HEPATITIS C VIRUS GENOTYPES ARE CHANGING IN THE SOUTHEAST OF TURKEY

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ABSTRACT

Hepatitis C virus (HCV) infection is a frequent cause of acute and chronic hepatitis, and may lead to the development of cirrhosis and subsequently hepatocellular carcinoma. It is estimated that about 150 to 200 million people have been in contact with HCV worldwide, and approximately 85% are chronically infected. HCV is a highly heterogenous virus. At least 6 genotypes and more than 50 subtypes marked with letters (e.g. 1a, 1b, 2a, 2b) of the virus, have been detected worldwide until now. HCV genotype 1 was detected as the most common genotype in the studies also done in our country. The aim of this study is to determine the HCV genotypes and reveal the change in HCV genotypes in the South-Eastern region of Turkey.

Between April 2007 and October 2008, the study evaluated serum samples of 74 patients (40 women) that were HCV RNA positive determined by polymerase chain reaction (PCR). HCV genotypes were determined with Inno-LIPA method. 5’ non-coding region (5’NCR) of HCV-RNA was amplified by reverse transcriptase polymerase chain reaction (RT-PCR) and genotyped by line probes in Inno-LIPA assay. The genotype results of HCV in the serum samples were as follows: 87.8% genotype 1b, 4.1% genotype 1, 2.7% genotype 2, 2.7% genotype 3 and 2.7% genotype 3a. Genotype 1 was determined predominantly in a total of 68 (91.9%) patients.

Keywords: Hepatitis C virus, polymerase chain reaction, genotype, Inno-LIPA

Abbreviations: HCV: Hepatitis C virus; RNA: Ribonucleic acid; PCR: Polymerase Chain Reaction; RT-PCR: Reverse transcriptase polymerase chain reaction; 5’NCR: 5’ non-coding region; ORF: Open reading frame


Introduction

Hepatitis C virus (HCV), is a systemic infection agent which primarily affects the liver. Studies show that 3% of the human population in the world, i.e. approximately 170 million people, is infected with HCV. HCV infection may cause various clinical forms varying from an asymptomatic to fulminant infection (6, 9, 14). The viral infection may also lead to the development of cirrhosis and subsequently hepatocellular carcinoma.

HCV is a lipid enveloped, approximately 50 nm in diameter, small RNA virus. It is classified in the Flaviviridae family, genus Hepacivirus with GB virus B (15). It is a single stranded RNA virus with a genome comprised of 9500 nucleotids. It contains one open reading frame (ORF) which encodes large polyproteins. ORF carries at both termini (5’ and 3’) highly structured nontranslated regions that provide indispensable signals for RNA translation and replication (5, 16).

Six HCV genotypes and a large number of subtypes have been identified in the studies done in different parts of the world. Genotypes 1, 2 and 3 are found throughout the world; but other genotypes are common in particular geographic regions (10). Genotype 1b is encountered as the most common genotype in the studies done in our country (1, 6). There are diversities in terms to response to interferone therapy among these genotypes. HCV genotype 1 and 4 are less likely to respond to interferon treatment than HCV genotype 2 or 3. Therefore genotyping, might help to direct the treatment management (10, 13).

Genotyping is based on determination of changes in the viral genome. For this purpose, a short region of viral genome is amplified by PCR. The amplified segment is mostly the 5’NCR region. Genotyping is made according to sequence analysis and a probe hybridisation of the amplified segment from the viral genome or according to the differences of the restriction region (13).

Detection of HCV genotypes in our region and determination of its evolution over time was aimed in this study.

Materials and Methods

Between April 2007 and October 2008, HCV RNA positive serum samples of 74 patients (40 women) were collected and included in this study. Samples were initially tested and determined as positive for HCV RNA by reverse transcriptase real-time PCR (COBAS Ampli-Prep/COBAS TaqMan HCV Test).
HCV genotypes were then identified with Inno-LIPA method (INNO-LiPA HCV II, Innogenetics, Belgium). 5’non-coding region (5’NCR) of HCV-RNA was amplified by reverse transcriptase polymerase chain reaction (RT-PCR) and genotyped by line probes in Inno-LIPA assay.

**Results and Discussion**

The genotyping results of HCV in serum samples were as follows: 87.8% genotype 1b, 4.1% genotype 1, 2.7% genotype 3, 2.7% genotype 3a. Genotype 1 was determined predominantly in total of 68 (91.9%) patients.

HCV genotyping and subtyping was done for 74 HCV RNA-positive serum samples of which genotype 1 was observed in 68 (91.9%), genotype 2 was observed in 2 (2.7%), and genotype 2 was observed in 2 (2.7%) subjects. Further subtyping analysis showed the genotype 1b was present in 65 (87.8%) subjects (Table 1).

**TABLE 1**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>4.1</td>
</tr>
<tr>
<td>1b</td>
<td>65</td>
<td>87.8</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2.7</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>2.7</td>
</tr>
<tr>
<td>3a</td>
<td>2</td>
<td>2.7</td>
</tr>
</tbody>
</table>

The distribution of HCV genotypes show differences throughout the world. While some genotypes are observed all over the world, some are seen in certain geographical regions (3). Genotype 1, 2 and 3 are frequently encountered in Europe, North America, China, Japan and Australia. Genotype 4 is the predominant genotype of Egypt, Middle East and Middle African countries. Genotype 5 is found particularly in South Africa, genotype 6 is seen in Southeast Asia (2, 7, 10).

There are also important differences in subtype distribution. Genotype 1b is the most frequent genotype in our country, as well as in South and East Europe. Recent studies done in Europe reported an increase in genotype 1a and 3a in parallel to decrease in genotype 2a, 2c and 1b (especially among young people) (7).

**TABLE 2**

<table>
<thead>
<tr>
<th>RESEARCHER</th>
<th>1a</th>
<th>1b</th>
<th>2a</th>
<th>3a</th>
<th>4</th>
<th>Indeterminate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altındiş et al. (1)</td>
<td>3.3%</td>
<td>96.7%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Altuğlu et al. (2)</td>
<td>9.9%</td>
<td>87.2%</td>
<td>0.9%</td>
<td>1.4%</td>
<td>0.6%</td>
<td>-</td>
</tr>
<tr>
<td>Yıldız et al. (18)</td>
<td>6%</td>
<td>91%</td>
<td>1.3%</td>
<td>-</td>
<td>1.3%</td>
<td>-</td>
</tr>
<tr>
<td>Selçuk et al. (12)</td>
<td>24.6%</td>
<td>68.5%</td>
<td>-</td>
<td>-</td>
<td>7%</td>
<td>-</td>
</tr>
<tr>
<td>Bozdayı et al. (4)</td>
<td>22.3%</td>
<td>77.7%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gökahmetoğlu et al. (8)</td>
<td>3.5%</td>
<td>96.5%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Savas et al. (11)</td>
<td>-</td>
<td>77.1%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>22.9%</td>
</tr>
</tbody>
</table>

Genotype 1b was found in approximately 90% of HCV positive samples as reported by genotyping studies from Turkey. The other genotypes (genotypes 2, 3 and 4) were less frequently encountered in HCV infections (Table 2).

Genotype 1b was the most frequent genotype in this study concordant with the other studies done in Turkey. Genotype 1, 2, 3 and 3a were less frequently encountered. Genotype 4 was not detected.

Genotype 1 was detected as a predominant genotype in this study, like previously documented in the other two studies from our region (Table 3). In addition to this, genotype 1b was detected in 100% of the cases as reported by a study done in 1999. In another study, that was performed in 2007, genotype 1a and 3a were detected in addition to genotype 1b. In this study we also found genotype 2 that was different from other studies.

**TABLE 3**

Previous studies from our reagion

<table>
<thead>
<tr>
<th>RESEARCHER</th>
<th>n</th>
<th>Year</th>
<th>1a</th>
<th>1b</th>
<th>2a</th>
<th>3a</th>
<th>4</th>
<th>Indeterminate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yalcin et al. (17)</td>
<td>28</td>
<td>1999</td>
<td>-</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cil et al. (6)</td>
<td>22</td>
<td>2007</td>
<td>22.7%</td>
<td>72.8%</td>
<td>-</td>
<td>4.5%</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Conclusions**

Detecting and reporting different genotypes is important epidemiologically. Therefore and because of the effect of genotyping on treatment, it is considered that genotyping might be required before treatment.

**REFERENCES**


HCV Genotypes from different centers of Turkey