b}

	Patients With Negative HBV DNA and HDV RNA at the End of Treatment	Patients With Negative HDV RNA and HBV DNA 6 Months After Treatment	Patients With HBV DNA Becoming Positive While HDV RNA Was Negative 6 Months After Treatment
Peg INF α 2a (n = 41)	16 (39)	9 (22) ^c	6 (14.6)
Peg INF α 2b (n = 15)	2 (13.3)	2 (13.3)	0
Total (n = 56)	18 (32)	11 (19.6)	6 (10.7)

^a Abbreviations: ALT, alanine aminotransferase; HBV; Hepatitis B virus; HDV RNA, Hepatitis delta virus RNA.

^b Data are presented as No. (%).

^c No statistical significance between two groups ($P > 0.05$).

5. Discussion

Chronic HDV is less common than HBV and HCV infections causing serious liver damage. Its prevalence is still high in eastern and south-eastern regions of Turkey and considered as a significant public health problem. HDV infection is associated with severe liver damage and usually leads to cirrhosis (16). Therapeutic options are limited. The only treatment with proven efficacy is interferons (14, 16, 17). There has been an increase in the number of studies conducted with pegylated-interferons in recent years. One of the earliest studies is that of Niro et al. (18). Thirty-eight patients with positive HBsAg and HDV RNA and ALT level 1.5 times of the upper normal limit were included in the study and randomized in two groups. One group received Peg IFN α 2b 1.5 mcg/kg alone, while the other group received Peg IFN α 2b 1.5 mcg/kg combined with ribavirin for 48 months. Virological response was found to be 19% (3/11) for monotherapy and 9% (2/16) for combination therapy 24 months after completion of the treatment. Discontinuation rate was 29% in the ribavirin arm. Hepatic flare occurred in two patients, but no hepatic decompensation occurred. Cirrhosis was found histologically in 28 of 38 patients included in the study. Thirty of them were patients with no response to standard interferon therapy. Response of naive patients to treatment was found to be better. Adding ribavirin to pegylated interferon treatment has been reported not to provide any contribution (18). In another study, 14 patients with chronic hepatitis delta infection received Peg IFN α 2b subcutaneously at a dose of 1.5 mcg/kg for 12 months and at the end of treatment, persistent response was found in eight (57%) patients and persistent virological response in six (43%) patients. For HDV RNA kinetics, HDV RNA levels were lowest in patients who showed persistent response compared to those who did not. Peg IFN α 2b was found to be reliable and effective in the treatment of chronic viral delta hepatitis (19). In our study, of 56 patients who completed the one-year treatment with Peg IFN α 2a and 2b, 32% were both HDV RNA-negative and HBV DNA-negative at the end of treatment, but this rate was 19.6% six months after the completion of treatment. When only HDV RNA negative status was considered, the rate was 30.3% at six months after the completion of study. Only one patient became HDV RNA-positive. On the other hand, we found positive results for HBV DNA in six (10.7%) patients six months after the treatment, although it was negative at the end of treatment. The response rates in the multicenter study by Wedemeyer et al. (20) were similar to our study for HDV RNA negativity. They found a persistent response rate of 25% and biochemical response rate of 40% with Peg IFN α 2a. Karaca et al. (21) found higher persistent virological and therapeutic response rates compared to our study. They found a virological response rate of 50% and persistent virological response rate of 47% at the end of treatment with Peg IFN α 2a (180 mcg) and α 2b (1.5 mcg/kg) for 24 months in 32 pa-

tients with chronic hepatitis Delta. Hepatitis Delta prevalence is high in Pakistan and in a study conducted there, persistent virological response was 29.4% in 238 of 277 patients who completed the study and treated with Peg IFN α 2a for 48 months (22). In many studies and in our study as well, persistent HDV RNA negativity occurred at a rate of 30%. For both HDV DNA and HBV DNA negativity, this rate was lower in our study (19.6%). The most important finding in our study was that HBV DNA became positive in 10.4% of patients six months after completion of study. Chronic delta hepatitis is a dual infection. Delta infection is dominant in most cases (16). Initially, HBV DNA positivity was 61% in the group of Peg IFN α 2a and 80% in the group of Peg IFN α 2b. Probably, suppression on replication of hepatitis B virus disappears when HDV RNA which is dominant becomes negative as a consequence of Peg IFN α treatment. When the treatment is terminated, HBV DNA becomes positive in some patients. We started and maintained oral antiviral treatment in these patients. Considering long-term use of oral antivirals, using Peg IFN for longer-term (for two years) appears more reasonable. Gulsun et al. (23) found that relapse was lower with two years of Peg IFN α treatment. Ormeci et al. (24) suggested no difference between the treatments administered for 12 and 24 months. Number of patients was low in the study by Ormeci et al. (24). Peg IFN α 2a demonstrated higher response rates compared to Peg IFN α 2b, but the difference did not reach statistical significance. The fact that the rate of cirrhosis was higher in patients receiving Peg IFN α 2b may be a factor affecting the response to treatment (53.3% vs. 34.1%). Samiullah et al. (22) found that significant positive response was a predictor of lower stage of fibrosis. Furthermore, the fact that viral load was lower in the group of Peg IFN α 2b may be explained by higher cirrhosis rate in this group. As in previous studies (18, 21), in the present study the positive response predictor was HDV RNA negativity at month six. This may be instructive in determining the duration of treatment. Significant decrease occurred in ALT levels at the end of treatment and six months after completion of the treatment. Biochemical response is usually associated with virological response. If there is persistent ALT elevation despite virological response, one should explore especially fatty liver and autoimmune hepatitis, because patients rapidly losing weight during Peg IFN α treatment show weight gain at the end of treatment. Main limitations of the present study were its small sample size and absence of a power analysis. One reason for this was limited number of chronic HDV patients receiving any type of treatment due to mainly lack of appropriate treatment strategies. However, using the treatment response rates, we analyzed the power of study and found it more than 0.8. In conclusion, treatment with Peg IFN α achieved HDV RNA negativity in about one third of patients. HBV is activated in one third (10.7%) of patients. Extending

the treatments to two years in patients with response to treatment may prevent activation of HBV.

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