PREVALENCE OF HCV GENOTYPES IN PATIENTS REPORTING IN TERTIARY HEALTH CARE HOSPITAL OF KARACHI

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ABSTRACT:
The present study was designed to see the variables which may affect the conventional treatment outcome by injection interferon in chronic hepatitis “C” patients. Among these variables hepatitis “C” genotype is most important, as it influences the duration and dosage of anti-viral therapy. To determine the frequency distribution of HCV genotypes, a study was conducted in a tertiary care centre of Karachi, the largest metropolitan city of Pakistan, where people of all ethnic origins are found. All the HCV positive patients, including inpatient admitted in hospital and outpatients, more than 18 years of age, reported for genotyping of HCV in the clinical laboratory of Dr. Ziauddin Hospital from May 2009 to April 2010 were studied. HCV genotyping was performed on a total of 457 patients who tested positive for presence of Hepatitis “C” viral RNA. The most prevalent genotype was type 3 with 392 (85.8%) cases, followed by type 1 with 51 (11.2%) cases. There were only two cases with a co-infection of type 1 & 3, while genotype 2, 4 and 5 were also seen in a few cases. Among the cases studied, there were higher proportion of females 276 (60.4%), dominating the HCV infected population. The present study revealed that genotype 3 was the most common genotype in patients with HCV infection in Karachi. Other genotypes were also present in the patients infected with HCV, but were of lesser frequency. To prevent the treatment failure it is advisable that before commencement of therapy, the genotype of the patient should be established so that appropriate treatment in correct dosage for optimal duration of time could be instituted.

Keywords: Hepatitis “C” virus, prevalence, genotype, Karachi

INTRODUCTION

Hepatitis “C” Virus (HCV) is the second most common cause of viral hepatitis (Idrees and Riazuddin 2008) and is responsible for infecting about 170 million patients throughout the world. Approximately 250,000 to 350,000 deaths per year are due to chronic hepatitis “C” infection mainly due to cirrhosis, end-stage liver disease, and hepatocellular carcinoma (Chevaliez, et al., 2007). Pakistan has a population of about 170 million people, and it is estimated that about ten million people or 6% of the population is infected with hepatitis C (Hamid et al., 2003; Waheed et al., 2009).

Hepatitis “C” is a major public health concern, and significant amongst all the hepatitis viruses. The most common ways of transmission in low resource countries are transfusion of inadequately screened blood and blood products, use of contaminated needles, syringes and other medical equipment (Ramarokoto et al., 2008) as well as needle sharing among intravenous drug users, unsafe tattooing and body piercing. Sexual and perinatal transmission can occur but are of minor importance (WHO Hepatitis “C” Factsheet No-164, 2000). HCV infection becomes chronic in about 85% cases, with a significant risk of progression to liver cirrhosis (Scot et al., 2007). Once cirrhosis is
Hepatitis “C” virus belongs to the family Flaviridae and genus Hepacivirus. It is a small enveloped single stranded linear, non-segmented, positive polarity ribonucleic acid (RNA) virus with an icosahedral nucleocapsid (Chevaliez and Riazuddin 2008).

The HCV is considered as essentially hepatotropic (Trimoulet et al., 2000), but the virus sequences have also been found in other important extrahepatic sites, including peripheral blood mononuclear cells (PBMCs), the central nervous system, and bone marrow from chronically-infected patients (Majda et al., 2006).

Hepatitis “C” virus due to its peculiar genomic variation is classified in to six major genotypes, 1 to 6, and a large number of subtypes (1a, 1b, 1c etc) (Chevaliez et al., 2007; Lee et al., 2008). The knowledge of genotypes in chronic hepatitis “C” is crucial for the choice of the therapeutic regimen and for the prediction of therapy outcome, because genotypes 2 and 3 are curable in more than 80%, whereas genotypes 1 and 4 are curable in only 40-50% of cases (Strader et al., 2004).

HCV genotype is the strongest predictive factor for sustained virological response (SVR), since patients with different HCV genotypes react differently to α-interferon therapy (Zein et al., 1996). The reported rates of SVR to interferon plus ribavirin combination therapy are 65% and 30%, in patients infected with HCV-2/3 and HCV-1 genotypes respectively (Poynard et al., 1998). The patient genotype has a vital role in treatment outcome therefore it should be done before starting treatment. Although the significance of genotype in treatment response is relatively clear, the influence of viral genotype on the severity of liver damage and the rate of disease progression has not been well defined (Kau et al., 2008).

The patterns for the distribution of different HCV genotypes globally are as follows:

1a is mostly found in North and South America, also common in Australia.
1b is mostly found in Europe and Asia.
2a is the most common genotype in Japan and China.
2b is the most common genotype in the US and north Europe.
2c is the most common genotype in western and southern Europe.
3a is highly prevalent in Australia (40% of cases) and south Asia.
4a is very common in Egypt.
4c is highly prevalent in central Africa.
5a is highly prevalent only in South Africa.
6a is found only in Hong Kong, Macau and Vietnam.
7a and 7b are prevalent in Thailand.
8a, 8b and 9a are common in Vietnam.
10a and 11a are found in Indonesia (Ramia et al., 2006).

MATERIALS AND METHODS

Patient selection:
All anti hepatitis “C” antibody positive patients, more than 18 years of age of either gender were enrolled in the study from May 2009 to April 2010 from the gastroenterology clinic and wards at Dr Ziauddin hospital North-Nazimabad and Clifton campuses Karachi. Those patients (n=457) who were HCV RNA positive were studied for genotyping. Venus blood samples were collected aseptically for genotyping in two
yellow top gel tubes. 3ml whole blood was separated within one hour and subsequently stored at -70°C to be run as one time batch analysis. These were run in groups of 24 tests with a positive and negative quality control with each batch.

**Sample Preparation:**

**RNA extraction and target amplification**

Viral RNA was extracted from patient’s plasma using QIAamp Viral RNA mini kit according to manufacture’s instruction. Two extraction negative (Known negative samples) and one extraction positive (Known positive sample) were included in each batch to assess the efficiency of extraction.

Amplicons for HCV genotyping were produced using Qiagen HCV RG real time PCR kit on Rotor Gene 3000 real time PCR platform.

**HCV Genotyping:**

HCV genotyping was done using the Invader HCV ASR based genotyping assay, developed by Third Wave Technologies, Inc. (TWT) USA. This assay can detect HCV genotypes 1 through 6. The third wave invader assays uses cleavase enzymes to recognize and cleave specific structures formed by the addition of two oligonucleotides (an invader oligo and a primary probe) to a nucleic acid

**Table 1**

Frequencies of various HCV genotypes in males and females of different age categories

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Age less than 40 years</th>
<th>Age more than 40 years</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male n = 77 (35.3%)</td>
<td>Female n = 141 (64.7%)</td>
<td></td>
</tr>
<tr>
<td>Genotype 1</td>
<td>12 (15.6%)</td>
<td>12 (8.5%)</td>
<td>51 (11.2%)</td>
</tr>
<tr>
<td>Genotype 2</td>
<td>1 (1.3%)</td>
<td>-</td>
<td>2 (0.4%)</td>
</tr>
<tr>
<td>Genotype 3</td>
<td>59 (76.6%)</td>
<td>125 (88.7%)</td>
<td>392 (85.8%)</td>
</tr>
<tr>
<td>Genotype 4</td>
<td>3 (3.9%)</td>
<td>4 (2.8%)</td>
<td>10 (2.2%)</td>
</tr>
<tr>
<td>Genotype 1&amp;3</td>
<td>2 (2.6%)</td>
<td>-</td>
<td>2 (0.4%)</td>
</tr>
</tbody>
</table>

Fig. 1: Distribution of genotypes (n=457).
target. A 1:800 dilution of amplified product was made in DNAse RNAse free water. The Invader HCV genotyping assay (TWT) uses eight separate HCV genotype-specific assays on a microtiter plate. The plate has 12 lanes: one devoted to a negative control and the remaining 11 for patient samples. For each case eight wells are utilized.

Samples were denatured at 98°C for 10 minutes followed by incubation with prepared master mix (A-H). The composition of each master mix are (5µl Invader buffer, 1µl Invader enzyme and 4µl respective Invader oligo mix). The mixture was incubated on thermal cycler at 63°C for half an hour followed by reading on ABI 4000 series flourimeter.

Each mixture generates two signals. One signal is a positive control for the presence of any HCV amplicons while the other signal is genotype specific. The values for the two fluorescent signals were entered into a Microsoft Excel based invader data analysis work sheet. The data analysis software compares the signal with a cutoff value and assigns a genotype result.

**Invader Principle:**

The third wave invader assays uses cleavage enzymes to recognize and cleave specific structures formed by the addition of two oligonucleotides (an invader oligo and a primary probe) to a nucleic acid target. The reaction conditions and sequences of the primary probe and invader oligo probe are selected so that the primary probe has a transient association with the target template to form an overlapping structure. The primary probe also contains a 5 prime flap sequence that is non complementary to the target nucleic acid. The 3 prime nucleotide of the invader oligo overlaps the primary probe, but need not hybridize to the target DNA. The cleavage enzyme recognizes this overlapping structure and cleaves off the unpaired 5 prime flap of the primary probe, releasing it as a target specific product. Thus, under the assay conditions, multiple primary probes associate with the target DNA isothermally. This allows for multiple rounds of primary probe cleavage for each target DNA, and amplification of the number of released 5 prime flaps.

**Table 2**

Comparison of various HCV genotypes in different studies of the region

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Genotype 1</td>
<td>n = 457</td>
<td>51 (11.2%)</td>
<td>386 (11.5%)</td>
<td>322 (23.6%)</td>
<td>6 (1.4%)</td>
<td>34.6%</td>
</tr>
<tr>
<td>Genotype 2</td>
<td>2 (0.4%)</td>
<td>282 (8.4%)</td>
<td>-</td>
<td>-</td>
<td>2%</td>
<td>-</td>
</tr>
<tr>
<td>Genotype 3</td>
<td>392 (85.8%)</td>
<td>2261 (67.5%)</td>
<td>806 (59.1%)</td>
<td>265 (63.9)</td>
<td>61.2%</td>
<td>35 (26.3)</td>
</tr>
<tr>
<td>Genotype 4</td>
<td>10 (2.2%)</td>
<td>50 (1.5%)</td>
<td>186 (13.6%)</td>
<td>-</td>
<td>-</td>
<td>2 (1.5%)</td>
</tr>
<tr>
<td>Genotype 1&amp;3</td>
<td>2 (0.4%)</td>
<td>161 (4.8%)</td>
<td>16 (1.2%)</td>
<td>28 (6.7%)</td>
<td>2%</td>
<td>3 (2.3%)</td>
</tr>
<tr>
<td>Genotype 5</td>
<td>-</td>
<td>6 (0.2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Genotype 6</td>
<td>-</td>
<td>4 (0.1%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Un-type able</td>
<td>-</td>
<td>201 (6.0)</td>
<td>34 (2.5%)</td>
<td>116 (27.9%)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
In the secondary reaction, each released 5 prime flap can serve as an invader oligo on a Fluorescence Resonance Energy Transfer (FRET) cassette to create another overlapping structure that is recognized and cleaved by the fluorophore (F) and quencher (Q). The released 5 prime flap and the FRET cassette cycle, result in amplified fluorescence signal.

The primary and secondary reactions run in the same well simultaneously. The bi-plex format of the invader DNA assay enables simultaneous detection of two reactions in a single well.

STATISTICAL ANALYSIS

Automated data analysis and HCV genotype determination were performed using a Microsoft Excel based HCV genotyping invader data analysis worksheet (IDAW) developed and produced by TWT. The data was entered and analyzed through the SPSS version 10.0 (SPSS Inc, Chicago, US). Descriptive statistics was used to summarize the continuous and categorical data. Results were expressed as mean ± standard deviation (SD), frequencies and percentages.

RESULTS

A total of 457 samples were tested for HCV genotyping in the department of molecular pathology, clinical laboratories, Dr Ziauddin Hospital from May 2009 till April 2010. Genotyping was performed on those patients who had earlier tested positive for HCV-RNA. Out of these 457 patients 276 (60.4%) were females and 181 (39.6%) were males. The overall mean age of all the patients was 42.33 years ranging from 18 to 75 years. In females the mean age was 42.13 years (±11.89), ranging from 18 to 70 years and in males the mean age was 42.65 years (±12.26), ranging from 19 to 75 years.

The patents were divided into two groups on the bases of age and the cutoff was taken as 40 years. Patients less than 40 years of age were 218 (47.70%) in which the mean age was 32.03 (±6.34) years. In this group there were 141 females and 77 were males. In this age category the most prevalent genotype was genotype 3, as 184 (84.4%) were genotype 3. The other genotypes in this category included; genotype 1, 24 (11.0%), genotype 4, 7 (3.2%), mixed genotype infection by 1 & 3, 2 (0.9%) and genotype 2, 1 (0.5%).

Patients more than 40 years of age were 239 (52.30%) in which the mean age was 51.73 (±7.41) years. In this group there were 135 (56.49%) females and 104 (43.51%) were males. In this age category the most prevalent genotype was genotype 3, as 208 (87.0%) were genotype 3. The other genotypes in this category included; genotype 1, 27 (11.3%), genotype 4, 3 (1.3%), and genotype 2, 1 (0.4%) (Table 1 & Figure 1).

DISCUSSION

This study was conducted in tertiary care hospital of Karachi. The results of the present study were compared with other studies of the region (Table 2).

Amongst the viral factors, the viral genotype is the most important independent parameter for response to IFN-α therapy. Viral genotype also determines the dose and duration of anti viral therapy. Genotype 3 was found to be the most prevalent type in this study which is in agreement with previously reported studies (Ahmad et al., 2010; Ali et al., 2010; Zarkesh et al., 2010). However, the prevalence rate was relatively higher than earlier studies. The prevalence rate of genotype 3 was 85.5% in the present study, whereas it was 67.5%, 59.1%, 63.9% and 61.2% respectively in other studies. In one of the studies from Iran the prevalence of genotype 3 was only 26.3% (Salim et al., 2010).

The second common genotype in present study was type 1 (11.2%), which is commonly
Prevalence of HCV Genotypes in patients

reported in studies from other parts of Pakistan and Iran, being 11.5%, 23.6% and 34.6% respectively (Ahmad et al., 2010; Zarkesh et al 2010). In one study from Iran however, type 1 was the most prevalent type (69.9%) (Chevaliez et al., 2007). Interestingly genotype 1 was found only in 1.4% patients from a study conducted in Khyber Pakhtoonkhwah (former NWFP province). This low prevalence may be due a large number of untypeable cases (27.9%) in that study (Ali et al., 2010).

In this study it was seen that there were a higher proportion of females than males infected with HCV. This is an interesting finding as studies have shown that female patients have better chances of achieving SVR (sustained viral response) as compared to the males (Kau et al., 2008).

It was also seen that HCV infection is equally prevalent in age groups above or below 40 years. In conclusion the present study revealed that genotype 3 is the most common genotype in patients with HCV infection in Karachi, the largest city of Pakistan, where people of various ethnic origins are found. Other genotypes were also present in the patients infected with HCV, but were of lesser frequency. It is recommended that before commencement of therapy, the HCV genotype of the patient should be established so that appropriate treatment in correct dosage could be started.

REFERENCES


