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Research Article

Relationship Between Interleukin-28B Gene Polymorphism and Chronic Hepatitis B Infection

Kronik Hepatit B Enfeksiyonu ile İnterlökin-28B Gen Polimorfizminin İlişkisi

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Abstract

Aim: Interleukin 28B (IL28B) gene polymorphism may play a role in hepatitis B virus (HBV) infection prognosis. We investigated the effects of IL28B gene polymorphism on viral clearance and viral load in chronic hepatitis B (CHB) patients.

Material and Methods: We included 146 individuals who applied to our center between October 2011 and October 2012. CHB (N=117) and control (N=29) groups were compared in terms of IL28B gene rs12979860, rs12980275 gene region polymorphisms, and IL28B gene expression (mRNA) levels.

Results: There was no statistically significant difference between groups in terms of rs12979860 gene region polymorphism rates ($\chi2=0.36$, $p=0.835$). But there was a significant association between groups in terms of rs12980275. In the CHB group, the A/A genotype was much more, ($\chi2=55.2$, $p<0.001$), G/A and G/G genotypes were less frequent. There was no statistically significant difference between the groups in terms of IL28B expression levels.

Conclusion: This study revealed the genotype profile of the IL28B gene of our region. It is the first study on this subject in our region. The association between CHB and rs12980275 polymorphism may be important. Our results will contribute to future studies.

Keywords: Chronic hepatitis B, IL 28B polymorphism, IL 28B expression, Turkey

Öz

Amaç: İnterlökin-28B (IL28B) geni polimorfizmi hepatit B virus (HBV) enfeksiyonunun prognozunda önemli olabileceğine dair yayınlar bulunmaktadır. Bu çalışmada IL28B geni ile HBV enfeksiyonunun viral klirens ve kronik HBV enfeksiyonunda viral yük arasındaki ilişkiyi araştırmayı planladık.

Materyal ve Metot: Ekim 2011 ile Ekim 2012 tarihleri arasında merkezimize başvuran 146 kişi çalışmaya dahil edildi. Kronik hepatit B (KHB) grubunda 117, kontrol grubunda 29 kişi vardı. Tüm gruplarda IL28B geni rs12979860, rs12980275 polimorfizmlerine ve IL28B geni ekspresyon düzeylerine (mRNA) bakıldı ve gruplar karşılaştırıldı.

Bulgular: Rs12979860 gen polymorfizmi oranları açısından gruplar arasında fark yoktu ($\chi2=0.36$, $p=0.835$). Ama rs12980275 bölgesi açısından KHB grubunda A/A genotipi daha fazla iken ($\chi2=55.2$, $p<0.001$), G/A ve G/G genotipleri istatiksel anlamlı olarak daha az bulundu. Gruplar arasında MRNA düzeyleri açısından fark yoktu

Sonuç: Öncelikle bu çalışma bölgemizdeki IL28 gen polimorfizm oranlarını ortaya koyan ilk çalışma olmasından dolayı önemlidir. KHB ile rs12980275 polimorfizmi arasındaki ilişkinin klinik önemi bulunduğu düşünülmektedir. İleride bu konuda yapılacak çalışmalara ışık tutmaktadır.

Anahtar Kelimeler: İL28 B polimorfizmi, İL28B ekspresyonu, kronik hepatit B, Türkiye

INTRODUCTION

More than 350 million people are diagnosed with chronic hepatitis B (CHB) in the world, annually. Around 600 thousand people die from CHB related complications worldwide per year (1). The most common complications of CHB are liver cirrhosis and hepatocellular carcinoma (HCC). Age, gender, blood TNF, IFN, vitamin D, estrogen receptor alpha level, and genetic variations in many HLA loci have been shown to correlate with the prognosis of CHB infection (2). There are many reports that IL28B gene polymorphisms are closely related to viral load and spontaneous clearance of hepatitis C infection. The IL28B gene encodes the cytokine IFN-lambda3 (INF-λ3), which belongs to the type III INF family (INF-λ) (3). The pathogenesis and the transmission characteristics of

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HBV and hepatitis C virus (HCV) are similar. Recently, an increasing number of studies have been published about the effects of the IL28B gene on HBV infection (4). In a study, the relationship between viral replication, spontaneous clearance rates of HBV, and IL28B polymorphism variants (rs12979860, rs12980275, rs8099917) were investigated. In that study, 203 patients with chronic hepatitis B, 203 hepatitis B carriers, and 203 healthy individuals had included. It was reported that there was no statistical difference between the three groups in terms of IL28B gene polymorphism and viral replication, and viral clearance (5). In another study, it was reported that HBV-related HCC has a statistically significant relationship with IL28B gene polymorphism (6).

In our study, we aimed to reveal the frequency of IL28B polymorphism and to investigate the relationship between IL28B polymorphism, and CHB infection, HBV viral load, viral clearance, and other factors.

MATERIAL AND METHOD

We included 146 volunteers in our study who applied to our center's outpatient clinic between October 2011 and October 2012. There were 117 CHB patients in the study group and 29 resolved hepatitis B patients in the control group. IL 28B gene rs12979860, rs12980275 polymorphism, and IL 28B gene expression level (mRNA level) were measured in all patients included in the study. Rs12979860 gene region has homozygous (CC), heterozygous (CT), and mutant (TT) genotypes. The Rs12980275 gene region has homozygous (AA), heterozygous (GA), and mutant (GG) genotypes. The study and control groups were compared for frequency of both gene region polymorphisms, IL28B gene expression levels, gender, HBsAg level, hepatosteatosis level (mild if <5% fat, moderate if <5%, severe if >30%), HBeAg positivity presence of liver cirrhosis, HBV DNA level (those with <104 copies/ml, those between 104-107 copies/ml and those >107 copies/ml), response to IFN treatment (those who became HBV DNA negative with IFN treatment before 1 year, after 1 year and those who were unresponsive to IFN therapy).

Eligibility criteria

1) Chronic Hepatitis B group was defined as, HBs-Ag positive for more than 6 months, anti-HBc IgG positive, and anti-HBs Ag negative. A liver biopsy must be done before. Anti-HCV, Anti-HIV, HBV-DNA, HBeAg, Anti-HBeAg, AST, ALT, liver ultrasound tests must be checked regularly.

2) For the control group; it must be HBsAg negative, Anti-HBs, and Anti-HBc IgG positive.

Exclusion criteria

1) HCV or HIV positive

2) Malignancy (except HCC)

3) Severe systemic disease

Genomic Isolation and Measurement Method

Approximately 3cc of blood was obtained from peripheral venous blood from the patients and control group included in the study. DNA isolation was performed with a DNA isolation kit (purelink™ genomic DNA kits) in the Medical Genetics laboratory. Patient DNA was stored at -20 degrees until the study was conducted. RNA isolation was performed with a Roche Magna compact magic isolation device in the medical genetics laboratory, and the samples were stored at minus 80 degrees. Genomic DNAs obtained from blood samples rs12979860 showing TNP in the IL28B gene, rs12980275 amplification mix, and "Light Cycler Fast Start DNA Master Hybridization" probes (Roche, Germany) using the LightCycler 2.0 (Roche Applied Science, Germany) instrument. The prepared mixtures and DNAs were mixed, and the results were uploaded to the Real-Time device, and the results were added to the data. The obtained RNAs were measured by nanodrop and brought to an equal position for obtaining cDNA. Obtained RNAs were mixed with 1 µl H2O, 1 µl Random hexamer primer, anchored-oligo (Dt) 18 primer on 10 µl and put into 0.2 Eppendorf tubes and waited for 10 minutes at 65 decimal points in the PCR protocol using the "transcriptor first-strand cDNA synthesis" kit. A mixture was prepared for overlaying the RNAs. The appropriate protocol was loaded into the Real-Time PCR device by adding cDNA to the prepared mixture following the protocol in the kit. Forward and Reverse Primers specific to the IL28B gene in Gene Expression Assay (GENEX-250, Suarge Biotechnology, Turkey) were prepared according to the AMPLIFYME SYBR Universal Mix (AM02, BLIRT, Poland) protocol by preparing qPCR experiments with the StepOnePlus™ Real-Time PCR System (ThermoFisher Scientific, USA) device, RNA expression levels were measured. RNA expression levels were determined by the ΔΔCт method according to normalization with G6PDH endogenous control. In this study, the "G6PDH detection mix" was used as a control. For each sample, one control was used and the study was repeated for control purposes. The obtained results were arranged and entered by the light cycler relative quantitation software program.

Statistical analysis

The statistical package program SPSS 16 (Statistical Package for the Social Sciences, version 16 (SSPS Inc, Chicago, IL, USA) was used for the analysis of the data set. Independent t-test and one-way ANOVA test were used in the evaluation of the data, while Pearson $\chi 2$ test was used for the evaluation of categorical data, and $p < 0.05$ was considered statistically significant.

Ethical issue

Approval was obtained from the ethics committee of our center. Detailed information was given to all participants and an informed consent form was signed.

RESULTS

The IL28B gene rs12979860 polymorphism was examined in 111 individuals (89 patients in the CHB group and 22 people in the control group) (Table 1). In CHB group there were 46 (51.7%) C/C, 38 (42.7%) C/T, 5 (5.6%) T/T genotype. In the control group, there were 11 (50%) C/C, 9 (40.9%) C/T, 2 (9.1%) T/T. There was no association between rs12979860 polymorphism and CHB ($\chi 2(2, N=111)=0.36$, p=0.835). Rs12979860 genotypes (C/C, C/T, and T/T) were compared in terms of gender, hepatosteatosis level, presence of HBe antigen, presence of liver cirrhosis in the CHB group, and no statistically meaningful difference was found between genotype groups.

Patients in the CHB group were divided into three subgroups in terms of HBV-DNA levels (<104, 104-107,> 107), and it was found that they had statistically similar genotypes in the subgroups. We didn't find a statistically meaningful difference between the genotype distributions of those who responded to interferon treatment before one year, after one year, and those who did not respond at all. Interleukin 28B gene rs12980275 region has 3 polymorphisms namely A/A, G/A, and G/G. In the CHB group, 52 patients (59.8%) were A/A, 34 patients (34.1%) were G/A, 1 patient (1.1%) was the G/G genotypes. In the control group, 1 patient (3.8%) was A/A, 11 patients (42.3%) were G/A, and 14 patients (53.8%) were G/G genotype. A/A genotype was much more in the CHB group than the control group, and the difference was statistically meaningful ($\chi 2(2, N=113)=55.2$, p<0.001). In addition, IL28B rs12980275 gene region A/A, G/A, and G/G genotypes were compared in terms of gender, hepatosteatosis, HBe antigen positivity, liver cirrhosis, HBV DNA level and, response to INF treatment There was no statistical difference between genotypes (Table 2).

Interleukin 28B gene expression level was measured in 117 patients (95 in the CHB group and 22 in the control group). The mean values in the groups were compared using the T-test and no statistically meaningful difference was found between the groups (Table 3). In addition, the rs12979860 gene polymorphisms, those with C/C genotype and those with C/T and T/T genotypes were compared separately and the total (C/T + T/T) IL28B gene expressions of the two, and there was no statistical difference between the groups (p=0.35).

Table 1. Comparison of Groups in Terms of IL 28B Rs12979860 Gene Polymorphism (Chi-Square Test)

rs12979860	C/C		C/T		T/T		P-value
	N		N		N		
Polymorphism Distribution							
Chronic Hepatitis B	46	51.7%	38	42.7%	5	5.6%	0.835
Control group	11	50.0%	9	40.9%	2	9.1%	
Sex							
Men	22	44%	24	48%	4	8%	0.295
Women	23	64%	12	33%	1	3%	
Hepatosteatosis							
<%5	33	76%	26	72%	4	100%	
%5-30	10	24%	9	25%	0	0%	0.615
>%30	0	0%	1	3%	0	0%	
HBeAg							
Negative	36	54%	26	39%	4	7%	0.701
Positive	9	45%	10	50%	1	5%	
Cirrhosis							
Yes	7	85%	33	92%	3	60%	0.145
No	38	15%	3	8%	2	40%	
HBV DNA levels							
$<10^4$	6	13%	6	17%	1	20%	
10^4-10^7	22	48%	17	48%	2	40%	0.891
$>10^7$	17	37%	12	35%	2	40%	
Response to IFN treatment							
<1 year	2	15%	1	11%	1	33%	
>1 year	1	7.5%	1	11%	1	33%	0.913
Unresponsive	10	77.5%	7	78%	1	33%	

Abbreviations; IFN: Interferon. HBeAg: Hepatitis B e antigen. HBV-DNA: Hepatitis B virus deoxyribonucleic acid

Table 2. Comparison of Groups in Terms of IL 28B Rs12980275 Gene Polymorphism (Chi-Square Test)

rs12980275	A/A		G/A		G/G		P-value
	N		N		N		
Polymorphism Distribution							
Chronic Hepatitis B	52	59.8%	34	39.1%	1	1.1%	<0.001
Control group	1	3.8%	11	42.3%	14	53.8%	
Sex							
Men	26	54.2%	21	43.8%	1	2%	0.295
Women	25	70%	11	30%	0	0%	
Hepatosteatosis							
<%5	37	77%	24	75%	1	100%	
%5-30	11	23%	7	21%	0	0%	0.763
>%30	0	0%	1	4%	0	0%	
HBeAg							
Negative	38	74%	23	71%	1	50%	0.807
Positive	13	26%	9	29%	1	50%	
Cirrhosis							
Yes	47	77%	28	78%	1	50%	0.915
No	7	23%	4	22%	1	50%	
HBV DNA levels							
$<10^4$	6	11%	6	19%	1	44%	
10^4-10^7	23	45%	15	48%	1	33%	0.147
$>10^7$	22	44%	10	33%	1	33%	
Response to IFN treatment							
<1 year	1	7%	1	11%	1	33%	
>1 year	1	7%	1	11%	1	33%	0.913
Unresponsive	12	86%	7	78%	1	33%	

Abbreviations; IFN: Interferon, HBeAg: Hepatitis B e antigen, HBV-DNA: Hepatitis B virus deoxyribonucleic acid

Similarly, no significant meaningful difference was found between those with the rs12980275 gene region A/A genotype and those with the G/A and G/G genotype in terms of IL28B gene expressions (p=0.31).

Table 3. Comparison of patient and control groups in terms of IL28B gene expression levels (T-test)

Groups	N	Mean	S.D	S.E
CHB	95	2.6823	8.39663	.86148
Control	22	1.8150	3.02216	.64433

Abbreviations; CHB: Chronic hepatitis B, SD: Standart deviation, SE: Standart error

DISCUSSION

Chronic hepatitis B is an important health problem. Several factors predict the prognosis of CHB infection and response to treatment. In several studies, the relationship between the IL28B gene and CHB showed, before. In our study, CHB (n=117) and control (n=29) groups were compared in terms of IL28B gene rs12979860 and rs12980275 gene region polymorphisms. There was a significant difference between these two groups in terms of rs12980275 but not in terms of rs12979860 gene region polymorphism rates (p=0.835). We found that the CHB group have more A/A genotype than the control group at the rs12980275 gene region (p<0.01). Also, the control group has more G/A and G/G genotype rates. We can say that if individuals do have not the G allele in the rs12980275 region they tend to be CHB infection. However, in a meta-analysis, in which 18 studies were conducted, it was reported that there is no relationship between rs12979860, rs12980275, rs8099997 polymorphism, and HBsAg clearance. The difference between this meta-analysis and our study may be attributed to the different genotype rates of the virus or the ethnic origin of the patients. Because, in the meta-analysis mentioned above, the patient population consists of Asians. In Asian CHB patients mostly infected genotypes other than D (genotypes A, B, C). But in Turkey, genotype D is by a percentage of 78% (7). Martin MP et al. (8) reported that the C/C genotype of rs12979860 was not associated with HBV recovery, but in that

study, 20% of the patients were HCV and 69% were HIV positive patients. In our study, HCV or HIV positivity was an exclusion criterion. In our study, the co-mutation of rs12980275 and rs12979860 gene regions was found to be very high (p<0.01). However, the clinical significance of this situation isn't known. In our study, it was planned to investigate the relationship between HCC and IL28B gene polymorphism, but it could not be evaluated due to only 7 patients had HCC. In our study, no significant relationship was found between IL28B rs12979860 and rs12980275 polymorphisms and HBV recovery. In the study of Sonneveld et al. (9), It was emphasized that especially patients with the rs12979860 C/C allele had 3 times more HBV recovery than those with C/T and T/T alleles. It has been reported that the C/C allele is high in genotypes A, B, C, while the C/C allele is less in the D genotype. In our study, IL28B rs12979860 CC allele frequency was found 56%. Since HBV genotype D is higher in the Turkish population, HBsAg seroconversion may not be affected by IL28B polymorphism. In a study, it has been reported that the prevalence of the C/C allele is 95% in Asians, 75% in Europeans, and 42% in Africans (9). In our study, neither rs12979860 nor rs12980275 was found the predictive factor of IFN treatment outcomes. It is well-known that hepatitis C patients who have rs12979860 CC genotype, respond to IFN therapy better and have a higher sustained virological response. However, there is no consensus for CHB on this issue. Lampertico et al. (10) reported that in a study conducted 101 HBeAg negatives (all patients HBV genotype D) CHB patients, the IL28B rs12979860 CC genotype has a significant positive effect on response to IFN treatment, persistent virological response, and HBsAg clearance compared to the CT + TT genotype. Sonneveld et al. (9) showed the CC genotype as a positive predictor of response to IFN treatment in 203 HBeAg positives (HBV genotype not specified) CHB patients. On the contrary, in three separate studies conducted with 512 CHB (HBV genotype B and C) patients in China, 115 HBeAg positives (HBV genotype B and C) patients in Taiwan and, 95 HBeAg positive or negative patients in Germany, a significant relationship between response to PegIFN treatment and IL28B polymorphism has not been found (11,12). The reason for these contradictions may be due to the inhomogeneity of the patient population, the effects of other genetic factors of the host, the genotype difference of HBV in the studies, and the fact that some studies were conducted with HBeAg positive patients and some with HBeAg negative and some without considering HBeAg. In future studies, we can have more clear information on this issue with homogeneous patient groups.

There were some limitations of our study. Because our study was single-center, the results cannot be generalized to the entire country population. The two most frequently studied gene regions in CHB were evaluated, but the effects of polymorphisms in other gene regions could not be compared. The false-positive and false-negative rates of the PCR method can be high. Next-generation sequencing might be more accurate for reliable results.

CONCLUSION

In conclusion, our study is the first study on this subject in our region. Our findings will be useful for future studies in terms of revealing the genotype profiles of the IL 28B gene. The high co-mutation rates of rs12979860 and rs12980275 may be important but we do not know clinical significance at the moment. Individuals with A/A or G/A genotype at rs12980275 were found significantly more in CHB patients. This polymorphism will be important in the prediction of the prognosis of HBV infection. In this study, no relationship was found in terms of sex, HBsAg level, percentage of hepatosteatosis, HBeAg, liver cirrhosis rates, and HBV DNA levels, for both gene regions, in CHB patients. In the future, IL28B may be useful in clinical practice for predicting the response to treatment and prognosis as in CHB infection. However, available data are not sufficient for this, yet.

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