



The Role of the Basal Core Promoter (BCP) and Precore Codon 28 Mutations in the Treatment Response to the Pegylated Interferon

Pegile-Interferon Tedavisine Yanıtta Bazal Kor Promoter (BCP) ve Prekor Kodon 28 Mutasyonlarının Rolü

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ABSTRACT

Objective: Pegylated interferon has an important place in chronic hepatitis treatment and there are many factors affecting treatment response. Among these factors, studies on viral genomic mutations increase recently. In this study we aimed to determine the effects of precore and basal core promoter (BCP) mutations on the response to peg-interferon treatment in patients received interferon treatment with the diagnosis of chronic hepatitis B.

Materials and Methods: Seventy eight chronic hepatitis B patients were enrolled in the study. Before the treatment precore codon and BCP mutations were studied by Multiplex PCR and Reverse Hybridization Methods (Inno-Lipa HBV DR v2. Innogenetics, Belgium). Biochemical and virological response rates were calculated for patients treated with peg-interferon therapy for 48 weeks.

Results: Twenty two patients had precore codon 28 mutations, 10 patients had BCP (A1762/T1764) or BCP (T1762/A1764) mutations, 19 had precore codon 28 mutations with BCP mutations, and 27 patients had no mutations. Virological response rates in the precore codon 28 mutation group were 68.1%, 80% in the BCP mutation group, and 73.7% in the group with both mutations, and 70.4% in the group with no mutations.

Conclusion: According to these results, although the presence of BCP mutations is an indicator of a better response to pegylated interferon treatment, it was not statistically significant. However, in order to demonstrate that it is effective in the response to interferon therapy, newer studies with much larger patients groups should be conducted. (*Viral Hepatitis Journal* 2014; 20(1): 19-22)

Key words: Hepatitis B, pegylated interferon, precore mutations

ÖZET

Amaç: Pegile interferon, kronik hepatit tedavisinde önemli bir yere sahiptir. Pegileinterferona tedavi yanıtını etkileyen birçok faktör bulunmaktadır. Bunlar arasında viral genomik mutasyonlarla ilgili yapılan çalışmalar son zamanlarda artmaktadır. Bu çalışmada, kronik hepatit B tanısıyla interferon tedavisi verilmiş hastalarda prekor ve bazal kor promoter (BCP) mutasyonlarının pegile interferon tedavisine yanıtındaki etkilerini saptamayı amaçladık.

Gereç ve Yöntemler: Çalışmaya kronik hepatit B tanılı 78 hasta dâhil edildi. Tedavi öncesi prekor kodon ve BCP mutasyonları nested PCR yöntemiyle çalışıldı. Pegile interferon tedavisi 48 hafta süreyle uygulanan hastaların virolojik ve biyokimyasal yanıt oranları hesaplandı.

Bulgular: Hastaların 22'sinde prekor kodon 28 mutasyonu, 10'unda BCP (A1762/T1764) ya da BCP (T1762/A1764) mutasyonu, 19'unda prekor kodon 28 mutasyonu ile birlikte BCP mutasyonu saptanırken, 27 hastada herhangi bir mutasyona rastlanmadı. Virolojik yanıt oranları prekor kodon 28 mutasyonu saptanan grupta %68,1; BCP mutasyonu saptanan grupta %80, her iki mutasyonun birlikte bulunduğu grupta %73,7; mutasyon saptanmayan grupta ise %70,4 olarak hesaplandı.

Sonuç: Bu sonuçlara göre BCP mutasyonlarının varlığı, pegile interferon tedavisine yanıtta daha iyi yanıt göstergesi olsa da, istatistiksel olarak anlamlı bulunmamıştır. Ancak interferon tedavisine yanıtta etkili olduğunun gösterilebilmesi için çok daha geniş hasta gruplarıyla birçok yeni çalışma yapılmasının gerektiğini düşünmekteyiz. (*Viral Hepatit Dergisi* 2014; 20(1): 19-22)

Anahtar Kelimeler: Hepatit B, pegile interferon, prekor mutasyonları

Introduction

Chronic hepatitis B virus (HBV) infection is an important health problem that can cause 25%-40% of the liver cirrhosis and hepatocellular carcinoma. Approximately 350 million people worldwide have chronic hepatitis B (1,2).

The goal of treatment is to protect patients from complications of hepatitis B. Pegylated-interferon has an important place in the treatment of chronic hepatitis. Approximately one third of patients with interferon therapy develops HBeAg seroconversion (3). Pegylated-interferon, has an important role in the treatment of chronic hepatitis, there are many factors that affect response to therapy. These include the role of viral genomic mutations even if it is not clarified yet, although the studies on this subject are increasing recently.

Precore stop codon mutations blocking HBeAg expression without preventing HBV replication, aggravate liver disease and cause fulminant hepatitis (4,5,6). The most frequent stop mutation in HBV precore side is located in G1896A. As a result of this precore mutation, codon 28 which is known as stop codon and thus the HBeAg synthesis is prevented (7,8,9). While the precore mutation is rare in patients with genotype A, it is especially is more common in patients infected with genotype-D (10,11). One of the other mutation groups affects the basal core promoter (BCP) region. The most common mutations in BCP are nt 1762 (A-T) and 1764 (G-A). This mutation was first reported by Okamoto et al (12). BCP mutations cause less transcription of RNA, while it does not affect significantly RNA transcription or translation of core proteins. The formation of these mutations resulted in an decrease the synthesis of HBeAg and viral load increased and they occur more often in patients with genotype-A, B and C (13).

In this study, we aimed to determine the effects of precore and BCP mutations on pegylated-interferon treatment response who followed in our hospital with a diagnosis of chronic hepatitis B patients treated with pegylated interferon.

Material and Methods

Seventy-eight patients treated 48 weeks with pegylated interferon for chronic hepatitis B infection were included in the study. The inclusion criteria; >18 year old, being more than 6 months of HBsAg positivity, HBV DNA ≥ 105 copies / mL in HBeAg positive patients, HBV DNA ≥ 104 copies / mL in HBeAg negative patients, patients who used pegylated interferon at least twelve months, patients whose serum HBV DNA levels measured before, 3, 6 and 12 months of treatment and cases with HBV precore and BCP mutations studied before the treatment. Exclusion criteria; who stopped the treatment before 12 months, pregnant, transplant patient, patients who had major depression, untreated hyperthyroid, uncontrolled hypertension, heart disease, diabetes mellitus, autoimmune diseases, decompensated liver disease and renal failure. Before the treatment, serum HBsAg, anti-HBs, HBeAg, anti-HBe serologic indicators were tested with EIA (Liaison, Diasorin, Italy) method in patient serum. Each patient was underwent liver biopsy and then staged by Knodell staging scoring system. HBV-DNA and liver function tests were performed before treatment, 12, 24, 36 and 48 weeks of the treatment. Real time PCR method (COBAS Ampliprep / COBAS Taqman 48, Roche, Branchburg, NJ, USA) for HBV-DNA test was used.

HBV precore and BCP mutations were studied before the treatment by multiplex PCR and reverse hybridization methods (Inno-Lipa HBV DR v2, Innogenetics, Belgium). HBV-DNA < 2000 IU / mL at 6 months of the treatment is accepted as virological response criteria and alanine aminotransferase (ALT) normalization is accepted as biochemical response (14).

Chi-square test was used for statistical analysis of mutations detected and undetected virologic response according to the state group.

Results

Forty-nine (62.8%) of the 78 patients were males, mean age was 38.8 year (range:20-58). All patients in the study were infected with hepatitis B genotype D. The number of the patients with pc codon 28 mutation, BCP (A1762/T1764) or BCP (T1762/A1764) mutation, pc codon 28 mutation with the BCP mutation were 22, 10 and 19, respectively. No mutation was determined in 27 patients.

In 22 patients with precore codon 28 mutation, mean HBV-DNA, mean ALT and virological response rate were $1,8E+7$ IU/mL, 57,5 IU/dL and 68,1%, respectively at the beginning of the treatment. The biochemical response rate was 72,7% at the end of the treatment. In 10 patients with BCP (A1762/T1764) or (T1762/A1764) mutations, mean HBV-DNA, mean ALT and virological response rate were $3,9E+5$ IU/mL, 36,4 IU/dL and 80%, respectively at the beginning of the treatment. In 19 patients with precore codon 28 mutation and BCP mutations, mean HBV-DNA, mean ALT, virological response rate and biochemical response rate were $6,7 \times 10^7$ IU/mL, 64,4 U/L, 73,7% and 78,9%, respectively at the beginning of the treatment. In 27 patients with no mutation, mean HBV-DNA, mean ALT, virological response rate and biochemical response rate were $4,4 \times 10^7$ IU/mL, 68,6 IU/L, 70,4% and 74,1%, respectively at the beginning of the treatment. All results were shown in (Table1).

According to the results obtained BCP presence of mutations, in response to treatment with pegylated interferon better response indicator, though, were not statistically significant. The presence of precore mutation in precore codon 28 group with virologic response was not statistically significant.

Discussion

The main goals in the treatment of chronic HBV are reduce or stop the replication of HBV and prevent to develop the complications such as cirrhosis, liver failure and hepatocellular carcinoma. For this purpose, the one of the main target is HBV-DNA reduction or disappearance (15,16). Among the drugs currently used in the treatment of chronic hepatitis B, the pegylated-interferon has an important role. In many studies conducted with pegylated-interferon treatment showed that there are some factors affecting the treatment response. These includes the initial ALT and HBV-DNA levels, genotype, age, sex and HBeAg status (17,18,19). -Genetic changes are thought to play an important role in the natural course of HBV infection. These mutations may also influence the development liver cancer, chronic liver disease and of fulminant hepatitis. First identified and the most emphasized HBV mutations are precore / core mutations (20). Studies have found higher

Table 1. Baseline treatment characteristics and response rates of patients according to the mutation status

Characteristics	Precore mutation group (n=22)	BCP mutation group (n=10)	Precor Codon 28+BCP mutation group (n=19)	Undetected mutation group (n=27)
Age	37.9	39.3	39.1	38.7
Gender (male/female)	11/11	8/2	14/5	16/11
ALT (U/L)	57.5	36.4	64.4	68.6
HBV-DNA (IU/mL)	1.8E+7	3.9E+5	6.7E+7	4.4E+7
HAI	10.1	9.2	9.6	11.5
Genotype	D	D	D	D
HBeAg (+)	4	2	4	9
Virological response (%)	68.1	80	73.7	70.4
Biochemical response (%)	72.7	80	78.9	74.1

prevalence of precore mutation in patients with HBeAg positive and showed that these mutations increase the risk of persistent hepatitis and liver cirrhosis (21). In USA studies performed in adults infected with chronic HBV, the prevalence of this mutation ranges from 20%-95% (22). In our country, Arslan et al. did not detect any precore / core mutations of 51 HBV DNA-positive chronic hepatitis B patients (23). Today, the effects of these mutations in the antiviral response to treatment are not clear. The most recent studies showed that the patients have no precore mutation chain before treatment with interferon alpha, had better treatment results (24,25). Unlikely, we found that virological response rates were similar in groups with or without precore mutation.

The core mutation affect BCP which includes c1742-c1849 nucleotide ad cause decreased transcriptional precore and core mRNA. BCP mutation usually is located in Nt 1744-1804 (21). In some studies with BCP mutations found that these mutations were associated with increased viral replication and viral load, suppressed expression of HBeAg. However, other studies were found no association with viral load level (26-29). Moreover, it has been considered that BCP double mutations increase viral replication, fulminant liver failure and hepatocellular carcinoma by upregulating pregenomic RNA (30). In this respect, BCP mutations have an important role to follow-up of chronic HBV. Although there is not much study on the effects of the mutations on the pegylated interferon response, it has been reported that especially the T1762, A1764 were advantage in response to pegylated interferon therapy (31,32). Similarly, in our study the virological response rate was higher in groups with BCP mutations than in the group have no mutations, but there was no statistically significant differences between the groups ($p>0.05$).

According to the results obtained BCP presence of mutations, in response to treatment with pegylated interferon better response indicator, though, were not statistically significant. The presence of precore mutation in precore codon 28 group with virologic response was not statistically significant. To clarify the effects of both the precore codon 28 and BCP double mutations on the response to pegylated interferon, the new studies with large patients groups are needed.

Conflict of interest: None declared.

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