Genotypic Analysis of Hepatitis C Virus in Khuzestan Province, Southwestern Iran

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ABSTRACT

BACKGROUND

Hepatitis C virus (HCV) infection is responsible for considerable morbidity and mortality worldwide. The HCV genotype has a geographic distribution and an important role in clinical and histological outcomes. This study determined HCV genotypes and their related risk factors among patients from Khuzestan Province, Southwest Iran.

METHODS

In a cross-sectional study, 223 patients infected with HCV who referred to Ahwaz Jundishapour University Hospitals (AJSUH) and Hepatitis Clinic were enrolled. Specific and nested polymerase chain reaction (PCR) and restriction fragment length polymorphisms (RFLPs) were performed to determine viral infection and genotype analysis. Liver enzymes including ALT and AST and the correlated risk factors were also determined.

RESULTS

The HCV genotype distribution was as follows: genotype 1a (41.7%); genotype 1b (2.7%); genotype 2 (4.1%); genotype 3a (31.4%); and genotype 4 (1.8%). There were 42 samples (18.84%) not classified into any of the known HCV subtypes. No patient was infected with more than one genotype. HIV was found in four (1.8%) cases, of which all were intravenous drug users. Univariate analysis demonstrated an independent association of intravenous drug use (IVDU) and genotypes 1a (60.7%) and 3a (30%).

CONCLUSION

Our findings suggest that genotypes 1a and 3a are the most common ones among Iranian patients with chronic hepatitis C infection in Khuzestan Province, Southwest, Iran.

KEYWORDS

HCV; Genotyping; Iran

INTRODUCTION

Hepatitis C virus (HCV) infection is responsible for considerable morbidity and mortality worldwide. HCV is a leading cause of liver failure and liver transplantation in adults.[1]

Genotyping of HCV based on geographic distribution has been described.[2] Moreover the HCV genotype has been shown to have an important role in clinical and histological features as well as the response to antiviral treatment.[3]

Most studies suggest that subtype 1b isolate possibly represent more aggressive variants of the virus and better responses to IFN are found in patients infected with either HCV genotypes 2 or 3 in comparison with those infected with genotypes 1 and 4.[4, 5]

Considering that approximately 1% of Iran's population is infected, chronic hepatitis C remains a serious medical problem with considerable burden on Iran's health care system.[6]

These observations have led us to determine HCV genotypes, particularly in the region of Khuzestan Province, Southwest Iran, which has no available data on HCV genotypes.

Herein, we report the results of our cross-sectional analyses of HCV genotype distribution and its association with certain clinical features in patients with chronic HCV infection in Khuzestan Province conducted from September 2006 to September 2009.

MATERIALS AND METHODS

During a three year period (Between September 2006 and September 2009), we conducted a cross-sectional study on 223 consecutive HCV positive individuals who referred to the Ahwaz JundiShapour University Hospitals (AJSUH) and Hepatitis Clinic. Analyses included medical history, physical examination and periodic evaluation, clinically and serologically. Liver enzymes including ALT and AST and the correlated risk factors were also determined. The presence of anti-HCV antibody was determined by an ELISA Test (ORTHO HCV 3.0 Diagnostics, Raritan, NJ, USA). The presence of HCV RNA in anti-HCV positive and indeterminate samples was detected by RT-PCR with the qualitative AMPLICOR HCV Test v.2.0 (Roche Molecular Systems, Branchburg, NJ, USA).

HCV genotyping/sub-typing was performed by two RT-PCR assays, restriction length polymorphism analysis (RFLP) of the 5´noncoding region (5´NCR) and nested PCR with type specific primers following primary RT-PCR with consensus primers designed for the core region of the HCV genome.

HCV genotyping by RFLP was carried out using the restriction enzymes AvaII and RsaI on PCR-amplified from 5´NCR as previously described.[7] Type-specific PCR assay was accomplished by means of the nested PCR assay originally developed by Okamoto et al.[8] for sub typing genotypes 1 and 2, and modified by Widell[9] for the identification of genotype 3. All samples positive for 5´NCR were analyzed. Sequences of the HCV core region were detected after synthesis of cDNA and nested PCR with conserved primers covering positions 439 to 751 of the HCV genome.[10]

Rank-sum and Kruskal-Wallis tests compared continuous variables such as age between the groups. Fisher Exact test assessed associations. Statistical analysis was performed using SPSS software (version 11.5, SPSS Inc., 1989-2002, Chicago, IL, USA) for Windows in addition to univariate analysis and multivariate logistic regression. $p < 0.05$ was considered statistically significant.

RESULTS

All 223 patients had documented positive anti-HCV antibodies for at least six months. There were 170 males (mean age: 34.6 years) and 53 females (mean age: 49.1 years) with an age range of 13-70 years. Samples comprised 42 patients with unknown risk factors and 179 with parenteral risk of infection [11 dialyzed, 10 hemophiliacs, 95 intravenous drug users (IVDU), 7 with tattoos, 54 polytransfused, and needle sticks in 2 cases]. There was a history of extramarital sexual contact in two cases.

Possible routes of infection were recognized in 181 (81.2%) patients. Among these, patients with a history of IVDU, blood transfusion recipients, undergoing chronic hemodialysis, history of tattooing, accidental inoculation and non-marital sexual contact comprised 42.6%,

23.3 %, 4.93%, 3.15%, 0.9% and 0.9% of samples, respectively (Table 1). RFLP identified HCV genotypes in 181 of 223 samples, whereas 42 cases could not be sub-typed with this method.

Table 1: Routes of infection in patients with chronic hepatitis C.

Routes of infection	No. (%)
IVDU	95 (42.6)
Blood transfusion	64 (28.7)
Hemodialysis	11 (4.9)
Tattooing	7 (3.15)
Accidental inoculation	2 (0.9)

Patients' ages were analyzed as a categorical variable (<40 and >40 years). Genotypic distribution was as follows: 1a (41.7%); 1b (2.7%); 2 (4.1%); 3a (31.4%) and 4 (1.8%). There were 42 samples (18.84%) not classified into any of the known HCV subtypes. None of the individuals was infected with more than one genotype. HIV was found in 4 (1.8%) cases, all were IVDU. Univariate analysis demonstrated an independent association between IVDU and genotypes 1 (60.7% 1a) and 3a (30%). Statistical analysis did not show a difference in the frequency distribution of genotypes according to gender and age ($p > 0.56$). In this study, 64 (28.7%) of 223 patients received blood transfusions before 1995, whereas 5 (2.25%) of 64 patients were positive for HCV RNA who received transfusions after 1995.

DISCUSSION

Hepatitis C infection is currently the most common cause of end-stage liver disease in many countries,[11] however the whole population, prevalence is less than 1% in Iran.[12] A comparison of HCV genome sequences from various geographical regions of the world has shown substantial heterogeneity of nucleotide sequences within several regions of the viral genome.

Based on these genomic differences, HCV has been classified into various genotypes. Six major genotypes with several subtypes have been identified and a nomenclature for these was given following a consensus proposal.[13]

In the first report on Iranian patients, Zali et al. in Tehran studied the prevalence of specific genotypes in 15 samples with the following results: type I /1a (7), type II/1b (3), type V/3a (4) and type 4 (1).[14] A recently published article by Samimi-Rad et al. in Iranian patients with anti-HCV Ab positive from Tehran and five cities from different locations of Iran showed a predominance of genotype 1a (47%), followed by 3a (36%), 1b (8%) and 4 (7%), respectively.[15] The current study has shown that in Khuzestan Province, Southwest Iran, genotype 1a accounted for the majority of HCV infections (41.7%).

This is the first study of the genotype distribution in this area which suggests a pattern similar to that reported in other parts of Iran,[14,15] where most cases studied thus far belonged to genotype 1. However the results of our study differed from other Middle-East countries such as the Republic of Yemen, Kuwait, Iraq and Saudi Arabia where genotype 4 has been identified as the most prevalent.[16] A study of Saudi patients detected HCV genotype 4 in 50% of cases and genotype 1b in nearly 40%.[17]

From other parts of the world, studies have revealed that genotype 3 is prevalent in South East Asia whereas genotype 1 is common in the USA and Western Europe.[18] These geographical differences may help in predicting the origin of HCV virus. Patients with risk factors for HCV infection enrolled in the present study were very few and therefore we were unable to reach any conclusion of genotype distribution within this group. The distribution of the two most common HCV viral types (1a and 3a,) were not statistically different in terms of mean age or gender.

The relative influence of HCV genotype, risk factors for infection, duration of infection,

and host factors on progression of liver disease seemed to be important. Many patients in this study were unaware of the duration of infection. Our study indicated that IVDU and blood transfusions were the leading risk factors for HCV acquisition (71.3%), of which IVDU was the most frequent risk factor (42.6 %,). All HCV infected IVDU patients were male, which was consistent with other reports.[12]

Univariate analysis demonstrated an independent association between IVDU and genotypes 1 and 3a. The route of HCV transmission mainly causes this epidemiological pattern in genotype distribution such that IVDU associated with genotype 1a and 3a is currently the primary risk factor of HCV infection.[19]

Our genotype study confirms the epidemiological change in IVDU patients in our area. Screening for HCV is routine for all blood donors since 1995 in Iran[20] but 5 (2.25%) out of 64 patients who received transfusions after 1995 were positive for HCV RNA. Therefore, more careful pretransfusion screening of blood for anti-HCV must be introduced in our blood banks. There were 42 cases (18.8%) unable to be classified for HCV genotype by RFLP technique. This may be due to multiple infecting genotypes in these patients, difficulties in sample handling or unusual HCV genotypes in this patient group which could not be classified by this method.

We can conclude that genotype 1, particularly the subtype 1a and genotype 3, subtype 3a are predominate in this geographic part of Iran. Blood transfusion and IVDU as the main mode of HCV transmission should be noted. Knowledge on the distribution of various genotypes in our area is essential for its prognostic implications in chronic hepatitis C infection. Further investigations with larger sample numbers are necessary to determine the major genotypes that cause HCV infection in various clinical conditions and potential associations with disease severity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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