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IL28B polymorphism To predict the response to chronic hepatitis C treatment

Kronik hepatit C tedavisine yanıtı tahmin etmede IL28B gen polimorfizmi

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Abstract

Purpose: Hepatitis C is an important medical problem which causes chronic liver disease. 170 million people are believed to be infected throughout the world. While spontaneous hepatitis C virus (HCV) clearance occurs in almost 30% of patients with hepatitis C, it becomes chronic in 70% which may progress to cirrhosis or hepatocellular carcinoma (HCC). In recent years, *IL28B* polymorphism on chromosome 19q13 has been suggested as the most important predictor among the host factors for sustained virologic response (SVR). In the present study, we aimed to evaluate the effect of *IL28B* gene polymorphism on conventional treatment response.

Materials and methods: Totally 95 patients treated with a combination of peginterferon and ribavirin during 48 weeks were included the study. Age, gender, AST and ALT levels, insulin and fasting glucose levels, BMI, fibrosis levels on liver biopsy and 0-4-12-24-48-72-week HCV RNA values were recorded.

Results: In the study group of 95 HCV-infected patients, the mean age was 52.4±11 years. SVR was observed in 54 (56.8%) of patients at the follow up. Demographical and laboratory variables were similar in SVR and non-SVR groups. *IL28B SNP rs12979860* was positive in CC 26 (47.2%), CT 25 (45.4%) and TT 4 (7.2%) patients with SVR group and in CC 4 (10%), CT 23 (57.5%), TT 13 (32.5%) patients with non-SVR group.

Conclusion: SVR was achieved more likely in HCV genotype 1 rs12979860, and treatment decision may be changed based on the degree of liver disorder. In this regard, new studies involving larger patient population from our country are needed.

Key words: Hepatitis C, interleukin-28B, genetic variation

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Özet

Amaç: Hepatit C, kronik karaciğer hastalığına neden olan büyük bir sağlık sorunudur. Dünya'da 170 milyon insanın enfekte olduğu düşünülmektedir. Hepatit C'li hastaların yaklaşık %30'unda spontan viral klirens meydana gelirken, %70'inde hastalık kronikleşmekte, bir kısmında siroz ve hepatosellüler kanser gelişebilmektedir. Konak faktörleri içinde kalıcı viral yanıtın en iyi belirleyicisi, son yıllarda ortaya konan kromozom 19q13 üzerindeki *IL28B* polimorfizmidir. Çalışmadaki amacımız bölgemizdeki hasta populasyonunda IL28B gen polimorfizminin tedaviye yanıta olan etkisini ortaya koymaktır.

Gereç ve yöntem: Çalışmaya 48 haftalık peginterferon ve ribavirin tedavisi ve sonrasındaki 24. haftada kontrole gelen 95 hasta alındı. Hastaların tedavi öncesi yaş, cinsiyet, AST, ALT değerleri, insülin ve açlık glukozu, karaciğer biyopsisinde fibrozis düzeyleri,-0-4-12-24-48-72.hafta HCV RNA değerleri kaydedildi.

Bulgular: Hastaların yaş ortalaması 52,4±11 idi. Çalışma sonunda hastaların 54'ünde (%56.8) kalıcı viral yanıt gözlendi. Tedaviye yanıta etki eden demografik ve laboratuvar özellikleri kalıcı viral yanıt ve kalıcı olmayan viral yanıt gruplarında benzerdi. Kalıcı viral yanıt olan hastalarda IL 28B SNP rs12979860 polimorfizmine bakıldığında; CC 26 (%47.2), CT 25 (%45.4), TT 4 (%7.2) hastada saptandı. Kalıcı viral yanıt olmayan hastalarda IL 28B SNP rs12979860 polimorfizmi; CC 4 (%10) ,CT 23 (%57.5), TT 13 (%32.5) hastada saptandı.

Sonuç:HCV genotip 1 *rs12979860* CC genotipte kalıcı viral yanıt oranının yüksek olması nedeniyle karaciğer hastalığının şiddetine dayalı tedavi kararını değiştirebilir. Bu konuda ülkemizde daha büyük hasta populasyonlarını içeren çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Hepatit C, interlökin-28B, gene polimorfizmi

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Introduction

Hepatitis C virus (HCV) infection is considered as a global health problem. World Health Organization (WHO) reported that 130-170 million people are chronically infected with HCV worldwide and that 3-4 million people are newly infected per year [1] Anti-HCV seropositive was detected in 1% in Turkey. [2]. While spontaneous hepatitis C virus (HCV) clearance occurs in almost 30% of patients with hepatitis C, it becomes chronic in 70% which may progress to cirrhosis or hepatocellular carcinoma (HCC) [3].

Hepatitis C is associated with mortality and morbidity related to cirrhosis and HCC. These complications can prevent with hepatitis C-oriented curative antiviral treatment. Genotype 1b is frequently seen in Turkey [4]. Sustained virologic response (SVR) can be provided in only half of the patients with genotype 1 HCV by peginterferon/ribavirin treatment. Several factors, viral or host, such as HCV genotypes, baseline viral load, age, gender, BMI, insulin resistance, hepatic steatosis and hepatic fibrosis have been reported to be linked to obtain sustained response.In recent years, IL28B polymorphism on chromosome 19q13 has been suggested as the most important predictor among the host factors for SVR. Although these factors inform about the degree of SVR possibility, their utilization is controversial for treatment decision [5-7]. The cure rate is up to 40% with standard therapy in patients with genotype 1 [7]. HCV RNA negativity defined as rapid virologic response and early virologic response has significant predictive value for outcome after 4 weeks of treatment [8-9].

Genetic identification studies were performed to establish the treatment response in genotype 1 hepatitis C patients treated with peginterferon and ribavirin combination [3]. A single nucleotide polymorphism coded IFN-lambda-3 near the *IL28B* gene on chromosome 19 has been described and it was associated to 2-fold increased sustained viral response in Caucasians, Afro Americans and Hispanics [3]. The importance of such generic region on the treatment response was demonstrated in two genome-related studies. It was shown that IL28B polymorphism is associated with spontaneous clearance of HCV infection [10, 11]. IL28B codes a protein known as interferon-lambda-3 (IFNλ-3). IFNλ is thought to play an important role in innate immunity and viral clearance. Although IFNλ and IFNλ-3 receptors are different, their intracellular pathways (jak/STAT) are similar and both of them express various IFN-stimulated gene [10]. *rs12979860* and *rs8099917* are single nucleotide polymorphism (SNP) variants of *IL28B* showing strongest relation with treatment response. *IL28B rs*12979860 is the well-known and most-investigated variant. There are more six SNPs showing correlation with SVR in IFN lambda gene cluster apart from *IL28B rs*12979860. However, their efficiency disappears after presence of *rs12979860* is adjusted. Following these, another variant is rs8099917 which correlates with SVR following the adjustment about *rs12979860* association. Although there are SNP variations between studies, strong association between these SNPs and SVR indicated the close association between IFN response and genomic region contained *IL28B* and its potential regulatory sequences [3, 5, 10]. In the present study, we aimed to evaluate the effect of *IL28B* gene polymorphism on conventional treatment response.

Material and methods

Totally 95 patients treated with a combination of peginterferon and ribavirin during 48 weeks and after followed for 24 weeks in İzmir Katip Çelebi University Atatürk Training and Research Hospital, Department of Gastroenterology between 2009-2011 were included the study. Age, gender, AST and ALT levels, insulin and fasting glucose levels, BMI, fibrosis levels on liver biopsy and 0-4-12-24-48-72-week HCV RNA values were recorded.

We knew that obtained results would show both allele T and allele C because our positive control was a heterozygote DNA. It allowed us to compare the samples and control with melting peaks from positive control. For real time PCR, an operational kit with primer and mixed probes included control DNA was used.

Primary and probe mix was prepared according to the manufacturer's instructions. 3 mM of Mg^{+2} concentration, concentration of enzyme mix of LightCycler® FastStart DNA Master HybProbe (03003248001) and 20 ul of final reaction volume was prepared. Because of a Hotstart enzyme is used according to

LightCyler Protocol, amplification was applied at 95^{0}C for 5 seconds, 60^{0}C for 10 seconds and 72^{0}C for 15 seconds during 45 cycles following pre-incubation at 95^{0}C for 10 minutes. Single fluometric reading was performed at 60^{0}C. Then, continuous reading was performed at 95^{0}C for 30 sec, 40^{0}C for 120 sec and 85^{0}C for 0 sec during 1 cycle but 3 readings were performed if any temperature increase would occur. Finally, device was cooled at 40^{0}C for 30 sec to protect the device. Mutation analyse was performed using hybridisation probe method. Sequences contained T nucleotide and C nucleotide were decided to compare the control DNA and melting results of samples. Since our probe is C nucleotide-specific, cases were termed as those with C nucleotide or T nucleotide if high or lower peak was observed, respectively.

Statistical analysis: Wilcoxon test and Man-Whitney U test for non-normally distributed data and chi-square test for categorical data were used to compare the groups. P<0.005 was considered as significant. Treatment response was evaluated using logistic regression. All analysis was performed by SPSS 15.0 for Windows.

Results

In the study group of 95 HCV-infected patients, the mean age was 52.4±11 years. The mean baseline viral load was 2809163±575348.6 IU/ml and 66.8% (*n*=56) of patients had high viral load (>400000 IU/ml). Sustained virologic response (SVR) was observed in 54 of patients at the end of the study. When demographic and laboratory characteristics which effect the treatment response were compared with SVR and non-SVR groups, the mean age was 51.2±11.2 years for SVR group and 53.9±10.6 years for non-SVR group and there was no significant difference (*p*=0.2). No gender difference was found between SVR and non-SVR groups (35.1% vs. 28.7%, *p*=0.15). Also, number of patients with AST>40 IU/ml (34.9% vs. 30.2%, *p*=0.49) were comparable in both groups. SVR and non-SVR groups were statistically similar for baseline viral load (2600989±766270.8 IU/ml vs. 3086730±881197.1 IU/ml, *p*=0.6). 33% of patients had high viral load (>400000 IU/ml) in both groups. In terms of histological stage, 9.7% of patients were pre-cirrhotic, 4.2% were cirrhotic in SVR group and 6.9% were pre-cirrhotic, 1.4% were cirrhotic in non-SVR group. Demographical, biochemical, histological and genetic characteristics of patients are summarized in Table 1.

IL28B SNP *rs12979860* was positive in CC 26 (47.2%), CT 25 (45.4%) and TT 4 (7.2%) patients with SVR group and in CC 4 (10%), CT 23 (57.5%), TT 13 (32.5%) patients with non-SVR group.

When treatment response was assessed for genotype subgroups, 66% of patients with CC genotype showed early virologic response (EVR) and 66.7% showed sustained virologic response (SVR). Early virologic response rate was 23.5% and sustained virologic response rate was 23.5 in patients with TT genotype (Table 2). Odds ratio was estimated for responders and non-responders; SVR was obtained in 26 of 30 patients with CC genotype (OR:8.06, *p*<0.001) No significant effect of CC genotype was determined on the treatment response. SVR was seen in 13 of 17 patients with TT genotype (OR:6.14, *p*=0.002 (Table 3). Genotypes of *IL28B* gene *rs12979860* single nucleotide polymorphisms (SNPs) were analyzed. CC genotype was associated with SVR and EVR; SVR and EVR were seen in 86.7% and 66.7% of patients with CC genotype, respectively. SVR and EVR were seen in 23.5% patients with TT genotype. For SVR, odds ratio was 8.06 (*p*<0.001) in patients with CC genotype and 6.14 (*p*=0.002) in patients with TT genotype.

Discussion

IL28B SNPs, *rs129479860* and *rs8099917* are known to be associated with treatment response. *IL28B rs12979860* is the well-known and most investigated variant. There are 6 more SNPs into IFN-lambda gene cluster related to SVR apart from *IL28B rs12979860*. However, their efficiency disappears after presence of *rs12979860* is adjusted. Following these, another variant is *rs8099917* which correlates with SVR following the adjustment about *rs12979860* association. There are SNP differences among the studies. In a study using multiple platform by Suppiah et al. [5] have reported that *rs8099917* (TT genotype) showed strongest association with SVR. Another independent genome-wide association study

Table 1. Demographic, biochemical, histological and genetic characteristics.

	SVR (*n*=55)	non-SVR (*n*=40)	*p*
Age	**51.2±11.2**	**53.9±10.6**	**0.2**
BMI	**26.5±4.23**	**27.8±4.58**	**0.15**
Female	**35.1%**	**28.7%**	**0.66**
AST>48 IU/ml	**19.8%**	**22.1%**	**0.13**
ALT>40 IU/ml	**34.9%**	**30.2%**	**0.49**
Baseline viral load (IU/ml)	**2600989±766270.8**	**3086730±881197.8**	**0.6**
High viral load (>400000 IU/ml)	**33.3%**	**33.3%**	**1**
METAVIR stage %			**0.55**
***pre-cirrhotic**	**9.7%**	**6.9%**	
***cirrhotic**	**4,2%**	**1.4%**	

Table 2. *IL28B* polymorphisms predict the response to treatment

	n	RVR	EVR	SVR
CC	**30 (31.5%)**	**8 (26.7%)**	**20 (66.7%)**	**26 (86.7%)**
C/T	**48 (50.5%)**	**3 (6.3%)**	**18 (37.6%)**	**25 (37.6%)**
TT	**17 (18.0%)**	**3 (17.6%)**	**4 (23.5%)**	**4 (23.5%)**

Table 3. Odds ratio of patients for treatment response

Allelefrequency	responder	non-responder	OR	%95 CI	*p*
C	**51 (65.3%)**	**27 (34.7%)**	**6.14**	**1.9-19.6**	**0.002**
T	**29 (52.7%)**	**26(47.3%)**	**8.09**	**2.7-24.6**	**<0.001**
Genotype frequency	**responder**	**non-responder**	**OR**	**%95 CI**	***p***
CC	**26(47.2%)**	**4(10%)**	**8.06**	**2.6-24.6**	**<0.001**
CT (responder)	**25(45.4%)**	**23(57.5%)**	**0.62**	**0.27-1.4**	**0.24**
(non-responder)			**1.62**	**0.72-3.7**	**0.30**
TT	**4 (7.2%)**	**13(32.5%)**	**6.14**	**1.9-19.6**	**0.002**

(GWAS) by Ge et al. [3] reported the strongest association of *rs12979860* (CC genotype) SNP with SVR. Tanaka et al. [6] was reported that no *rs12979860* but *rs8099917* and *rs12980275* correlated with SVR. Ultimately, numerous studies have been shown an association between SNPs and SVR. Also, genomic area consisted of *IL28B* and its potential regulatory sequences had been associated with IFN response [12]. A study which compared two SNPs, *rs12979860* and *rs8099917* showed that individuals with *rs12979860* C allele were more likely to achieve SVR than those who carried the T allele and individuals with rs8099917 T allele were more likely to achieve SVR than those who carried the G allele. In addition, the positive predictive value (PPV) was found lower for *rs8099917* [12]. In IDEAL study included patients with HCV genotype 1 treated with peginterferon/ribavirin, SVR was achieved in 69% of patients who carry two positive allele (CC genotype), in 33% of patients with CT genotype and in 27% of patients with TT genotype for *rs12979860* [3]. In another study assessed HCV genotype 1 patients, SVR was reported as 85% in CC carriers, 45% in CT carriers and 41% in TT carriers for *rs12979860* [12]. Various studies were performed to compose a local guideline in many countries [13-15].

Our results were comparable with previous studies. *IL28B* polymorphism may be important for deciding to wait new antiviral drugs but it shouldn't be considered alone for treatment decision because of waiting new antiviral drugs

but it shouldn't be considered alone for treatment decision because of lower positive and negative predictive values. Since, SVR was achieved more likely in HCV genotype 1 *rs12979860*, treatment decision may be changed based on the degree of liver disorder. In this regard, new studies involving larger patient population from our country are needed.

Conflicts of interest: No conflicts of interest exist.

References

1. Mohamed AA, Elbedewy TA, El-Serafy M, El-Toukhy N, Ahmed W, Ali El Din Z. Hepatitis C virus: A global view. World J Hepatol 2015;7:2676-2680.
2. Tozun N, Ozdogan O, Cakaloglu Y, et al. Seroprevalence of hepatitis B and C virus infections and risk factors in Turkey: a fieldwork TURHEP study. Clin Microbiol Infect 2015;21:1020-1026.
3. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399-401.
4. Altuglu I, Soyler I, Ozacar T, Erensoy S. Distribution of hepatitis C virus genotypes in patients with chronic hepatitis C infection in Western Turkey. Int J Infect Dis 2008;12:239-244.
5. Suppiah V, Moldovan M, Ahlenstiel G, et al. *IL28B* is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 2009;41:1100-1104.
6. Tanaka Y, Nishida N, Sugiyama M, et al. Genome-wide association of *IL28B* with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C Nat Genet 2009;41:1105-1109.
7. Thompson AJ, Muir AJ, Sulkowski MS, et al. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. Gastroenterology 2010;139:120-129.
8. Yu JW, Wang GQ, Sun LJ, Li XG, Li SC. Predictive value of rapid virological response and early virological response on sustained virological response in HCV patients treated with pegylated interferon alpha-2a and ribavirin. J Gastroenterol Hepatol 2007;22:832-836.
9. Jensen DM, Morgan TR, Marcellin P, et al. Early identification of HCV genotype 1 patients responding to 24 weeks peginterferon alpha-2a (40 kd)/ribavirin therapy. Hepatology 2006;43:954-60.
10. Thomas DL, Thio CL, Martin MP, et al. Genetic variation in *IL28B* and spontaneous clearance of hepatitis C virus. Nature 2009;461:798-801.
11. Rauch A, Kutalik Z, Descombes P, et al. Genetic variation in *IL28B* is associated with chronic hepatitis C and treatment failure: a genome-wide association study Gastroenterology 2010;138:1338-1345.
12. Stattermayer AF, Stauber R, Hofer H, et al. Impact of *IL28B* genotype on the early and sustained virologic response in treatment-naïve patients with chronic hepatitis C. Clin Gastroenterol Hepatol 2011;9:344-350.
13. Echeverría N, Chiodi D, López P, et al. IL28B gene polymorphism rs12979860, but not rs8099917, contributes to the occurrence of chronic HCV infection in Uruguayan patients. Virol J 2018;15:40.
14. Ramos JA, Ramos AL, Hoffmann L, et al. A single nucleotide polymorphism, *rs129679860*, in the *IL28B* locus is associated with the viral kinetics and a sustained virological response in a chronic, monoinfected hepatitis C virus genotype-1 Brazilian population treated with pegylated interferon-ribavirin. Mem Inst Oswaldo Cruz 2012;107:888-892.
15. Świątek-Kościelna B, Kałużna E, Strauss E, et al. Prevalence of IFNL3 *rs4803217* single nucleotide polymorphism and clinical course of chronic hepatitis C.World J Gastroenterol 2017;23:3815-3824.