

Hepatitis C Virus genotypes detected in Erciyes University

Erciyes Üniversitesi'nde saptanan Hepatit C Virüs genotipleri

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SUMMARY

Hepatitis C virus is an important cause of chronic liver disease worldwide. There has been at least 6 genotypes of HCV and genotype 1b has been associated with more severe liver disease and poor response to therapy. The purpose of this study was to investigate the distribution of HCV genotypes among patients with chronic hepatitis C in Kayseri. Fifty seven patients with chronic hepatitis C were included in this study. HCV RNA viral load were determined by real time PCR method. HCV RNA positive sera were genotyped by restriction fragment length polymorphism method. Type 1b and type 1a were detected in 55 (96.5 %) of patients and 2 (3.5 %) of the patients respectively. HCV genotype 1b is the predominant genotype in patients with chronic hepatitis C in Kayseri. The results of our study contribute important information to the management and treatment of chronic hepatitis C in our region.

Key words: Hepatitis C virus, hepatitis C genotypes, chronic hepatitis C.

ÖZET

Hepatit C virüsü tüm dünyada kronik karaciğer hastalığının önemli bir etkenidir. Altı HCV genotipi bulunmaktadır. Genotip 1b ile enfekte hastalarda ciddi karaciğer hastalığı görülür ve interferon tedavisine yanıt düşüktür. Bu çalışmanın amacı Kayseri'de kronik hepatit C'li hastalarda HCV genotipini araştırmaktır. Kronik hepatit C'li 57 hasta çalışmaya dahil edilmiştir. HCV RNA düzeyi gerçek zamanlı PCR yöntemi ile araştırılmıştır. HCV genotipleri 'Restriction Fragment Length Polymorphism' metodu ile belirlenmiştir. HCV genotiplerinin 55 (%96.5)'i tip 1b, 2 (%3.5)'si tip 1a olarak bulundu. Sonuç olarak Kayseri bölgesinde kronik hepatit C'li hastalarda genotip 1b en sık genotiptir. Bu çalışmanın sonuçları bölgemizdeki kronik hepatit C'li hastaların takip ve tedavisi açısından önemli bilgi sağlamaktadır.

Anahtar kelimeler: Hepatit C virus, hepatit C virus genotip, kronik hepatit C.

INTRODUCTION

Hepatitis C virus (HCV) infection is a major cause of morbidity and mortality worldwide. HCV infection frequently persists and may cause cirrhosis and hepatocellular carcinoma (1,2).

The virus has a positive sense single stranded RNA genome approximately 10 kb in length. Although most of the patients infected with HCV can be diagnosed by using both standard serological assays for anti-HCV and PCR for HCV RNA, it has become apparent that isolates

of HCV may vary considerably in the nucleotide sequences of their genomes. These variations fall into a series of specific patterns, which have been defined genotypes. At least six major genotypes of HCV, each comprising multiple subtypes have been identified worldwide (2). Interferon responsiveness, the pathogenesis of infection and the performance of diagnostic assay all may vary according to genotype (3,4).

HCV subtypes 1a and 1b are the most common genotypes in United States (5). These subtypes

also are predominant in Europe (3,6). In Japan, subtype 1b is responsible for up to 77 % of cases of HCV infection (7). Although HCV subtypes 2a and 2b are relatively common in North America, Europe and Japan, subtype 2c is found commonly in Northern Italy (8). HCV genotype 4 appears to be prevalent in Egypt (9) and genotypes 5 and 6 seem to be confined to South Africa and Hong Kong respectively (8). HCV genotype 1b is the most common one in Turkey (10,11).

In this study our aim was to investigate the genotype distribution of HCV in Kayseri.

MATERIALS AND METHODS

Patients. Fifty seven patients (35 Female, 22 Male) with chronic hepatitis C were included in the study. In all patients serum aminotransferase levels, (ALT, AST) and histopathological examinations of liver were determined. All of the patients were anti-HCV positive. All sera were stored at -70°C until use.

Real Time PCR quantification of HCV RNA. HCV RNA was extracted by a RNA extraction kit (Viogene, USA) and detected by PCR (ABI PRISM 7700 sequence detection system) (12).

HCV Genotyping. The RNA pellet was reverse transcribed to complementary cDNA using random hexanucleotide mix and 10 units of AMV RT in a final volume of 20 μL . This mixture was incubated in a thermal cycler at 42°C for 1 hour. Five microliters of the cDNA mixture was added to PCR mixture containing 5 pmol of primers derived from the 5' non-coding region of the virus. The first round of PCR was performed using external sense and antisense primers (5' ATACTCGAAGGTGCAGTCTACGAGACCT 3'; 5' CTGTGAGGAACTACTGTCTT 3'). A thermal cycler program with 30 cycles; following initial denaturing at 94°C for 5 minutes, denaturing at 94°C for 1 minute, annealing at 60°C for

1 minute, extension at 72°C for 2 minutes, followed by 7 minutes final extension at 72°C was performed. In the second round of the nested PCR, the same cycle program was performed using internal sense and antisense primers (5'CTGTGAGGAACTTCTG TCTT 3' ;5' TTCACGCAGAACGTCTAG 3'). The second PCR products were analyzed on a 2% agarose gel stained with ethidium bromide, and the expected 256 base pair length was confirmed. Amplified RT-PCR products from the 5' non-coding region were used for restriction fragment length pattern analysis. Secondary PCR products were double digested with the restriction enzyme pairs HaeIII and RsaI; MvaI and HinfI. The contents of each digest were 20 μL PCR products, 2.5 μL of 10X enzyme buffer and 10 units of each enzyme. Reactions were incubated at 37°C for 4-16 hours. Subtypes 1a/b were distinguished by incubating the mixture at 60°C for overnight using the restriction enzyme BstUI. Digestion products were visualized under UV light after electrophoresis through an ethidium bromide stained 6% polyacrylamide gel in 1X Tris-borate-EDTA buffer at 200 V for 4 hours (13).

RESULTS

HCV genotypes of 55 (96.5 %) patients were found as type 1b, 2 (3.5 %) of them were found as type 1a. Thirty five of type 1b patients were female and 20 of them were male and all of genotype 1a patients were male.

Mean ALT levels and AST levels of type 1b patients were 62 IU/mL and 50 IU/mL respectively. Mean serum HCV RNA levels were found as 5×10^5 IU/mL in type 1b patients and 9×10^5 IU/mL in type 1a patients. Liver histopathologies were found as chronic active hepatitis C in 53 (96 %) of type 1b patients and 2 (100 %) of type 1a patients (Table 1). Transfusion history was present in 7 % of the all cases.

Table 1. Demographic, clinical, serum biochemical and histological features of 57 patients with chronic hepatitis C.

Features	Type 1b (n:55)	Type 1a (n:2)
Gender (F/M)	35/20	0/2
Mean age (± sd) years	48±10.5	47±9.9
Mean ALT(± sd) IU/ml	62±27 IU/mL	58±50 IU/mL
Mean AST(± sd) IU/ml	50±21 IU/mL	40±8 IU/mL
Mean serum HCV RNA (IU/mL)	5 x10 ⁵ ±5.8 10 ⁵	9 x10 ⁵ ± 2.7 10 ⁵
Transfusion history	3	1
Liver histopathology		
CAHC*	53	2
Cirrhosis	2	-

*Chronic active hepatitis C

DISCUSSION

Hepatitis C virus is the major cause of hepatitis, cirrhosis and hepatocellular carcinoma. Substantial divergence in nucleotide sequences occur among isolates of HCV. Phylogenetic analysis classify HCV into at least six major genotypes on the basis of overall sequence similarity in both coding and noncoding regions of the viral genome (14). The molecular methods of HCV genotyping are direct sequencing, line probe assay and restriction fragment length polymorfism (8). Restriction fragment length polymorfism could be insufficient for subtyping of HCV genotypes (15).

In our country, Abacıoğlu et al (16) found the ratio of HCV genotypes as 75.3 % for genotype 1b, 19.1 % for genotype 1a, 3.4 % for genotype 2 and 2.2 % for genotype 4. Sönmez et al ¹⁰investigated HCV genotypes in Turkish patients with a multicenter study and found type 1b ratio as 69.5 %. In southern part of Turkey, the prevalence of HCV genotypes were detected as 82.2 % for genotype 1b, 14.5 % for genotype 1a and 3.3 % for 2a (11). Tuncer et al (17) reported that the most common genotype was 1b (72 %) among patients. Yalçın et al (18) found that the ratio of genotype 1b was 100 % in chronic hepatitis C patients. Kılıç et al (19) found that 88 % of the hemodialysis patients had genotype 1b.

In this study HCV genotypes of 55 (96.5 %) patients were found as type 1b, 2 (3.5 %) of the patients were found as type 1a. Abacıoğlu et al (16) reported the transfusion history as 39.5 % in chronic liver disease patients. In this study transfusion history was present in 7 % of all cases.

Several investigators reported that genotype 1b is associated with older patient age, longer duration of infection, acquisition of infection by routes other than injection drug use, poor responsiveness to interferon therapy, higher levels of viremia and more severe liver disease, including cirrhosis and hepatocellular carcinoma (5,20,21). In agreement with this, most of the type 1b infected patients in our series have responded poorly or not at all to interferon (unpublished observations).

As a conclusion in this study the genotype 1b was found as the most common genotype among chronic hepatitis C patients. The genotypic distribution of the samples in this study reflects that of in Turkey. Therefore these results should be confirmed by larger series containing samples with different genotypes.

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