



Distribution of Hepatitis C Virus Genotypes in Patients with Major β -Thalassemia in Mashhad, Northeast Iran

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ABSTRACT

BACKGROUND

Hepatitis C virus (HCV) is considered to be the major cause of post-transfusion hepatitis in patients with thalassemia. We aimed to determine the HCV prevalence, genotypes, and viral load among patients with major β -thalassemia in Mashhad, Iran.

METHODS

Medical records of all 550 patients with major β -thalassemia who referred to Thalassemia-Hemophilia Center of Mashhad (Sarvar Clinic) were reviewed from October to November 2011. Plasma samples of the patients were tested for the presence of anti-HCV antibodies by enzyme linked immunosorbent assay. Real-time polymerase chain reaction (PCR) was used to determine viral genotype and HCV RNA titer.

RESULTS

HCV antibodies were detected in 37 individuals (6.73%) including 17 men and 20 women with mean age of 25.2 ± 8.4 years. The PCR analysis was performed for 27 patients, of whom HCV RNA was detected in 17 patients (63.0%). Viral titers were investigated in 14 subjects and a high viral load more than 600000 copies/mL was observed in 6 patients (42.9%). The most prevalent genotypes were 3a (50.0%) followed by 1a (37.5%). No significant correlation was found between genotype and age, sex, serum ferritin, liver tests, and HCV RNA titer.

CONCLUSION

HCV infection among patients with thalassemia is more common than general population in Mashhad, northeast Iran. The dominant HCV subtype is 3a followed by 1a. These findings could help health authorities to provide preventive measures, and practitioners to choose the right protocol of treatment for the patients.

KEYWORDS:

Hepatitis C, Genotype, Prevalence, Viral load, Beta-Thalassemia, Iran

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INTRODUCTION

Hepatitis C virus (HCV) infection is a major global healthcare problem with more than 350000 deaths each year, most of which are related to chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). According to World Health Organization estimate, 170 million people are chronically infected with HCV infection worldwide.^{1,2}

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HCV has been classified into seven genotypes and more than 67 different subtypes based on genomic heterogeneity.³ Distribution of HCV genotypes varies geographically and genotypes differ from each other based on response to antiviral therapy.⁴ Genotypes 1 to 3 have a global distribution, however; genotypes 4 to 6 are restricted to certain geographical regions. Genotype 4 is a predominant subtype in North Africa and the Middle East.^{5,6} On the other hand, 1a and 3a are the most reported HCV subtypes from Iran.^{5,7} HCV genotyping could indicate the route of acquisition and may have important clinical implications for prognosis. It could be also considered as a significant predictor of the response to the antiviral therapy in affected patients.^{5,8}

HCV infection is an important transfusion-transmitted condition and the main cause of liver injury in multi-transfused patients (MTPs).⁹ Although screening of the donated bloods have reduced the risk of transfusion-transmitted infections in patients with thalassemia, blood transfusion seems to be the main mode of HCV transmission among this group due to its transfusion from seronegative blood collected during the window period. The prevalence of HCV infection in the general population of Iran is relatively low, less than 0.5%,¹⁰ however, the infection is common in MTPs such as hemophilia, thalassemia, and hemodialysis patients, and in intravenous drug users.¹¹ Anti-HCV antibody seropositivity ranges from 9.4% to 27% among patients with thalassemia in various areas of the country.¹²

Based on a systematic review of 21 studies, the prevalence of HCV infection among 5229 patients with thalassemia from different provinces in Iran has been estimated to be 18% (95% CI 14-21).¹³

Genotypes 3a followed by 1a were introduced as the most prevalent genotypes in Hormozgan province, southern Iran⁷ and Mazandaran province, northern.¹ However, the frequency of genotype 1a was found to be higher than genotype 3a in Markazi and Fars provinces in the center of country.^{14,15} There is inadequate data about genotype distribution of HCV among patients with thalassemia in our region. The present study was done to identify common genotypes among patients with thalassemia in Mashhad, northeast Iran.

MATERIALS AND METHODS

Patients and study design

In this cross-sectional study, all 550 patients with major β -thalassemia admitted to Thalassemia-Hemophilia Center of Mashhad (Sarvar Clinic), Iran were included. The medical records of the patients were reviewed from October to November 2011. Serum samples of this group were checked routinely for common blood borne viruses. Cases with sero-positivity for HCV antibody detected by enzyme-linked immunosorbent assay (ELISA) were further confirmed by polymerase chain reaction (PCR). Demographic and clinical data including age, sex, hematological and liver tests, HCV RNA titer, and genotypes were collected. This study was approved for scientific and ethical issues by Iranian Academic Center for Education, Culture, and Research (ACECR), Mashhad Branch.

Laboratory assays

ELISA method was used to detect anti-HCV antibodies (4th generation ELISA, Dia.Pro Diagnostic Bioprobes srl, Milan, Italy). RNA virus was extracted from plasma samples using QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized using RevertAid First Strand cDNA Synthesis Kit (Fermentas, Ontario, Canada) and amplified by allele specific PCR method. HCV viral load was determined by real-time PCR assay using a commercial kit (artus® HCV RG RT-PCR, QIAGEN GmbH, Germany) and HCV was genotyped by Genotype Assay Kit (Novin Gene, Iran), according to the manufacturer's specifications. Liver tests including serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), as well as ferritin were done by spectrophotometric analysis using a multipurpose autoanalyzer. Upper normal limits for the above markers were considered as follow: ALT; 40 mg/dL, AST; 40 mg/dL, ALP; 140 mg/dL, and ferritin; 240 ng/mL, respectively.

Statistical analysis

Data on demographic characteristics and clinical data were analyzed by SPSS software version 18.0. Data were described as percentages, mean, and standard deviations. Association between viral load and independent variables was evaluated using t test, and if needed, Mann-Whitney

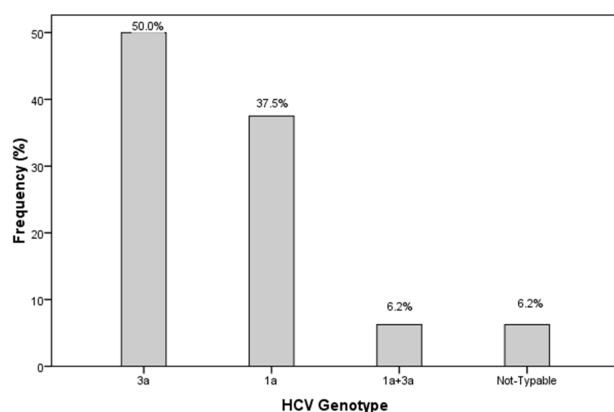


Fig.1: Distribution of hepatitis C virus genotypes in patients with major β -thalassemia in Mashhad, northeast Iran

U test. Assessment of HCV genotypes distribution based on HCV RNA titer and other independent variables was performed using χ^2 test and Fisher's exact test when appropriate. A $p < 0.05$ was considered as statistically significant.

RESULTS

Out of 550 patients, HCV antibodies were detected in 37 individuals (6.73%) including 17 men and 20 women with mean age of 25.2 ± 8.4 years (range: 9-57 years). Serum level of AST, ALT, ALP, and ferritin was abnormally elevated in 16:33 (48.5%), 17:33 (51.5%), 22:26 (84.6%), and 26:29 (89.7%) of the patients, respectively.

Prevalence of HCV infection

The PCR analysis result was available in 27 patients, of whom HCV RNA was detected in 17 cases (63.0%). No difference was observed between HCV RNA-positive and -negative groups regarding the patients' age ($p = 0.669$) and sex ($p = 0.656$). The frequencies of abnormal serum ALT and AST in HCV RNA-positive patients were higher than those in HCV RNA-negative group, however, the differences were not statistically significant ($p = 0.191$ and $p = 0.069$, respectively). Viral load data were available for 14 patients, of whom 6 patients (42.9%) had high viral titers (> 600000 copies/mL). The frequency of abnormal serum levels of AST and ALT was not significantly different in the HCV RNA-positive and -negative patients (56.3% vs. 20%, $p = 0.109$ and 56.3% vs. 30%, $p = 0.248$, respectively).

HCV genotyping

Viral genotyping data were available in 16 out of 17 HCV RNA-positive patients (figure 1). The most prevalent genotypes were 3a (50.0%) followed by 1a (37.5%). Mixed genotype (1a & 3a) was found in one patient (6.2%) and unclassified genotype was reported in another patient (6.2%). There was no significant relation between HCV RNA titer and the virus genotypes ($p = 0.570$). Furthermore, no significant correlations were found between HCV genotypes and age, sex, serum ferritin, and liver tests.

DISCUSSION

HCV is considered as the principal etiologic agent of post-transfusion hepatitis in patients with thalassemia.¹⁶ In the present study, HCV antibodies were detected in 6.73% of the patients with thalassemia, which was significantly higher than those reported previously in general population of Mashhad (0.67%).¹⁷ Routine screening of donated bloods for anti-HCV in the country have been started since 1996.¹² It seems that stricter blood screening besides other preventive strategies should be taken into account.

HCV genotypes and subtypes determination may help epidemiologists to discover the source of HCV infection in a specified population and also it might be useful for clinical management and development of an effective HCV vaccine.¹⁶ The most prevalent genotypes in the present study were genotype 3a (50%) followed by 1a (38%), mixed (6%), and unclassified (6%). Significantly, the predominance of genotypes 1a and 3a in this study was in agreement with other reports on HCV infected patients from different groups in Iran.¹⁸⁻²⁰ A study by Vossughinia and colleagues¹⁸ on 382 clinical specimens obtained from patients with hepatitis C in Mashhad showed that the most common genotypes were genotype 3a (40%) and 1a (39%). Similarly, a research for determination of HCV genotypes in Iranian patients with thalassemia and chronic hepatitis C showed that genotypes 1a (52%) was the most common type and genotypes 3a (35%) was the other frequent genotype.²¹ On the other hand, HCV genotypes among patients in other Middle Eastern countries and Europe were predominantly type 4 followed by 1.²²⁻²⁶

Moreover, in the current study 6% of the patients had

a mixed genotype. Infection with two or more distinct HCV genotypes have been reported in high risk groups with multiple exposure to the virus such as patients with hemophilia, thalassemia, hemodialysis patients, and intravenous drug abusers.⁴ Detection of mixed genotypes are important regarding the clinical management of the patients due to the risk of antiviral therapy failure, infection relapse or progression to more severe conditions such as cirrhosis and HCC.^{1,15} Furthermore, one of our patients had an unclassified genotype, which could be explained by either presence of a new variant or mixed infection undetectable by the genotyping kit used in the study.

HCV genotype was not significantly associated with age, sex, serum ferritin, and liver tests in the current study. Similarly, some reports from Iran and other countries could not find any relation between genotype and age, and serum liver enzymes in HCV infected patients from different groups.^{7,11,27} In a survey on Iranian patients with congenital bleeding disorders, no significant association was observed between serum liver enzymes and HCV viral load and genotypes.¹¹ However, one study conducted on 280 patients with thalassemia and chronic hepatitis C showed a significant higher serum ferritin concentration in genotype 1a compared with those infected with genotype 3a and mixed genotypes. In addition, ALT levels in patients with genotype 1a were significantly higher than those with genotype 3a.²¹ Factors such as ethnicity and duration of HCV infection¹¹ as well as different studied populations and sample size could partly explain this difference.

Taken together, the prevalence of HCV infection among patients with major β -thalassemia is higher than general population in this area. Similar to other provinces in Iran, the dominant HCV subtypes are 3a and 1a. There is no correlation between virus genotypes and age, sex, liver tests, serum ferritin, and HCV RNA titer. These findings could help health authorities to provide preventive measures and practitioners to choose the right protocol of treatment for the patients.

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ETHICAL APPROVAL

There is nothing to be declared.

CONFLICT OF INTEREST

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

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