ABSTRACT

Background and Objective: This study was conducted to evaluate epidemiology of risk factors for hepatitis C virus (HCV) transmission, and determine the association of its genotypes with viral load and response to treatment in patients referred to the Infectious Disease Clinic of Khorramabad.

Methods: The study included patients infected with hepatitis C referred to the Infectious Disease Clinic of Khorramabad during 2011-14. Those who met the inclusion criteria were referred to counseling centers for risky behaviors. After collecting demographic and epidemiologic data on the modes of transmission, blood samples were collected for more accurate testing. The serum of patients with hepatitis C was evaluated in terms of HCV genotype and viral load using PCR.

Results: Among 120 patients studied, 102 (85%) were male and 18 (15%) were female. The most prevalent genotypes were 3a (63.8%), 1a (25%), 1a/1b (5.2%) and 2 (1.7%). Intravenous drug abuse was the main route of transmission of hepatitis C in the study. The genotypes 3a and 1a had a clear correlation with intravenous drug abuse and history of blood transfusion (P<0.05). It was also found that the patients with genotype 3a show a better initial response to treatment with interferon.

Conclusion: The study shows that intravenous drug abuse is the main route of transmission, which highlights the need for intervention and education in this group of patients to prevent disease transmission.

Keywords: Hepatitis C, Genotype, Intravenous Drug Abuse, HIV, Interferon

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Received: 10 Aug 2014
Revised: 08 Oct 2014
Accepted: 20 Oct 2014
INTRODUCTION

Nowadays, infectious disease caused by hepatitis C virus (HCV) is a major public health problem worldwide and a main cause of chronic liver disease (1). Some studies show that about three percent of the world’s population (170 million people) is infected with hepatitis C. Moreover, it is currently the most common cause of advanced hepatic malignancy (1, 2). The prevalence of HCV infection in Iran is less than one percent of the normal population (3). This indicates the lower incidence of the disease compared to neighboring countries such as Yemen (1.1%), Saudi Arabia (8.1% in young population) and Pakistan (4% in blood donors) (4). HCV genome contains a positive-sense, single-stranded, 9.6 kb long RNA molecule (5). Various HCV strains have been isolated from different regions of the world that differ in their nucleotide sequences (6). Based on heterogeneity (diversity) of the isolates’ nucleotide sequences, HCVs are classified into different genotypes and subtypes (7). In addition to clinical and epidemiological importance, HCV genotyping has a prognostic value that can be used to help determine the progressive course of the disease (8). For instance, since peginterferon + ribavirin (standard therapy) and sustained viral response depend on the genotype, the genotypes 2 and 3 respond better to treatment (6, 9, 10). Currently, intravenous drug abuse is the main route of transmission in developing countries. Sexual transmission of HCV is also possible, but uncommon. Other modes of transmission include frequent contact with infected blood among healthcare personnel and perinatal transmission from mother to infant. Transmission via tattooing, cupping and sacrificing rituals is also possible, but extremely rare (11). Previous studies have been on the epidemiology of HCV transmission risk factors, distribution of different genotypes based on variables such as gender, age and viral load, and response to therapy. Contradictory results in different countries based on different geographical regions and ethnicities indicate the need for such studies in other countries such as Iran. Therefore, we aimed to perform an epidemiologic study of risk factors for hepatitis C transmission, and evaluate the relationship between genotypes and viral load and response to therapy in patients admitted to the Infectious Disease Clinic in city of Khoramabad, Iran.

MATERIAL AND METHODS

This cross-sectional study was performed on all patients with HCV referred to the Infectious Disease Clinic in Khoramabad during 2011-14. All participants were informed about the research project, and written consent was obtained from all of them. A trained physician interviewed all patients, and clinical information and history were collected via relevant information forms. Diagnosis of HCV-positive patients in the ELISA method was confirmed by western blotting. These patients were referred to the counseling centers for risky behaviors in the city. Then, demographic and epidemiological data such as age, gender and possible route of transmission were collected from each patient. They were referred to pathobiology laboratories for further laboratory testing. Polymerase chain reaction (PCR) was used to determine the genotype and viral load. For this purpose, 5 ml of blood was taken from each one and mixed with two drops of 7.5% EDTA solution. The mixture was centrifuged and the plasma was transferred to 1.5 ml microtube. Then, viral RNA was extracted using high pure viral nucleic acid kit (Roche, Germany) according to the manufacturer’s instructions. The HCV Real Time PCR kit (Rotor Gene Q) was used to determine the viral load. For genotyping, NS5B and 5UTR viral regions were amplified by RT-PCR and prepared for sequencing. The genotyping results were collected in form of codes. ELISA method was used to evaluate coinfection with HIV among the subjects. All patients received treatment with peginterferon alfa-2b (180μg weekly) + 800 mg of ribavirin for genotypes 2 and 3. However, the dosage ranged between 800-1200 mg per day for other genotypes based on weight of subjects. The treatment continued for six and three months for patients with genotypes 2 and 3 that showed reduced viral load of more than one log and zero in the first
The study population (Table 2) showed that the highest frequency of HCV genotype in males (59.4%) and females (94.7%) was related to the genotype 3a. There was no significant relationship between genotype and gender ($P=0.42$). In this study, 98 patients (81.67%) were married and only two couples were both infected. Coinfection with HIV was found in 22 patients (18.33%) who also had history of intravenous drug abuse. No statistically significant association was found between coinfection with HIV and gender, age and viral load. The patients had history of intravenous drug abuse (48.33%), tattooing (18.33%), dental procedures (25.38%) and blood transfusion (20.83% (15% for surgery and 5.83% for haemoglobinopathy)). In addition, 30% of patients had a history of unsafe sex.

In this study, the genotypes 3a and 1a were the predominant genotypes among intravenous drug abusers and the patients with the history of blood transfusion ($P<0.05$). No significant relationship was found between genotypes and other risk factors. The results also showed that the people with genotype 3a show better initial response to treatment with interferon (IFN). The results also showed that the participants with genotype 1a had higher viral load compared to other genotypes (Table 3). Given the non-normality of data, non-parametric tests were used to compare genotypes’ viral load. The Kruskal-Wallis test showed a significant difference in the viral load of genotypes ($P=0.007$). The Mann-Whitney test showed that the viral load of genotype 1a was significantly higher than genotypes 3a ($P=0.043$) and 1a/1b ($P=0.048$). Moreover, the viral load of genotype 2 was significantly lower than other genotypes.

### RESULTS

Of 120, 102 (85%) were male and 18 (15%) were female. The average age was $10.29 \pm 36.54$ (range of 18-70 years). The predominant genotypes were 3a (63.8%) and 1a (25%), followed by 1a/1b (5.2%) and 2a (1.7%). Moreover, the genotype of five samples (4.3%) were not typeable. Evaluation of frequency distribution of HCV genotypes based on age (Table 1) showed that the genotype 3a was dominant in all age groups. The prevalence of this genotype in the patients under 25 years, 25-40 years, 40-55 years and older than 55 years was 50%, 65.75%, 66.67% and 77.78%, respectively. Moreover, the highest frequency of genotype 1a (68.96%) and 3a (61.54%) was observed in the ones aged 25-40 years. The highest frequency of genotype 2a (100%) was observed in under 25 years. However, there was no statistically significant association between genotype and age groups ($P=0.11$). Evaluation of frequency distribution of HCV genotypes for each gender in the study population (Table 2) showed that the highest frequency of HCV genotype in males (59.4%) and females (94.7%) was related to the genotype 3a. There was no significant relationship between genotype and gender ($P=0.42$). In this study, 98 patients (81.67%) were married and only two couples were both infected. Coinfection with HIV was found in 22 patients (18.33%) who also had history of intravenous drug abuse. No statistically significant association was found between coinfection with HIV and gender, age and viral load. The patients had history of intravenous drug abuse (48.33%), tattooing (18.33%), dental procedures (25.38%) and blood transfusion (20.83% (15% for surgery and 5.83% for haemoglobinopathy)). In addition, 30% of patients had a history of unsafe sex.

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### Table 1 - Frequency distribution of HCV genotypes based on age of patients with hepatitis C

<table>
<thead>
<tr>
<th>Genotype</th>
<th>&lt;25</th>
<th>25-40</th>
<th>40-55</th>
<th>≥55</th>
<th>Total</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>4 (13.79)</td>
<td>20 (68.96)</td>
<td>3 (10.34)</td>
<td>2 (6.89)</td>
<td>29 (100)</td>
<td>$P=0.11$</td>
</tr>
<tr>
<td>1a, 1b</td>
<td>0 (0)</td>
<td>1 (16.67)</td>
<td>5 (83.33)</td>
<td>0 (0)</td>
<td>6 (100)</td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>2 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (100)</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>7 (8.97)</td>
<td>48 (61.54)</td>
<td>16 (20.51)</td>
<td>7 (8.97)</td>
<td>78 (100)</td>
<td></td>
</tr>
<tr>
<td>Non-typeable</td>
<td>1 (30)</td>
<td>4 (30)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>5 (100)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14 (11.67)</td>
<td>73 (60.83)</td>
<td>24 (20)</td>
<td>9 (7.5)</td>
<td>120 (100)</td>
<td></td>
</tr>
</tbody>
</table>
on the relationship between genotype and serum viral load. In a study on 379 patients in Germany, the patients with genotype 3 had significantly lower viral load compared to the ones with genotype 1 (25). This is coordinated with the results of the present study that showed genotype 1a has higher viral load compared to other genotypes. A study in Spain showed that patients with genotype 3 has higher viral load, which is inconsistent with our results (26). In another study in Iran, the highest viral load was reported for genotypes 1b, 1a and 3a (27). The present study also found that the people with genotype 3a show better initial response to treatment with IFN, which is partially consistent with the results of the study conducted by Bjoro et al. in Norway. In the mentioned study, patients with genotype 3a, low viral load and no cirrhosis showed better initial response to treatment with IFN (28). However, another study in Japan showed that suitable response to IFN treatment is usually observed in patients with genotype 2a and high viral load (29).

CONCLUSION
Drug addiction was found as the main mode of HCV transmission in this study. Therefore, preventive strategies are required with greater emphasize on this group to prevent the progression of an epidemic in this group of people.
ACKNOWLEDGMENTS

The authors wish to acknowledge the staff of Central and Noor Pathobiology Laboratories in Khorramabad and Department of Research at Lorestan University of Medical Sciences.

CONFLICT OF INTEREST

All contributing authors declare no conflicts of interest.


