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Original Article

Evaluation of the long-term outcomes of patients with hepatitis delta

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Abstract

Objective: To evaluate the long-term outcomes of hepatitis delta patients, including cirrhosis and hepatocellular carcinoma (HCC), based on clinical and laboratory data.

Methodology: A retrospective evaluation was conducted on patients diagnosed with hepatitis delta. The patients were formed into four groups: no-treatment, pegylated interferon, oral antiviral, and combined treatment.

Results: A total of 93 patients, 48 women and 45 men, were evaluated in the study. The mean follow-up time was 4.38 ± 2.7 years. Of those, 43 were in the no-treatment group, 22 received combination therapy including pegylated interferon and oral antiviral treatment for chronic hepatitis B (the combined treatment group), 19 received only oral antiviral treatment for chronic hepatitis B (the oral antiviral group), and nine received pegylated interferon (the pegylated interferon group). HDV-RNA negativity was observed in 67% (6/9) of patients in the pegylated interferon group and 33% (5/15) of patients in the combined treatment group. HDV-RNA became spontaneously negative in one of the two patients in the no-treatment group, while no patient in the oral antiviral group became HDV-RNA negative. Seven patients were diagnosed with cirrhosis and one with HCC. Three patients had undergone liver transplants. There were no fatalities among patients.

Conclusions: Pegylated interferon therapy has been demonstrated to have partial efficacy in the treatment of delta hepatitis, while oral antivirals have been shown to offer no additional benefit. Although negative HDV-RNA was achieved in some patients treated with pegylated interferon, pegylated interferon treatment could not eliminate the risk of cirrhosis and HCC.

Key words: Hepatitis delta virus; cirrhosis; hepatocellular carcinoma; pegylated interferon; antiviral treatment; liver transplantation.

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Introduction

In 1977, Rizzetto *et al.* found a distinct antigen in the liver biopsies of certain hepatitis B patients. Later research revealed that this structure-dubbed delta antigen- belonged to a virus. hepatitis delta virus (HDV) was the later nomenclature for this antigen [1]. A damaged RNA virus with a preference for the liver is called HDV which cannot induce disease in the absence of hepatitis B virus (HBV) [2]. According to recent HDV prevalence research, 4.5% to 13.6% of hepatitis B surface antigen (HBsAg) positive individuals globally have anti-HDV antibodies [3-5].

Liver-related events and deaths, such as cirrhosis, and hepatocellular carcinoma (HCC) are significantly increased by HBV infection, but delta hepatitis causes more liver damage compared to HBV [6]. HDV is the cause of 1/5 of diagnoses for HCC and 1/6 of diagnoses for cirrhosis linked to HBV [3]. Pegylated interferon alpha 2a or 2b is the only approved and effective treatment for delta hepatitis. It was reported that 25% of patients benefit from a virological response following pegylated interferon therapy. Oral antiviral treatments for chronic hepatitis B combined with pegylated interferon have not shown any further advantage in the treatment of hepatitis delta [6]. Patients with hepatitis delta must be closely monitored due to its rapid progression to cirrhosis and HCC. Patients have no other choice than to undergo a liver transplant when they develop decompensated cirrhosis or HCC.

This study aimed to retrospectively evaluate the long-term consequences of hepatitis delta, including cirrhosis and HCC, using clinical and laboratory data of patients.

Methodology

Study Design

A retrospective analysis was conducted on patients diagnosed with hepatitis delta and followed up at the department of Infectious Diseases and Clinical Microbiology, Bakirkoy Dr. Sadi Konuk Training and Research Hospital from January 1, 2015, to June 1, 2022. The diagnosis of hepatitis delta was defined as a positive anti-delta total test (reported as IgM or IgG antibody positivity or both) and HBsAg positivity for

more than six months.

Patients who discontinued the follow-up at our department or were diagnosed with hepatitis C or human immunodeficiency virus (HIV) co-infection, alcohol-related liver disease, or autoimmune hepatitis were not included in the study. Four patient groups were formed: no-treatment group, pegylated interferon group (patients who received pegylated interferon alpha 2a or 2b in the follow-up), oral antiviral group (patients who received only oral antiviral treatment for chronic hepatitis B in the follow-up), and combination therapy group (patients who received both oral antiviral therapy for chronic hepatitis B and pegylated interferon alpha 2a or 2b in the follow-up). Laboratory data and clinical courses of patients were compared for those four groups. Patients taking oral antivirals were given one of the following options for hepatitis B treatment: lamivudine 100 mg/day, entecavir 0.5 mg/day, tenofovir disoproxil fumarate (TDF) 245 mg/day, or tenofovir alafenamide fumarate (TAF) 25 mg/day. Patients receiving pegylated interferon treatment were administered 180 mcg pegylated interferon alfa-2a or pegylated interferon alfa-2b 1.5 μg/kg per week.

Oral antiviral treatment was administered in accordance with the Turkish National Chronic Hepatitis B Treatment Guide criteria. Pegylated interferon treatment was recommended to patients who tested positive for HDV-RNA. Some patients refused to receive pegylated interferon treatment due to potential side effects of the treatment. Pegylated interferon treatment was recommended to some patients with high liver enzymes despite being HDV-RNA negative, in accordance with the Turkish National Hepatitis Delta Treatment Guide criteria. This treatment was applied to the patients with their signed consent.

The primary aim of the study was to compare the development of cirrhosis and HCC, the need for liver transplantation, mortality and laboratory results during the follow-up period in these four groups to evaluate hepatitis delta progression. The secondary aim was to determine the parameters associated with the development of cirrhosis by comparing the laboratory results of individuals who developed cirrhosis and HCC with other patients who did not develop. Clinical symptoms, laboratory tests, and liver imaging were used to determine cirrhosis [7]. HCC was diagnosed by histopathological examination or dynamic abdominal magnetic resonance imaging (MRI) with contrast.

Data Collection

Data about the patients were obtained retrospectively from the hospital information management system. The follow-up period commenced on the date when a positive delta antibody was found. Patient data for each year were recorded separately. Laboratory findings after six years were excluded from the statistical analysis, as the number of patients with a follow-up period longer than five years was insufficient for statistical evaluation. The standardized normal ranges of the laboratory tests described by the hospital laboratory and taken into consideration in the assessment of patients in our study were: 0-37 U/L in men and 0-32 U/L in women for aspartate aminotransferase (AST); 0-41 U/L in men and 0-33 U/L in women for alanine aminotransferase (ALT); 0-60 U/L in men and 0-36 U/L in women for gamma glutamyl transferase (GGT); 40-129 U/L in men and 35-104 U/L in women for alkaline phosphatase (ALP); < 1.2 mg/dL for total bilirubin; 11-16 seconds for prothrombin time (PT); 0.9-1.2 for international normalized ratio (INR); 3.5-5.2 mg/dL for albumin; 0-7 mcg/L for Alpha-Fetoprotein (AFP); and 155.000-366.000 cells/μL for platelet count. HDV-RNA polymerase chain reaction (PCR) assay was performed using the Rotor-Gene Qiagen system®. The Real-Time PCR kit is calibrated in accordance with the first World Health Organization International Standard for HDV-RNA. Plasma samples were analyzed with the HDV-RNA PCR kit with a measurement range of 200 to 20.000.000.000.000 IU/mL. In Turkey, a routine liver biopsy is not performed on patients with delta hepatitis because the social insurance system requires liver biopsy only for those who will receive oral antiviral treatment for chronic hepatitis B.

Statistical Analysis

Statistical analysis was performed using the SPSS 27.0 (Statistical Package for the Social Sciences) software. The research data was evaluated using descriptive statistical approaches such as mean, standard deviation, median, frequency, and ratio. The decimal figures were rounded. The one-way ANOVA and Bonferroni tests were used to compare three or more normally distributed groups. Student t-test or Mann-Whitney U test was used to determine which group caused the difference in two-group mean evaluations. The chi-square test was used to compare nominal data, but Fisher's exact test was utilized when the sample size was less than 15. At the $p < 0.05$ level, statistical significance was defined.

Ethics

Our study, whose research protocol code 2022/395, was found ethically appropriate by the Clinical

Research Ethics Committee of Sadi Konuk Training and Research Hospital with the date of December 5, 2022, and the decision number 2022-03-06.

Results

Retrospective evaluations were conducted on 93 patients with hepatitis delta. Of these patients, 48 (52%) were female and 45 (48%) were male. At the diagnosis, the mean age was 41.74 ± 14.47 years for all patients, 39.42 ± 13.13 years for the female patients, and 44.22 ± 15.53 years for the male patients. The mean follow-up period of the patients was 4.38 ± 2.7 years. Of the 93 patients, 85 (91%) were hepatitis B envelope antigen (HBeAg) negative and anti-HBe positive, as eight (9%) were HBeAg positive and anti-HBe negative. At the beginning of the follow-up, the HBV-DNA level was less than 2000 IU/mL in 73 (78%) patients. Of the 20 patients with HBV-DNA values higher than 2000 IU/mL, 14 (70%) received oral antivirals for the treatment of chronic hepatitis B. The no-treatment group included 43 (46%) patients, the combination therapy group had 22 (24%), the oral antiviral group had 19 (20%), and the pegylated interferon group had nine (10%). During the follow-up period, seven patients (8%) were diagnosed with cirrhosis, one patient (1%) was diagnosed with HCC, and three patients (3%) had liver transplants. There were no deaths among the 93 patients.

In the no-treatment group, there were 25 (58%) women and 18 (42%) men. The mean age was 45.19 ± 12.14 years. The mean follow-up period was 3.42 ± 1.61 years. Of those patients, two (5%) were HBeAg positive and six had a liver biopsy on their initial visit. The mean fibrosis score was 1.33/6 and the mean histological activity index (HAI) was 3.67/18. HDV-RNA was negative in 39 patients (91%) and positive in two patients (5%) with a mean of 9257.5 ± 13052.48 IU/mL. ALT levels remained within the normal range in 30 patients (70%) throughout the follow-up period, as eight patients (19%) recovered and five patients (11%) continued to have high ALT levels. Spontaneous negative HDV-RNA was achieved in one of two patients. HBsAg seroconversion developed in nine patients (21%), as anti-HBs seroconversion developed in four patients (9%). Only this group achieved an HBsAg seroconversion and no patients developed cirrhosis or HCC.

In the combined treatment group, there were 22 patients including 15 (68%) males and seven (31%) females with a mean age of 37.18 ± 19.42 years. The mean follow-up period was 6.64 ± 3.67 years. HBeAg was positive in three patients (14%). In the first follow-up year, 19 of 22 patients underwent a liver biopsy. The mean fibrosis score was 2.95/6, and the mean HAI score was 8.37/18. HDV-RNA was positive in 15 (68%) patients with a mean of 2505657 ± 5571022 IU/mL and seven (32%) were HDV-RNA negative. Oral antiviral treatment lasted an average of 6.32 ± 3.96 years (range: 1-12 years), as pegylated interferon lasted an average of 14.64 ± 7.61 months (range: 3-31 months). While 13 (59%) patients received TDF, four (18%) received entecavir, four (18%) received TAF, and one (5%) received lamivudine. During the follow-up, ALT levels remained high in 12 patients (55%), recovered in four patients (18%), and remained within normal limits in four patients. Negative HDV-RNA was achieved in five of 15 patients (33%). No recurrence was observed in these five patients during follow-up. Negative HDV-RNA and ALT normalization were achieved in four patients (18%). In this group, two out of three patients diagnosed with cirrhosis underwent liver biopsy in the first year of follow-up for receiving oral antiviral treatment for chronic hepatitis B. One of both patients had a fibrosis score of 2/6 and a HAI of 9/18, while the other had a fibrosis score of 3/6 and a HAI of 10/18.

The oral antivirals group included 12 (63%) women and seven (37%) males, with a mean age of 40.26 ± 13.55 years. The mean follow-up period was 4.05 ± 2.07 years. The mean period of oral antiviral medication was 2.95 ± 2.20 years (range: 1-8 years). Among them, six patients (32%) received TDF, five (26%) received entecavir, four (21%) received TAF, and four (21%) received lamivudine. In this group, three patients (16%) were HBeAg positive, 16 (84%) were HDV-RNA negative, and two (11%) were HDV-RNA positive (mean: 221123 ± 202136.4 IU/mL). Two patients remained to be positive for HDV-RNA. In the first follow-up year, liver biopsies were done on 16 out of 19 patients, with mean scores of 1.94/6 for fibrosis and 6.44/18 for HAI. Following therapy, ALT levels remained high in five patients (26%) within normal ranges in four patients (21%), and recovered in seven patients (37%). Cirrhosis was diagnosed in two patients (11%), and two patients (11%) underwent liver transplantation due to cirrhosis and cirrhosis + HCC, respectively. The fibrosis score of the patient diagnosed with cirrhosis was 1/6, and the HAI was 6/18 in the liver biopsy performed five years before the diagnosis of delta hepatitis due to the treatment for chronic hepatitis B.

The pegylated interferon group included five (56%) males and four (44%) females, with a mean age of 39.56 ± 9.91 years. The mean follow-up period was 4.11 ± 2.52 years.

Table 1. Comparison of laboratory results between groups.

		No-treatment Group		Combination Therapy Group		Oral Antiviral Group		Pegylated Interferon Group		*p*
		n	**Mean**	**n**	**Mean**	**n**	**Mean**	**n**	**Mean**	
AST (U/L)	1st year	43	26.49	15	228.07	18	46.5	7	39.57	**0.000**
	2nd year	28	28.11	14	38.07	13	39.46	5	60.8	**0.002**
	3rd year	22	26.09	11	41.73	12	31.75	6	33.17	**0.035**
	4th year	20	20.35	12	48.17	11	40	5	31.8	**0.000**
	5th year	13	21.69	11	48.27	8	32.75	3	60.67	**0.002**
	6th year	12	18.92	11	52.36	6	38.33	2	62	**0.001**
ALT (IU/L)	1st year	43	29.93	15	188.93	18	62.94	7	54	**0.002**
	2nd year	28	33.96	14	36.5	13	48.54	5	51.8	0.145
	3rd year	22	30.82	11	48.73	12	36.25	6	32.33	0.210
	4th year	20	20.65	12	59.42	12	54.75	5	53.4	**0.000**
	5th year	13	23.31	11	51.27	8	38.5	3	85.33	**0.010**
	6th year	12	22.75	11	61	6	35.83	2	130	**0.011**
GGT (U/L)	1st year	30	25.93	12	56.5	14	45.5	5	51.2	**0.013**
	2nd year	23	33.39	10	41.6	12	22.17	4	76	0.111
	3rd year	21	33.38	10	45.9	10	27.2	5	71.2	0.273
	4th year	17	21.94	11	37.27	11	37.55	5	84.4	0.054
	5th year	10	21.6	11	40	7	39.29	3	72.33	0.341
	6th year	11	23.55	10	32.4	6	53.33	2	60	0.461
ALP (U/L)	1st year	15	72.4	9	83	5	107.8	3	65	0.170
	2nd year	14	76.21	8	83	9	94.22	2	57	0.351
	3rd year	12	87.5	9	83.44	8	103.38	3	75.33	0.792
	4th year	14	88.07	8	86.5	10	104.8	4	75.25	0.304
	5th year	7	72.43	8	86.75	4	103	3	87	0.487
	6th year	10	73.1	9	122.89	5	113	2	74.5	0.192
Total Bilirubin (mg/dL)	1st year	20	0.44	10	1.58	13	0.7415	6	0.62	**0.003**
	2nd year	18	0.44	11	0.69	10	0.736	4	0.78	**0.027**
	3rd year	14	0.55	10	0.59	10	0.61	5	0.64	0.778
	4th year	14	0.51	10	0.79	8	0.93	5	0.56	0.286
	5th year	5	0.41	9	0.55	7	0.64	2	0.60	0.593
	6th year	10	0.41	10	0.83	5	0.49	2	0.5	0.090
PT (sec)	1st year	33	12.47	11	13.99	12	13.43	5	12.58	**0.007**
	2nd year	17	12.65	10	13.04	8	13.65	3	11.33	0.159
	3rd year	11	13.79	4	13.28	7	13.96	5	13.32	0.699
	4th year	11	13.42	7	13.57	9	14.68	5	12.7	0.411
	5th year	8	12.97	5	13.56	6	14.35	2	14.8	0.175
	6th year	7	13.91	8	14.51	5	13.84	1	14.5	0.869
INR	1st year	32	1.02	11	1.11	12	1.08	5	1.01	0.053
	2nd year	17	0.99	10	1.07	8	1.07	3	0.92	0.320
	3rd year	11	1.08	4	1.08	7	1.08	5	1.03	0.672
	4th year	11	1.03	7	1.1	9	1.18	5	0.96	0.119
	5th year	8	0.99	5	1.06	6	1.11	2	1.17	0.093
	6th year	7	1.05	7	1.18	5	1.09	1	1.09	0.438
Albumin (mg/L)	1st year	29	4.34	13	4.12	12	3.92	4	4.31	0.088
	2nd year	20	4.32	11	3.87	11	4.23	4	3.93	**0.035**
	3rd year	15	4.28	6	3.94	11	4.20	5	4.37	0.260
	4th year	17	4.53	10	4.08	11	4.32	5	4.04	**0.015**
	5th year	8	4.57	6	3.98	8	4.45	3	4.36	0.163
	6th year	12	4.73	11	4.00	6	4.33	2	4.4	**0.005**
AFP (ng/mL)	1st year	35	3.50	13	5.79	15	6.57	4	5.67	0.494
	2nd year	16	3.28	12	8.60	10	4.27	4	5.61	0.223
	3rd year	14	3.90	9	6.87	10	5.04	5	82.45	0.185
	4th year	14	4.03	10	3.09	10	8.28	5	5.13	0.399
	5th year	6	2.76	8	10.35	6	2.73	3	5.40	0.589
	6th year	10	4.69	7	20.11	5	2.64	2	8.53	0.325
Platelet Count (cell/uL)	1st year	42	231047.62	15	154400	17	218470.59	6	162166.67	**0.000**
	2nd year	28	220346.43	13	135076.92	13	238230.77	3	202666.67	**0.002**
	3rd year	22	220454.55	10	143900	11	227636.36	5	220000	**0.030**
	4th year	20	226650	11	180909.09	11	205000	5	178200	0.083
	5th year	12	211333.33	9	154666.67	8	193250	3	169333.33	0.089
	6th year	12	247666.67	11	197545.45	6	220166.67	2	131500	0.061
HDV RNA (IU/mL)	1st year	24	1.17	11	1039319.64	11	7108.28	5	2502952.2	**0.000**
	2nd year	12	1594.5	11	34330.73	4	0	5	2183263.8	0.055
	3rd year	8	0	9	2434087	7	52007.86	6	2171.67	**0.006**
	4th year	11	0	6	1184211.67	6	35603.5	4	0	**0.000**
	5th year	8	0	4	2858563.25	2	0	2	28405.5	**0.007**
	6th year	10	0	7	139763.25	4	0	2	32870.5	**0.007**
HBV DNA (IU/mL)	1st year	42	2199.55	15	22993210.87	18	8468571.89	7	672.29	0.146
	2nd year	21	2126.29	12	3702.17	12	273782.33	5	74.8	0.453
	3rd year	16	7589.25	12	1558.75	11	1961.55	6	754	0.073
	4th year	20	214.85	11	9636383.91	10	7624.9	5	452.6	0.752
	5th year	11	3438.45	8	16.5	8	48.63	3	21.33	0.485
	6th year	11	2941.55	9	9.33	6	2.33	3	3.33	**0.000**

AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma glutamyl transferase; ALP: alkaline phosphatase; PT: prothrombin time; INR: international normalized ratio; AFP: alpha-feto protein.

The pegylated interferon therapy lasted 13.78 ± 5.33 months on average (range: 12-28 months). In this group, all patients tested negative for HBeAg. HDV-RNA was positive in six patients (67%; mean 13795453 ± 2781482 IU/mL) and negative in three (33%). After the pegylated interferon treatment, four out of six patients (67%) tested negative for HDV-RNA. Recurrence was observed in only one patient (25%) during follow-up. A liver biopsy was performed in six of nine patients with a mean fibrosis score of 2.33/6 and a mean HAI of 8.17/18. ALT remained high in four patients (44%). Both negative HDV-RNA and ALT normalization were achieved in two patients (22%) without recurrence. ALT level remained within the normal range in one patient (11%). Two patients (22%) were diagnosed with cirrhosis, and one patient (11%) underwent liver transplantation. In one of the two patients diagnosed with cirrhosis in this group, the fibrosis score was determined as 2/6, and the HAI was determined as 9/18 in the liver biopsy that had been performed in the third year of follow-up.

Statistically significant differences were found between the four groups for the mean ALT values in years 1, 4, 5 and 6; the mean AST values in all years; the mean platelet values in years 1, 2 and 3; the mean HBV DNA values in year 6; the mean HDV RNA values in years 1, 3, 4, 5 and 6; the mean GGT values in year 1; the mean PT values in year 1; the mean total bilirubin values in years 1 and 2; and the mean albumin values in years 2, 4 and 6 ($p < 0.05$; Table 1). There was no statistically significant difference between the four groups in terms of gender, the mean age at diagnosis, HBeAg serological status, the mean values of ALP, INR and AFP in each follow-up year ($p > 0.05$; Table 1).

Among the patients diagnosed with cirrhosis, five (71%) were male and two (29%) were female. The mean age at the diagnosis of cirrhosis was 48.9 years. When the AST, ALT, INR, albumin, platelet count, HDV-RNA, and HBV-DNA values of seven patients diagnosed with cirrhosis were compared with other patients, it was significantly determined that their AST,

Table 2. Comparison of laboratory results between cirrhotic and non-cirrhotic patients.

		Non-Cirrhotic Patients		Cirrhotic Patients		*p*
		n	Mean	n	Mean	
AST (U/L)	1st year	78	67.21	5	86.40	**0.014**
	2nd year	55	31.96	5	75.80	**0.004**
	3rd year	47	29.91	4	51.75	**0.034**
	4th year	43	30.05	5	58.40	**0.003**
	5th year	30	33.07	5	53.00	0.081
	6th year	28	33.50	3	73.00	**0.038**
ALT (IU/L)	1st year	78	66.56	5	88.00	**0.043**
	2nd year	55	36.49	5	69.00	**0.033**
	3rd year	47	35.34	4	45.50	0.240
	4th year	44	39.55	5	62.00	**0.032**
	5th year	30	38.30	5	56.40	0.144
	6th year	28	43.57	3	66.33	**0.044**
INR	1st year	55	1.04	5	1.12	0.141
	2nd year	33	1.01	5	1.12	0.109
	3rd year	25	1.06	2	1.25	0.610
	4th year	29	1.05	3	1.34	0.196
	5th year	18	1.02	3	1.29	**0.008**
	6th year	18	1.06	2	1.56	**0.023**
Albumin (mg/L)	1st year	53	4.22	5	3.97	0.305
	2nd year	41	4.23	5	3.52	**0.006**
	3rd year	33	4.27	4	3.69	0.063
	4th year	38	4.38	5	3.86	**0.027**
	5th year	21	4.39	4	4.21	0.394
	6th year	27	4.48	4	3.66	**0.023**
Platelet Count (cell/uL)	1st year	75	216000.00	5	101400.00	**< 0.001**
	2nd year	52	216148.08	5	78200.00	**< 0.001**
	3rd year	44	215863.64	4	98750.00	**0.006**
	4th year	43	216511.63	4	89750.00	**0.003**
	5th year	27	193444.44	5	151800.00	0.177
	6th year	28	231250.00	3	84666.67	**0.008**
HDV RNA (IU/mL)	1st year	47	481872.85	4	344368.00	**0.001**
	2nd year	29	389065.21	3	10066.67	0.909
	3rd year	26	842328.27	4	95833.25	0.079
	4th year	25	71343.56	2	2767651.00	**0.006**
	5th year	15	762283.53	1	56811.00	0.147
	6th year	21	46571.71	2	33038.50	**0.034**
HBV DNA (IU/mL)	1st year	77	6460123.68	5	4.20	**0.005**
	2nd year	46	73365.87	4	2.50	**0.033**
	3rd year	40	4144.52	5	90.60	0.126
	4th year	41	2587391.02	5	0.00	**0.017**
	5th year	26	1476.23	4	6.50	0.246
	6th year	27	1202.41	2	0.00	0.220

AST: aspartate aminotransferase; ALT: alanine aminotransferase; INR: international normalized ratio

ALT, and INR values were high and their platelet count, albumin and HDV-RNA values were low (Table 2). Liver biopsy was performed in 4 of 7 patients who developed cirrhosis during follow-up. At the time of biopsy, these patients had not yet developed cirrhosis. The mean fibrosis score was 2.00/6 in patients with cirrhosis and 2.36/6 (n = 44) in patients without cirrhosis (p = 0.552). The mean histological activity index was 8.50/18 in patients with cirrhosis and 6.95/18 in other patients (p = 0.225).

In our study, HDV-RNA was positive in 25 patients. The biochemical markers of these 25 patients were compared with those of HDV-RNA-negative patients. It was noted that in HDV-RNA-positive patients, the average AST, ALT, GGT, and AFP values were higher in all follow-up years, while the average AFP and platelet count were lower. A statistical comparison of laboratory parameters between these two groups is shown in Table 3. Liver biopsy was performed in 19 HDV-RNA positive patients and 29 HDV-RNA negative patients. The mean fibrosis score was 2.84/6 in the HDV-RNA positive group and 2.00/6 in the HDV-RNA negative group, and there was a statistically significant difference between both groups (p = 0.013). The mean histological activity index was 7.89/18 in the HDV-RNA positive group and 6.59/18 in the HDV-RNA negative group (p = 0.068).

Discussion

The primary aim of delta hepatitis treatment is to achieve negative HDV-RNA. Although negative HDV-RNA was obtained in four of six patients (67%) in the pegylated interferon group, the small number of patients in this group may have contributed to the high rate. On the other hand, the results of our study revealed that current treatments have limited success. In the studies using pegylated interferon to treat delta hepatitis, HDV-RNA negative was reported in 19% of participants in Kamal *et al*., 30% in Heller *et al*., 33% in Gheorge *et al*., and 17% in Bockmann *et al.* [8-11]. The findings of these studies indicate that pegylated interferon is a drug with limited effect on the replication of hepatitis delta virus (HDV). In our study, together with other studies, the partial response to treatment for HDV-RNA negativity suggests that pegylated interferon is not a fully effective treatment. Regarding the incomplete efficacy of pegylated interferon therapy, some patients may exhibit persistent or elevated HDV-RNA levels despite treatment, even if HDV-RNA initially decreases. Therefore, it is clear that new treatments for hepatitis delta are required.

In a study conducted by Wedemeyer *et al.* with 90 patients to evaluate the effect of combined treatment using pegylated interferon and one of the oral antivirals used in the treatment of hepatitis B, HDV-RNA became negative in seven of 31 patients (23%) who received pegylated interferon and adefovir, and in seven of 29 patients (24%) who received pegylated interferon and placebo. Positive HDV-RNA persisted in all 30 patients who received adefovir alone [12]. Other studies reported that lamivudine and entecavir monotherapies also did not achieve negative HDV-RNA [13-15]. In another study, HDV-RNA became negative in 28 of 59 patients (48%) receiving pegylated interferon + TDF and in 20 of 61 patients (33%) receiving pegylated interferon + placebo [16]. Similarly, lamivudine and pegylated interferon combined therapy was not found to be superior to pegylated interferon monotherapy [13]. In our study, oral antiviral treatments for chronic hepatitis B did not make an additional contribution to pegylated interferon treatment and did not achieve negative HDV-RNA either.

Kamal *et al.* examined the impact of HDV-RNA positivity on major liver-related clinical events by comparing 233 HDV-RNA-positive patients with 91 HDV-RNA-negative patients. Positive HDV-RNA did not significantly increase the development of HCC. However, liver decompensation, death, and liver transplantation were observed to be significantly higher in patients with positive HDV-RNA. The risk of developing any liver-related clinical event (decompensation, HCC, liver transplantation, and death) increased three times in HDV-RNA-positive patients and 11 times in patients with cirrhosis. Studies on the long-term consequences of chronic delta hepatitis show that the presence of cirrhosis is very important in the development of liver-related clinical events [8]. On the other hand, in a study examining liver biopsies of patients diagnosed with delta hepatitis, no correlation was found between serum HDV-RNA value and inflammation in the liver [17]. Liver inflammation and intrahepatic HDAg expression were found to be positively correlated in the Negro *et al.* investigation [18]. The damage at the cellular level can continue in delta hepatitis even if serum HDV-RNA levels are low or negative.

A meta-analysis of 93 studies found that patients with delta hepatitis had a modestly increased risk of HCC compared to those with HBV [19]. In another study involving 200 cirrhosis patients, a threefold higher rate of HCC development was observed in anti-HDV positive individuals compared to those with HBV mono-infection [20].

Table 3. Comparison of laboratory results between HDV-RNA positive and HDV-RNA negative patients.

		HDV-RNA Positive Patients		HDV-RNA Negative Patients		*p*
		n	Mean	n	Mean	
AST (U/L)	1st year	21	182.29	62	29.77	**< 0.001**
	2nd year	17	50.41	43	29.77	**< 0.001**
	3rd year	15	44.87	36	26.11	**< 0.001**
	4th year	15	42.87	33	28.52	**0.016**
	5th year	12	49.08	23	29.04	**0.040**
	6th year	12	56.58	19	25.16	**< 0.001**
ALT (IU/L)	1st year	21	158.62	62	37.11	**0.001**
	2nd year	17	46.18	43	36.44	0.099
	3rd year	15	47.33	36	31.47	**0.018**
	4th year	15	49.40	34	38.50	0.073
	5th year	12	55.50	23	33.26	0.144
	6th year	12	76.25	19	26.53	**0.002**
GGT (U/L)	1st year	16	65.56	45	28.89	**< 0.001**
	2nd year	13	54.54	36	29.03	**0.014**
	3rd year	15	54.00	31	31.55	0.064
	4th year	15	51.07	29	29.38	**0.036**
	5th year	12	39.58	19	35.42	0.180
	6th year	11	39.00	18	33.00	0.105
ALP (U/L)	1st year	10	91.50	22	75.09	0.329
	2nd year	9	97.78	24	75.54	1.000
	3rd year	11	94.09	21	86.62	0.796
	4th year	11	93.82	25	89.68	0.904
	5th year	9	83.33	13	86.46	0.570
	6th year	11	114.09	15	86.40	0.917
Total Bilirubin (mg/dL)	1st year	16	1.28	33	0.53	**< 0.001**
	2nd year	15	0.86	28	0.47	**0.006**
	3rd year	15	0.69	24	0.52	**0.050**
	4th year	14	0.90	23	0.55	0.069
	5th year	10	0.55	13	0.55	0.352
	6th year	12	0.77	15	0.44	**0.014**
PT (sec)	1st year	16	13.65	45	12.69	**0.010**
	2nd year	14	13.21	24	12.66	0.163
	3rd year	9	14.07	18	13.47	0.959
	4th year	11	13.77	21	13.66	0.905
	5th year	7	13.99	14	13.53	0.313
	6th year	8	14.71	13	13.81	0.425
INR	1st year	15	1.10	45	1.03	**0.037**
	2nd year	14	1.06	24	1.00	0.150
	3rd year	9	1.11	18	1.05	0.303
	4th year	11	1.09	21	1.07	0.525
	5th year	7	1.08	14	1.05	0.166
	6th year	7	1.19	13	1.06	**0.043**
Albumin (mg/L)	1st year	17	4.06	41	4.26	0.074
	2nd year	15	3.80	31	4.32	**< 0.001**
	3rd year	11	3.88	26	4.35	**0.003**
	4th year	13	3.91	30	4.49	**< 0.001**
	5th year	8	4.09	17	4.49	0.062
	6th year	12	4.01	19	4.60	**0.002**
AFP (ng/mL)	1st year	17	9.18	50	3.26	0.185
	2nd year	15	9.54	27	2.88	**0.030**
	3rd year	15	33.55	23	3.30	0.139
	4th year	14	7.82	25	3.45	0.736
	5th year	11	8.87	12	2.86	0.782
	6th year	9	17.53	15	4.01	0.387
Platelet Count (cell/uL)	1st year	20	147150.00	60	229400.00	**< 0.001**
	2nd year	15	126066.67	42	231897.62	**< 0.001**
	3rd year	14	146071.43	34	230823.53	**< 0.001**
	4th year	14	146214.29	33	230969.70	**< 0.001**
	5th year	12	151250.00	20	208350.00	**0.002**
	6th year	12	187333.33	19	235842.11	**0.027**
HBV DNA (IU/mL)	1st year	20	6732077.45	62	5851419.27	0.117
	2nd year	17	2638.88	33	100908.45	0.090
	3rd year	18	70.56	27	6109.78	**0.036**
	4th year	15	109.80	31	3421980.16	0.115
	5th year	11	15.45	19	2012.53	0.161
	6th year	11	8.55	18	1798.39	**0.013**

AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma glutamyl transferase; ALP: alkaline phosphatase; PT: prothrombin time; INR: international normalized ratio; AFP: alpha-feto protein.

Wranke *et al.* examined the 10-year follow-up of the patient group in the study of Wedemeyer *et al.* comparing pegylated interferon and adefovir treatments. Of the 90 patients in the first study, 60 were followed long-term (the median value of the follow-up period was 8.9 years). Liver-related clinical events occurred in a total of 12 patients. It was reported that 10 of these 12 patients developed liver decompensation, two patients developed HCC, and a total of six patients underwent liver transplantation. Among these patients, four patients died, two due to HCC, one due to variceal bleeding, and one due to decompensated cirrhosis. In this study, the average time for a liver-related clinical event to occur was 5.9 years [21]. Since the average follow-up period in our study was relatively short, 4.38 years, the development rates of cirrhosis and HCC were found to be lower than in other studies. The absence of death due to liver damage in our study suggests that it is primarily due to the low rates of cirrhosis and HCC development, as well as limited liver damage in terms of complications due to the low activity of the disease.

Chronic hepatitis C, alcohol-related liver disease, non-alcoholic fatty liver disease, hemochromatosis, Wilson's disease, α1-antitrypsin deficiency, autoimmune hepatitis, primary biliary cholangitis, and primary sclerosing cholangitis are among the risk factors for the development of cirrhosis [22]. Therefore, patients with delta hepatitis with the mentioned comorbid conditions and diseases should be followed more closely for the development of cirrhosis and HCC.

Surana *et al*. studied biochemical indicators in 1027 patients with chronic viral hepatitis (HCV: 701, HBV: 240, HDV: 86) that can be utilized to predict cirrhosis. The values of albumin, total bilirubin, AST, ALT, ALP, PT, and platelet counts were related to the stage of fibrosis in liver biopsy. The most reliable of these five indicators was reported to be the platelet count, especially a platelet value below 143000/μL had a negative predictive value of 94% in the prediction of cirrhosis [23]. As in published studies, poor prognostic indicators for the development of cirrhosis in our patients were persistently elevated AST and ALT, INR prolongation, thrombocytopenia, and low albumin levels. If these prognostic tests are not normal, patients with delta hepatitis should be actively monitored for cirrhosis and HCC and, if necessary, prepared for liver transplantation.

The following can be stated as the limitations of our study: the study has a cross-sectional nature since only patients who applied to our department were evaluated. In general, HDV-RNA negative patients from the young age group who were followed up without treatment constitute the majority of the study population. The number of patients receiving pegylated interferon treatment was low. The follow-up period is not very long, such as 10-15 years, the number of patients with long follow-up periods is low, and the distribution of patients in the groups is not similar.

Conclusions

In our study with shorter follow-up period and lower HDV-RNA positivity, the incidence of cirrhosis, HCC development and liver transplantation was lower compared to other studies. Despite the partial effectiveness of pegylated interferon treatment on HDV-RNA negativity, the development of cirrhosis and HCC in the pegylated interferon and combined treatment groups in our study shows that this treatment does not completely prevent the development of cirrhosis and HCC. HDV-RNA negativity was not obtained in the oral antiviral group. The combined treatment group did not exhibit greater HDV-RNA negativity than the pegylated interferon group. Therefore, the addition of oral antivirals to pegylated interferon therapy did not appear to provide any additional benefit in terms of HDV-RNA negativity. Annual follow-up of patients who develop cirrhosis reveals notable increases in liver damage and inflammation markers including AST, ALT, and INR. On the contrary, there are decreases in albumin and platelet counts. The degree of deterioration in biochemical markers can predict disease progression. A liver biopsy was performed in patients diagnosed with cirrhosis at the beginning of the follow-up period, and fibrosis and histological activity index scores were found to be relatively low. Liver biopsies performed at the onset of delta hepatitis may be inadequate to predict disease progression.

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Conflict of interests

No conflict of interests is declared.

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