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Original Article

Impact of interleukin 28B rs12979860 C/T polymorphism on severity of disease and response to treatment in hepatitis delta

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Abstract

Introduction: Pegylated-interferon alpha (Peg-IFN α) is the therapy most commonly used to treat chronic hepatitis delta virus (HDV) infection. In the present study, we planned to investigate effect of IL28B polymorphism on response to Peg-IFN α therapy and disease progression in patients with chronic HDV.

Methodology: A total of 47 patients who received Peg-IFNα therapy for at least one year were investigated. The patients were divided into three groups based on their response to treatment: sustained viral response (SVR) (32%), unresponsive (53%), and relapse (15%). The groups were compared in terms of age, gender, blood biochemistry (albumin, total bilirubin, lactic acid dehydrogenase, ALT, AST, ALP, GGT), complete blood count, HBeAg, HBsAg, HBV-DNA, HDV-RNA, IL28B genotypes (CC, CT, TT), and results of liver biopsy.

Results: Regarding the investigation of IL28B genotype, the prevalence of CC, CT, and TT showed no difference among the three groups. In the SVR group, the prevalence of CC was 53%, CT was 47%, but there was no patient with TT. In the unresponsive group, prevalence of CC was 52%, CT was 32%, and TT was 16%. In the relapse group, prevalence of CC was 43%, CT was 57%, but there was no patient with TT genotype. No significant difference was found among the groups with sustained response, no response, and relapse in terms of CC and CT polymorphisms ($p>0.05$).

Conclusions: No relationship was found between IL28B rs12979860 polymorphism and response to treatment and disease severity in patients with chronic HDV infection.

Key words: Delta hepatitis; interleukin 28B; interferon.

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Introduction

Hepatitis delta virus (HDV) infection is widely distributed all over the world. It is believed that about 15 million people are infected with HDV [1]. HDV is a defective RNA virus. It requires the helper function of hepatitis B virus (HBV) for transmission and replication [2]. Clinically, HDV is usually defined as superinfection in the individuals with viral hepatitis B. HDV infections defined in Europe and in Turkey are usually associated with severe liver damage [3-5].

HDV is a serious health problem with high prevalence rates in the eastern and southeastern Anatolian regions of Turkey [6,7]. The prevalence rate, however, is lower in Western countries, with higher prevalence observed in hepatitis B surface antigen (HBsAg)-positive intravenous drug addicts [8,9]. Delta infections displaying serious and rapid disease progression make HDV a highly pathogenic virus. Although 70% of HDV cases develop cirrhosis, this process may range from several to 10 years.

Pegylated-interferon alpha (Peg-IFN α) is currently used in the treatment of chronic delta hepatitis with a sustained virological response rate ranging between 20% and 40% [10,11]. It is important to know the factors that predict response to treatment because interferon, which is also used in patients with chronic hepatitis C virus (HCV) and chronic hepatitis B virus (HBV) infections, has serious adverse events, is expensive, and has a limited success rate. The studies performed with IL28B, which is a member of interleukin-10, determined that interleukin-28B gene polymorphism with interferon λ3 expression has strong association with spontaneous clearance and response to treatment in HCV patients [12]. Studies agreed that IL28B polymorphism is a strong prognostic marker in estimating response to treatment in the patients with hepatitis C. A study on patients with chronic hepatitis B demonstrated a similar relationship, although not definite [13].

The role of IL28B in chronic HDV infection, which is a dual infection, is not well established. We planned

to investigate the relationship between interleukin-28B polymorphism and response to Peg-IFN α therapy and disease severity in patients treated for chronic delta hepatitis.

Methodology

A total of 47 patients, who had been followed between 2007 and 2012 in the Firat University Faculty of Medicine, Department of Gastroenterology, and received Peg-IFN α (2a or 2b) therapy for at least one year, were enrolled in the study after providing informed consent. The patients were grouped based on their response to treatment.

Criteria for response to treatment in chronic HDV infection are not well-defined. Considering the European Association for Study of the Liver (EASL) [14] guidelines, criteria were established as follows. Early viral response (EVR) was defined as at least two log drop or negative HDV-RNA level on the 12th week of treatment. Sustained viral response (SVR) was defined as HDV-RNA negativity persisting both at the end of treatment and at the end of 24-week follow-up period after treatment. End-treatment viral response (ETVR) was defined as HDV-RNA negativity obtained during discontinuation of treatment. Relapse was defined as obtaining virological response at the end of treatment but HDV-RNA becoming positive again after discontinuation of therapy. Biochemical response was defined as serum ALT levels regressing to the normal limits.

Inclusion criteria included age >18 years, anti-HDV and HDV-RNA (polymerase chain reaction [PCR]) positivity for longer than 6 months, known HBsAg or HBV DNA positivity for longer than 6 months, and presence of liver biopsy result consistent with chronic hepatitis. Exclusion criteria included patients with concomitant autoimmune, metabolic, and viral chronic liver disease (autoantibody positivity, cases with positive anti-HAV IgM, anti-HCV, anti-HIV); and patients with hepatocellular carcinoma detected at admission.

Measurement of biochemical parameters

In all cases, biochemical parameters (albumin, total bilirubin, lactic acid dehydrogenase [LDH], alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP], and gamma glutamyltranspeptidase [GGT]) were measured using anOlympus AU 600 (Olympus Optical Co. Ltd,, Tokyo, Japan) autoanalyzer using Olympus brand commercial kits. CELL-DYN 3700 (Abboth Laboratories, Lake Forest, USA) device was used to measure hematological (hemoglobin, platelet) parameters.

Measurement of serological parameters

Hepatitis Be antigen, HBsAg, and anti-delta-total tests were performed using the enzyme-linked immunosorbent assay (ELISA) method. Quantitative HBV DNA test was performed using COBAS AmpliPrep/COBAS Taqman 96 system (Qiagen, Hilden, Germany). The dynamic range of the kit is between 20 IU/mL and 1.7×10^8 IU/mL. Quantitative HDV RNA test was performed using real-time PCR (LightCycler System, Salt Lake City, USA. The dynamic range of the kit was between 400 and 4×10^7 copies/mL.

Sample collection and DNA extraction

Blood samples were collected in Eppendorf tubes and stored at -80°C until the time of analysis. DNA extraction was performed using a High Pure PCR Template Preparation Kit (Roche Diagnostics, Manheim, Germany) in accordance with the recommendations of the manufacturer.

Detection of IL28B rs12979860 genotypes

Genomic DNA obtained from blood samples was reproduced by LightCycler 2.0 (Roche Applied Science, Germany) device using LightMix (TIB Molbiol GmbH, Berlin, Germany) amplification mixture including primary sequences that detect rs12979860 genotype, which shows single nucleotide polymorphisms (SNPs) in the IL28B gene, and LightCycler Fast Start DNA Master Hybridization probes (Roche Diagnostics, Manheim, Germany). The mixture was prepared using 5 µL of sample DNA,1.6 µL Mg^2Cl^2 solution (25 mM), 2 µL reagent mix (primary and probe), 2 µL Roche master mix, (Roche Diagnostics, Manheim, Germany) and 9.4 µL sterile deionized water to a final volume of20 µL. Lyophilized control DNAs (IL28B allele C, allele T, and allele C/T) were diluted with sterile deionized water to include DNA equal to 10^5 targets for each reaction.

Subsequently, PCR conditions in the LightCycler device were completed as follows: denaturation at 95°C for 10 minutes; 45 cycles of target DNA reproduction (5′ at 95°C, 10′ at 60°C, and 15′ at 72°C); melting curve analysis as the result of the heat rising to 85°C together with continuation of fluorescent radiation for 20′ at 95°C and 120′ at 40°C; and cooling at 40°C for 30′. Melting curve and melting point (Tm) analyses of the products were performed using the same device. PCR results for rs12979860 genotype region were analyzed

by Simple Probe (Roche Diagnostics, Manheim, Germany) in channel 530. Samples for rs12979860 SNPs were evaluated together with the standards that peak at 51.4±2.5°C for wild-type homozygote allele (T/T), at 51±2.5–59±2.5°C for heterozygote allele (C/T), and at 59.2±2.5°C for homozygote variant allele (C/C) in channel 530.No peak was observed in the negative controls used for rs12979860 SNPs. As seen in Figure 1, the green graph that peaks at 55°C indicates TT, the black graph that peaks at 55°C and 63°C indicates CT, and the red graph that peaks at 63°C indicates the CC genotype.

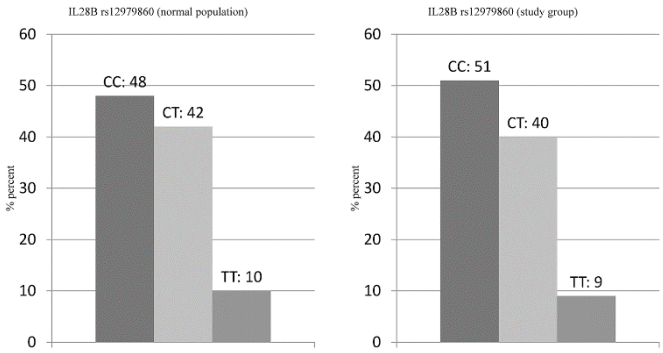
Histopathological evaluation

Staging and grading were done based on the modified Knodell Ishak scoring system [15].

Statistical analysis

Statistical analyses were done using SPSS version 18.0 (IBM, Armonk, USA). Statistical differences among the groups were evaluated using the Kruskal-Wallis test, and subsequent intergroup differences were analyzed using the Mann-Whitney U test for nonparametric data. A Chi-squared test was used to compare the categorical variables. Correlation between parameters was determined by Pearson's correlation analysis. The results were expressed as mean ± standard deviation, and $p < 0.05$ was considered to be statistically significant.

Figure 1. Prevalence of IL28Brs12979860 genotype in the normal population and study group.



Results

A total of 47 patients, each of whom had received Peg-IFN α therapy for at least 12 months, were examined. The overall mean age was 46.5±10 (range 23–69). Thirty-six percent (n=17) of the patients were female and 64% (n=30) were male. Of the patients, 10 (22%) were in a cirrhotic stage and 37 (78%) had chronic hepatitis. Laboratory and demographic characteristics of the study patients are summarized in Tables 1 and 2.

Table 1. Demographic and laboratory characteristics of the cases with chronic HDV infection.

	Mean + standard deviation (range)
Age (years)	46.5 ± 10 (23–69)
Leukocyte (mm^3)	$5 \times 10^3 \pm 1.6 \times 10^3$ (2.7×10^3–8.3×10^3)
Hemoglobin (mg/dL)	13.72 ±1.74 (10–16)
Thrombocyte (10^3/µL)	$1.49 \times 10^5 \pm 6.75 \times 10^4$ (5.4×10^4–3.39×10^5)
AST (IU/L)	86.12 ± 81.99 (21–397)
ALT (IU/L)	94 ± 95.3 (14–539)
ALP (IU/L)	94 ± 47.44 (25–289)
GGT (IU/L)	74 ± 78.22 (12–398)
Total bilirubin (mg/dL)	0.98 ± 0.549 (0.4–2.7)
Albumin (g/dL)	4.09 ± 0.64 (2–5)
HBsAg (IU/mL)	1,115 ± 1,043 (34–3,997)
HBV-DNA (copies/mL)	$55 \times 10^4 \pm 25 \times 10^3$ (1.1×10^7–1.14×10^7)
HDV-RNA (copies/mL)	$3 \times 10^5 \pm 7.13 \times 10^5$ (1.26×10^6–4.26×10^6)

HDV: hepatitis delta virus; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gamma glutamyl transpeptidase; HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus.

Table 2. Demographic and laboratory characteristics of the cases with chronic HDV infection.

HBeAg - / + (% total patients)	**8 (18%) / 39 (82%)**
Peg-IFN α 2a (% total patients)	32 (68%)
Peg-IFN α 2b (% total patients)	15 (32%)
Mean Ishak scores (HAI/fibrosis) of totalpatients	(10/3)
Chronic HBV, n (% total patients)	37 (78%)
Cirrhotic, n (% total patients)	10 (22%)

HDV: hepatitis delta virus; HBeAg: hepatitis B e antigen; Peg-IFN α 2a: pegylated-interferon alpha 2a; HAI: histological activity index; HBV: hepatitis B virus

Table 3. Prevalence of EVR and ETVR in the patient groups with permanent viral response and relapse.

	EVR	ETVR	Total (%)
SVR, n (%)	11 (73%)	4 (27%)	15 (32%)
Relapse, n (%)	6 (85%)	1(15%)	7 (15%)
Unresponsive, n (%)	-	-	25 (53%)

EVR: early viral response; ETVR: end-treatment viral response; SVR: sustained viral response

The mean duration of Peg-IFN α therapy was 12.51 (12–24) months. Of the patients, 45 (95%) received therapy for 12 months, while 2 patients in the SVR group received therapy continuously for 24 months. On the other hand, 2 patients in the relapse group received treatment for 24 and 36 months at one-year intervals. SVR was achieved in a total of 15 (32%) patients, whereas7 (15%) patients had relapse after discontinuation of the given therapy. Mean time for relapse was found to be 18 (12–24) months. Of the patients with SVR, 7 (46%) also developed a biochemical response. EVR was observed in 11 (73%) and ETVR in 4 (27%) of the 15 patients with SVR. In the patients who relapsed, EVR was observed in 6 (85%) patients and ETVR was observed in only1 (15%) patient (Table 3).

The prevalence of the IL28B rs12979860 genotype in all patients was found to be similar to that of the normal population. A total of 24 (51%) CC homozygote, 19 (40%) CT heterozygote, and 4 (9%) TT patients were identified (Figure 1). The prevalence of genotypes was similar in the male and female patient groups (p = 0.888). There were 15 (50%) CC, 12 (40%) CT, and 3 (10%) TT genotypes in the male patient group and 9 (53%) CC, 7 (41%) CT, and 1 (6%) TT genotypes in the female patient group.

The frequency of genotypes were also examined based on the response to treatment; there were 8 (53%) patients with CC genotype, 7 (47%) patients with CT genotype, and no patients with TT genotype in the SVR group comprising 15 patients; there were 13 (52%) patients with CC genotype, 8 (32%) patients with CT genotype, and 4 (16%) patients with TT genotype in the unresponsive group comprising 25 patients; and there were 3 (43%) patients with CC genotype, 4 (57%) patients with CT genotype, and no patients with TT genotype in the group comprising 7 patients with relapse. All the patients with the TT genotype were in the unresponsive group. However, statistical analysis revealed no significant difference among the SVR group, the un responsive group, and the relapse group in terms of prevalence of IL28B rs12979860 genotypes (Table 4).

When the patients were grouped as those carrying the C allele (groups with CC and CT genotypes) and those not carrying the C allele (group with TT genotype), regardless of being heterozygote or homozygote, they were divided into two groups: one with the C allele, consisting of 43 (91.5%) patients (polymorphism +), and the other without the C allele, consisting of4 (8.5%) patients (polymorphism -). Polymorphism was determined in all patients in the SVR group, in 21 (84%) patients in the unresponsive group, and again in all patients in the relapse group. The prevalence of polymorphism (+) patients was not different among each of the three groups (p = 0.146).

On the other hand, the prevalence of patients without polymorphism (TT genotypes) in the SVR, unresponsive, and relapse groups could not be calculated statistically because of the limited number of patients without polymorphism (TT genotype), all of whom were in the unresponsive group. No significant

Table 4. Comparison of the prevalence of IL28B rs12979860 genotypes between groups.

IL28B genotypes	SVR (n = 15)	Unresponsive (n = 25)	Relapse (n = 7)	p
CC genotype	8 (53%)	13 (52%)	3 (43%)	
CT genotype	7 (47%)	8 (32%)	4 (57%)	**> 0.05[a]**
TT genotype	0 (0%)	4 (16%)	0 (0%)	

SVR: sustained viral response; [a] Chi-squared p values.

Table 5. Comparison of the prevalence of polymorphism between groups.

IL28B rs1297986	SVR (n = 15)	Unresponsive (n = 25)	Relapse (n = 7)	p
Polymorphism (+)(CC or CT genotype)	15 (100%)	21 (84%)	7 (100%)	**0.146[a]**
Polymorphism (-)(TT genotype)	0	4 (16%)	0	**p***

SVR: sustained viral response; [a]Chi-squared p values; * The prevalence could not be calculated in the groups for those without polymorphism.

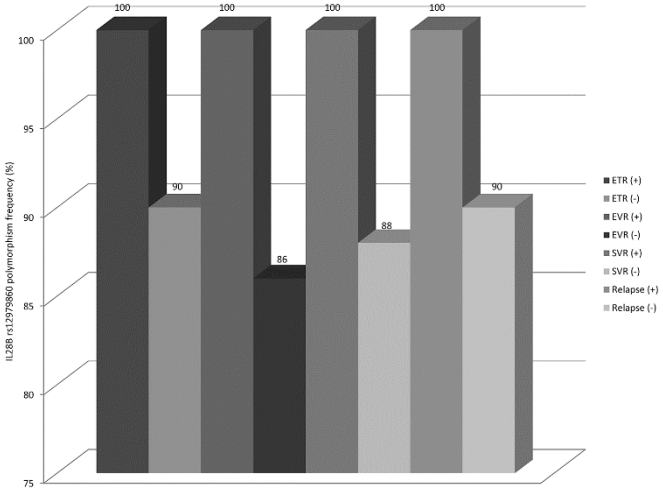
difference was found among the three groups concerning the frequency of polymorphism in the patients with ETVR, EVR, and relapse (p>0.05). The prevalence of patients without polymorphism (the group with the TT genotype) could not be calculated, again because of limited number of patients, all of whom were in the unresponsive group (Tables 5 and 6). While the frequency of polymorphism was 100% in the ETVR group, it was 90% in those without ETVR, with no difference between the groups (p>0.05). The prevalence of polymorphism was not different among the groups when a comparison was done between those with and without EVR, between those with and without SVR, and between those with and without relapse (p>0.05) (Figure 2).

The baseline mean liver fibrosis score and mean histological activity index (HAI), prevalence of HBeAg positivity, and the mean HDV-RNA levels were compared between the patients with different genotypes, no difference was determined between the groups. However, mean HBV-DNA level was found to be significantly higher in the patients with the CT genotype (p = 0.011) (Table 7).

Discussion

Chronic delta hepatitis infection is difficult to treat. Unless treated, it shows rapid progression and leads to cirrhosis and hepatocellular carcinoma more frequently than other viral agents. Forms of severe acute hepatitis develop in 50%–70% of super infections, and 80% become chronic [16]. Nevertheless, interferon is currently the only therapeutic option, with an SVR rate of 20%–40%. Various studies demonstrated that

Figure 2. Comparison of the prevalence of polymorphism-positive patients between ETVR, EVR, SVR, relapse, and no-relapse groups.



response to treatment is enhanced with duration of interferon therapy [11]. As side effects and cost of Peg-IFN α therapy are high, it remains debatable for how long it would be preferred in which patients. Of the 47 patients in the present study, 45 (95%) received 12-month treatment regularly, whereas 2 received treatments continuously for 24 months. SVR was achieved in these 2 patients who received continuous treatment for 24 months. However, 2 patients who received treatment for a total of 24 and 36 months at one-year intervals experienced relapse.

Many studies have been conducted to investigate a marker for response to treatment and prognosis of disease in patients with HBV or HCV infection. In recent years, studies have focused on the IL28B gene,

Table 6. Comparison of prevalence of polymorphism in EVR, ETVR, and unresponsive groups.

IL28B rs12979860	Early viral response	End-treatment viral response	Unresponsive	P
Polymorphism (+) (n = 43)	17	5	21	p > 0.05[a]
Polymorphism (-)(n = 4)	0	0	4	p*
Total (n = 47)	17	5	25	

[a]Chi-squared p values; ***** The prevalence could not be calculated in the groups for those without polymorphism.

Table 7. Comparison of baseline histopathological and serological data between patient groups with different IL28 genotypes.

	IL28B rs12979860 genotypes			
	CC genotype	CT genotype	TT genotype	p
Histological activity index (mean)	10.9 ± 2.7	10.46 ± 3.5	9.6 ± 3.7	0.75[b]
Fibrosis (mean)	2.65 ± 0.87	2.83 ± 0.93	2.67 ± 0.57	0.85[b]
HBsAg IU/mL(mean)	1,172 ± 1,121	1,059 ± 1,031	1006 ± 678	0.98[b]
HBV DNA copies/mL (mean)	$41 \times 10^5 \pm 18 \times 10^6$*	$83 \times 10^4 \pm 34 \times 10^5$	250 ± 208	0.016[b]*
HDV RNA copies/mL (mean)	$44 \times 10^4 \pm 91 \times 10^4$	$17 \times 10^4 \pm 36 \times 10^4$	$51 \times 10^3 \pm 88 \times 10^3$	0.32[b]
HBeAg (+) n (%)	4 (17%)	4 (22%)	0 (0%)	0.63[a]

HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; HDV: hepatitis delta virus; * p = 0.011 when the group with CC genotype was compared with the group with CT genotype (Mann-Whitney U test); [a]Chi-squared p value; [b]Kruskal-Wallis p values.

which is found on chromosome 19 and codes a protein known as lambda 3 (IFNλ-3) on the host genome. It was reported that SNPs (rs8099917, rs12979860), which are found in close localization to this IL28B, have a strong relationship with response to interferon. In one study, the prevalence of the C/C allele was found to be 95% in Eastern Asians and 42% in Africans. Further studies investigating the response to HCV treatment in different ethnic groups revealed that therapy success was higher in the population in which the prevalence of the rs12979860 C/C allele was higher [17,18].

In the present study, prevalence of the genotypes in all the patient groups was similar to that of the normal population. The C/C allele was found to be higher in spontaneous clearance of HCV. While spontaneous clearance was 52% in those with the C/C allele, it was 26% in those with the C/T allele and 22% in those with the T/T allele. In this respect, IL28B polymorphism has been suggested to be the most critical and strongest genetic factor associated with the spontaneous clearance of HCV infection [12,19].

The C allele versus the T allele reflected a strong correlation with better response in the IL28B rs12979860 polymorphism, and the T allele versus the G allele reflected a strong correlation with better response in IL28B rs8099917 polymorphism. Today, IL28B polymorphism, as well as HCV genotype, baseline histological findings, and baseline HCV-RNA and ALT levels, are considered to be strong prognostic markers in estimating prognosis of HCV [20]. On the other hand, studies concerning the relationship between IL28B polymorphism and hepatitis B yielded contradictory outcomes. In one of these studies, Kandemir *et al.* [21] examined three groups that consisted of 74 subjects receiving Peg-IFN α and/or oral antiviral therapy, 61 asymptomatic carriers, and 40 controls who were similar in terms of age and gender. They found that IL28B rs12979860 polymorphism showed no difference in the prognosis of disease and response to treatment. In a more recent Korean study, the patients were divided into three groups as healthy controls, patients with natural immunity against HBV, and patients with hepatocellular carcinoma (HCC) secondary to HBV. Interestingly, the rs12979860 CC, rs12980275 AA, and rs8099917TT alleles were found to be higher in the patients with HCC as compared to the control and natural immunity groups [22]. In a recent meta-analysis, it was suggested that IL28B rs8099917 AA genotype might be associated with low risk of HCC but that IL28B rs12979860 CC genotype might be associated with enhanced risk of developing cirrhosis in chronic HBV [23]

Starting from this point, we thought that IL28B polymorphism may have a predictive role in prognosis and response to treatment also in patients with chronic HDV infection. Two recently published studies investigated IL28B polymorphism in delta hepatitis. In one of these studies, the relationship of IL28B polymorphism with spontaneous and interferon-induced clearance of hepatitis delta virus RNA was investigated in a group comprising 55 patients; it was determined that the prevalence of IL28B rs12979860 (CC) and rs8099917 (TT) alleles was not different [24]. Yilmaz *et al.* [25] also found no relationship between the prevalence of rs12979860 and rs8099917and response to treatment.

In the present study, we compared the prevalence of IL28B genotypes (CC, CT, TT) separately in three different response profiles, including SVR, unresponsive, and relapse. In addition, we created two different groups: patients with the C allele (CC and CT) and patients without the C allele (TT). The prevalence among these two groups was explored in the patients with ETVR, EVR, SVR, and with or without relapse. However, we could not find any significant difference among response groups in terms of frequency of genotypes in either investigation.

All the TT genotypes were in the unresponsive group, but the total number of patients with the TT genotype and the number of patients in the groups (SVR, no response, relapse) were not sufficient for statistical analysis; hence, calculation could not be performed to compare the TT genotype between the SVR, unresponsive, and relapse groups.

We also could not find a significant difference in terms of fibrosis stage, which is one of the most important markers that reflect histological severity of disease in different genotypes and HDV-RNA level. Different factors likely play a role in poorer prognosis compared to HBV and HCV.

Conclusions

In our study was not highlighted a significant difference among the groups with sustained response, no response, relapse and fibrosis stage in terms of CC and CT polymorphisms.

The present study identified no relationship between IL28B rs12979860 polymorphism and response to treatment and prognosis of the disease in patients with chronic delta hepatitis.

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