DISTRIBUTION PATTERN OF HCV GENOTYPES AND SUB-GENOTYPES AND THEIR ASSOCIATION WITH VIRAL LOAD IN A GROUP OF IRAQI PATIENTS WITH CHRONIC HCV INFECTION

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ABSTRACT

Background: Hepatitis C Virus (HCV) is one of the major causes of viral hepatitis. Approximately 170 million people are infected with HCV worldwide. There are no published population based studies about the prevalence of HCV sub-genotypes in Iraq. Objectives: The aim of the study was determination of HCV sub genotypes among a group of Iraqi patients with chronic HCV infection. Patients and Methods: All the samples were subjected to RNA extraction and "real-time RT-PCR" for the quantification of HCV RNA. Genotyping and sub-genotyping were done by using "Versant-LiPA HCV Genotyping assay". The statistical analysis of the data was done by using SPSS version 18. Results: The predominant sub-genotype among Iraqi patients was 1a (20%), followed 4c and 4h (12% each) and then 1b (10%). The frequency of HCV sub-genotypes 1a was higher in male patients compared to female, while female found to be higher in sub genotypes 4 and 2. There was no significant difference of HCV RNA level and genotype and sub-genotype. Conclusions: The most common HCV sub genotype in Iraq was subtype 1a followed by 4c, 4h and 1b.

KEYWORD: HCV sub-genotypes in Iraq.

1- INTRODUCTION

It is estimated that approximately 170-300 million people worldwide are infected with the hepatitis C virus (HCV). The HCV accounts for 20% of all cases with acute viral hepatitis
and 70% of all cases with chronic viral hepatitis.\cite{1} Infection with HCV is associated with high morbidity and mortality, due to the development of cirrhosis and hepatocellular carcinoma and approximately 350000 people die every year, globally, due to HCV infection.\cite{2} HCV is a single stranded positive sense RNA virus of Flavivirus family, which contains two short untranslated region at each end (5’UTR and 3’UTR) and a single open reading frame that is translated to yield the viral proteins in the form of a polyprotein, identified in 1989.\cite{3,4}

HCV can transmit through many sources, such as re-use of syringes, improper blood screening before transfusion, sharing of razors, unsafe sex.\cite{5} Due to genetic variation in nucleotide sequences, HCV is further classified into six principal genotypes and almost 80 subtypes.\cite{6}

The HCV genotypes 1, 2 and 3 are circulating worldwide while the remaining genotypes vary from region to region. Genotype 1 is the dominant genotype in Jordan, Iran and Turkey, HCV subtypes 1a and 1b are most commonly distributed throughout the world and especially in Europe and USA.\cite{7,8}

2. OBJECTIVES
This is an original study in Iraq that involved the searching of HCV genotypes and sub-genotypes among chronic HCV patients. There are few published data about the prevalence of HCV genotypes in Iraq, but there are no published population based studies about the prevalence of HCV sub-genotypes in Iraq. Therefore, the aim of the study was determination of HCV sub-genotypes among patients with chronic HCV infection in Iraq.

3. MATERIALS AND METHODS

3.1. Source of Clinical Samples
All the serum samples were collected from Hepatology and Gastroenterology Teaching Hospital and Viral Hepatitis Referral Laboratory (Baghdad), from December 2015 to March 2016. This study involved 95 patients were screened for anti-HCV and only (50) patients were detected when tested with HCV RNA quantification test (Viral Load).

3.2. Samples Collection and HCV RNA Extraction
About (5-10) ML of venous blood was collected from patients and sera were separated and stored at \(-20^\circ\text{C}\) until tested for HCV genotyping. HCV RNA was extracted from (140 μL)
of serum using the QIAamp viral RNA extraction kit (Qiagen/ Germany) according to the manufacturer’s protocol. The samples were collected from different ages, ranging from (5 - 72) years.

3.3. HCV complimentary DNA (HCV cDNA) by RT-PCR

The Versant HCV amplification 2.0 kit (NLM/ Italy) was used according to the manufacturer’s protocol to amplify the 5'UTR and the core regions of the HCV genome. Master mix was prepared using 2 sets of primers, RT-enzyme, Tag polymerase, dNTPs, MgCl₂ and Nuclease free distilled water. Reactions were run in individual tubes containing (6 µL) of master mix and (14 µL) of the RNA extract of each sample. The PCR was performed as follows; hold 1 (50°C for 15 minutes), hold 2 (95°C for 20sec) and cycle 45 (95°C for 15 sec).

3.4. HCV Genotyping

Detection of HCV genotypes/ subtypes was performed using Versant HCV genotype assay kit (LiPA) 2.0 (NLM/ Italy). This kit is a reverse hybridization LiPA designed to identify HCV genotypes 1 to 6 in the 5'UTRs and core regions of HCV genome. The probes are blotted to a nitrocellulose strip by a poly (T) tail. After hybridization of the biotinylated PCR products to the probes, unhybridized PCR products were washed from the strips. Next, alkaline phosphatase-labeled streptavidin (conjugate) was added to the biotinylated hybrid and incubated. After washing the strips, 5-bromo-4-chloro- 3-indolylphosphate-nitroblue tetrazoliumchromogen (substrate) was added, which forms a purple/brown precipitate in the form of a visible line pattern on the strip specific for each genotype. Each strip has 3 control lines and 22 probe lines specific for HCV genotypes 1 to 6. HCV genotypes are determined by aligning the strips with a reading card and comparing the line patterns from the strip with the patterns on the interpretation chart.

3.5. Statistical Analysis

Some variables were presented in percentage (%). Associations between categorical variables were measured using mean and stander deviation. The SPSS version 18 was used for data analysis and P values of 0.05 or less were considered significant.

RESULTS

In the present study, only those patients were included that were found positive for HCV RNA by RT-PCR. Out of 50 HCV positive patients, 25 (50%) were males and 25 (50%) female; with ratio (1:1) (Table 1).
All the patients belong to different regions of Iraq. Ages of these patients are from (5 _72) years. The results showed that (14) (28%) of the patients were located in age groups between (41-50) years with Mean ± SD (47.0 ± 3.7) while, (12) (24%) of them were distributed in age interval (31-40) with Mean ± SD (36.6 ± 2.7) years and (9) (18%) of them were distributed in age groups (21-30) years with Mean ± SD (25.6 ± 3.3) and (> 50) years with Mean ± SD (59.2 ±5.5), while six of the patients were found in age (< 21) (12%) year constitutes the least number with Mean ± SD (11.8±6.3) as shown in table (2).

The prevalence of HCV genotypes and sub-genotypes distributed among a group of Iraqi patients with CHC virus infection in table (3) which show that the most frequently detected genotype was (4) (42%), with predominant sub-genotypes 4c (12%), 4h (12%), 4a(2%), 4b (2%), 4d (2%) and 4f (4%), while genotype (1) was (38%) with predominant subtype 1a (20%) and 1b (10%), but genotype (6) was (12%) with predominant sub-genotypes 6a (1%), 6c (2%). The lowest frequency was found in genotype (2) (8%) with predominant sub-genotypes 2a (2%), 2b (4%), 2c (2%).
Table (3): The Genotypes and Sub-genotypes of HCV in a group of Iraqi patients with chronic HCV infection according to the gender.

<table>
<thead>
<tr>
<th>Genotype and Sub-genotypes of HCV</th>
<th>Male (%)</th>
<th>Female (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>2 (4%)</td>
<td>2 (4%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>1b</td>
<td>8 (16%)</td>
<td>2 (4%)</td>
<td>10 (20%)</td>
</tr>
<tr>
<td>Genotype 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>0 (0%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>2b</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Genotype 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>2 (4%)</td>
<td>2 (4%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>4b</td>
<td>0 (0%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>4c</td>
<td>2 (4%)</td>
<td>2 (4%)</td>
<td>6 (12%)</td>
</tr>
<tr>
<td>4d</td>
<td>0 (0%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>4e</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>4f</td>
<td>3 (6%)</td>
<td>3 (6%)</td>
<td>6 (12%)</td>
</tr>
<tr>
<td>Genotype 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6a</td>
<td>0 (0%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>6c</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Total</td>
<td>25 (50%)</td>
<td>25 (50%)</td>
<td>50 (100%)</td>
</tr>
</tbody>
</table>

Viral load and its association with HCV genotypes

The Viral load (HCV RNA) of the 50 detected patients out of the 95 anti-HCV ELISA positive patients was compared between the four groups of genotypes as shown in table (4). In the present study the viral load in patients with genotype 4 was significantly higher than those with genotypes 1, 2 and 6, with the Mean ± SD (4.7×10^6 ± 1.9×10^7). The HCV viral load was found to be significantly reduced in genotype (6) Mean ± SD (5.2×10^5 ± 8.9×10^5). While viral load in genotype 1 was (1.8×10^6 ± 4.3×10^6) and in genotype 2 was (7.7×10^5 ± 1.3×10^6). This might be due to more efficient viral replication of genotype 4 as compared to the others. Whereas, there was no significant difference of HCV RNA level and genotypes/sub-genotypes (p value = 0.826).

Table (4): The association between viral load and different HCV Genotypes

<table>
<thead>
<tr>
<th>Genotype and Sub-genotypes</th>
<th>Number (%)</th>
<th>Viral Load Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 1a, 1b</td>
<td>19 (38%)</td>
<td>1.8×10^6 ± 4.3×10^6</td>
</tr>
<tr>
<td>2, 2a, 2b, 2c</td>
<td>4 (8%)</td>
<td>7.7×10^5 ± 1.3×10^6</td>
</tr>
<tr>
<td>4, 4a, 4b, 4c, 4d, 4f, 4h</td>
<td>21 (42%)</td>
<td>4.7×10^6 ± 1.9×10^6</td>
</tr>
<tr>
<td>6, 6a, 6c</td>
<td>6 (12%)</td>
<td>5.2×10^5 ± 8.9×10^5</td>
</tr>
</tbody>
</table>

P value = 0.826 (NS)
DISCUSSION
The epidemiological studies on Hepatitis C Virus genotypes have gained major attention all over the world as they appear to play an important role in elucidating the clinical status of such infection. They have shown to be of great benefit in guiding therapeutic decision and implementing proper preventive strategies. Genotyping of HCV may be a useful epidemiological marker particularly in establishing suspected unconventional routes of HCV transmission such as vertical, intraspousal, or interfamilial transmission (Alfaresi, 2011).[9]

Four different genotypes were reported in this study including genotypes, 1, 2, 4 and 6. The most prevalent genotype was genotype 4 and then genotype 1, though genotypes 2 & 6 were less prevalent. Different sub-genotypes were found among HCV genotypes studied. These include 13 HCV subtypes as genotype (1) with sub-genotype (1a) and (1b) genotype (2) (2a), (2b) and (2c), genotype (4) (4a, 4b, 4c, 4d, 4f and 4h ) and genotype (6) (6a and 6c).

This is the first study in Iraq that involved the searching of HCV genotypes and sub-genotypes among CHC virus infected patients. Although the sample size was small, but the results monitored that the predominant subgenotype of this study is 1a (20%) followed by 4c, 4h (12% each) and 1b (10%). These result was in agreement with the study in Iran and Jordan which found that the sub-genotype 1a is the most common.[10][11] On the other side, in Turkey 1b is the predominant sub-genotype (70%) followed by sub-genotype 1a, also this pattern was seen in Eastern and Southern Europe (Ramia and Eid-Fares, 2006).[13]

The subtype 1a was also the predominant sub-genotype in North America and Northern Europe (Katayama, 1996).[12]

Debojjyoti Bhattacharjee et al., (2015)[14] founds non statistical significance differences was observed in association between viral load and various HCV RNA genotypes.

CONCLUSIONS
In the present study, we concluded that the HCV sub-genotype 1a then 4h, 4c and 1b were the predominant among HCV patients in Iraq.

REFERENCE
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