Original Article

PREVALENCE OF HEPATITIS-C VIRUS (HCV) AMONG THALASSEMIA PATIENTS IN KHUZESTAN PROVINCE, SOUTHWEST IRAN

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ABSTRACT

Objective: The aim of this study was to determine the prevalence of HCV infection among thalassemia patients in Khuzestan province, southwest Iran.

Methodology: A retrospective cross-sectional study was conducted on 206 thalassemia patients referred to the Research Center of Thalassemia and Hemoglobinopathy (RCTH) of Ahvaz Shafa Hospital during March 2006 to April 2007. Demographic data were obtained from the patient files at the hospital. Serum specimens were tested with anti-HCV assays and a nested-PCR technique to assess HCV infection.

Results: Out of 206 patients, 97 (47.1%) and 109 (52.9%) were male and female, respectively, with a mean ±SD age of 16.4±6.42 years. The overall prevalence rate of anti-HCV was 28.1% (58/206, 95% CI: 22.4-34.6). Forty six of anti-HCV positive patients (46/58, 79.3%) were also HCV RNA positive. HCV–positive patients were significantly older from HCV-negative ones ($p<0.001$). In addition, the results indicate that higher prevalence of anti-HCV or HCV RNA were significantly associated with longer duration of transfusion ($p<0.003$ and $p<0.001$, respectively).

Conclusion: Although it seems blood donor screening project reduced HCV infection, using more accurate technique is necessary in order to find viral infection and treat thalassemia patients with HCV infection more carefully.

KEY WORDS: Prevalence, Thalassemia, Hepatitis C, Khuzestan.

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INTRODUCTION

Hepatitis C virus (HCV) is the most common cause of post-transfusion hepatitis (PTH) and end-stage liver disease in many countries. Regular blood transfusion in patients with hereditary hemolytic anemia, particularly thalassemia, has improved their overall survival, but carries a definite risk of acquisition of blood-borne virus infections, especially viral hepatitis.[1,2] Moreover, with respect to marked liver iron overload, which is often inevitable in patients on regular blood transfusion, HCV infection have been shown to have a potenti-

ating effect on hepatic fibrogenesis in thalassemic patients.[3] Khuzestan province is located in the southwest of Iran, a tropical area with an approximate population of 4.5 million (Census 2004). Khuzestan Province shares a land, river, and sea border with Iraq and Arabian countries along Persian Gulf. Khuzestan has suffered the heaviest damage of all Iranian provinces during a 28-year period including the Iran-Iraq War (1980-1988), the Gulf War (1990-1991), and the 18-year crisis in Iraq (1990-2008).[4] Moreover, thalassemia is an important health problem throughout Iran, particularly in Khuzestan province. Therefore, the aim of the present study was to identify the prevalence of HCV infection in thalassemia patients in Khuzestan province.

METHODOLOGY

Patients: This cross-sectional study was performed from March 2006 to April 2007 in Khuzestan Province on thalassemia patients attending the Research Center of Thalassemia and Hemoglobinopathies (RCTH) of Ahvaz Shafa Hospital in Khuzestan province. Whole blood specimens were collected from a total of 206 patients, after obtaining an informed consent. Serum samples were separated from the whole blood, aliquotted and stored at -70Ú C. Demographic data, such as age, duration and number of blood transfusions were obtained from patient records.

Laboratory assays: All sera were screened using anti-HCV assays with third-generation commercial ELISA microplate kits (DIA.PRO, Italy) according to the manufacturer's instructions. The samples were considered positive when the sample absorbance/cut-off (SA/C) ratio was higher to 1.1 and negative when the SA/C ratio was <0.9 (values given by the manufacturer). Positive samples were confirmed using nested-RT PCR for HCV.

Two hundred microliters of serum was used for HCV-RNA extraction using high pure viral nucleic acid kits (Roche, Germany) according to the manufacturer's instructions. HCV-RNA was immediately transcribed into cDNA using random primers (Fermentas, Lithuania). cDNA was targeted by a nested-PCR with specific primers for the conserved sequences in the 5` non-coding region (5`-NCR) of HCV.[5-7]

Statistical analysis

Prevalence and the corresponding 95% confidence interval (95% CI) were calculated with SPSS software version 13.0 (SPSS Inc., Chicago, IL, USA). Data comparisons were performed using the Chi-square, Fisher's exact test and Student's *t*-test. The differences were considered significant if $p<0.05$.

RESULTS

Two hundred six thalassemia patients were tested. There were 97 (47.1%) males and 109 (52.9%) females (Table-I); their mean age (±SD) was 16.4±6.42 years (range 2-34 years). Fifty eight of the 206 patients were anti-HCV positive by ELISA corresponding to a 28.1% (58/206, 95% CI: 22.4-34.6). The mean age of the fifty eight anti-HCV positive patients was higher than that of negative patients (18.98±6.65 *vs.* 15.47±6.06, p<0.001). No significant statistical difference in anti-HCV seropositivity was observed between males and females (p=1.0). The prevalence of anti-HCV seropositivity (47/136, 34.6%) was significantly higher (p<0.003) among patients who had started to receive transfusions before 1996 when serological screening for anti-HCV antibody had been in introduced to blood banks in Iran, than among patients (11/70, 15.7%) who had started to receive transfusions after 1996 (Table-II). The results indicate the incidence of anti-HCV was significantly associated with longer duration of transfusion (p<0.003).

The prevalence of HCV RNA was 22.3% (46/206, 95% CI: 17.1-28.5). Among the fifty eight anti-HCV positive patients, forty six individuals were positive for HCV RNA. Mean age was higher among HCV RNA-positive patients than among HCV

RNA-negative patients (20.17±6.32 vs. 15.39±6.05, p<0.001). The prevalence of HCV RNA was higher among females (22.9%) than among males (21.6%), although the difference

Table-I: Characteristics of HCV RNA-positive and-negative thalassemia patients

Factors	*No. of patients* (n=206)	*Anti-HCV Positive* (n=58) %	*p value*	*HCV RNA Positive* (n=46) %	*p value*
Sex			1.0		0.8
Male	97	27 (27.8)		21 (21.6)	
Female	109	31 (28.4)		25 (22.9)	
Age groupsd			0.01		0.001
<10	41	5 (12.2)		1 (2.4)	
11-21	121	35 (28.9)		29 (24)	
>22	44	18 (40.9)		16 (36.4)	

was not significant (p = 0.8) (Table-I). There was a significant difference in the prevalence of HCV RNA (p< 0.001) between subjects who had started to receive transfusions before 1996 (30.1%) and those who had started to receive them after 1996(7.1%). The prevalence of HCV RNA was higher among the patients who had received more than 100 units of blood components (91.3%) than among those who had received less than 100 units (8.7%) (p<0.01) (Table-II).

DISCUSSION

There are more than 25,000 patients with thalassemia major in Iran.[8] It is an important health problem throughout Iran particularly in Khuzestan province. This study showed that the prevalence of anti-HCV in thalassemia patients is 28.1%. A recent report from northern Iran showed a 63.8% prevalence of HCV antibody in thalassemics compared to 0.5% in blood donors. In this report confirmatory immunoblotting test was employed using HCV-positive cases, which showed that 92.6% of samples were positive.[9] Karimi et al.,[9] from Shiraz, southern Iran, reported 73 of 466 thalassemic children with a history of multiple transfusions (15.7%) positive for anti-HCV. Results from another study on Iranian thalassemic patients, revealed 24.2% of them were anti-HCV positive.[1] Previous single-center studies on Iranian thalassemic patients revealed a wide range of 16 - 64% for prevalence of HCV infection.[8-10]

This is the first study to report on the prevalence of HCV infection among thalassemia patients in a war stricken area, Khuzestan province, in Iran. The prevalence of HCV infection in thalassemia patients ranges 33 to 67.3% in some neighboring Arabic countries, [11-13] which share a sea boarder with Khuzestan province. Therefore, comparing our result with these countries, the frequency of HCV infection in our thalassemia population is relatively low.

Table-II: HCV infection among thalassemia patients receiving multiple transfusions

Factors	*No. of patients (n=206)*	*Anti-HCV Positive (n=58) %*	*p value*	*HCV RNA Positive (n=46) %*	*p value*
Duration of transfusion (years)			0.003		0.001
<11	65	12 (18.5)		6 (9.2)	
12-22	120	34 (28.3)		29 (24.1)	
23	21	12 (57.1)		11 (52.3)	
First transfusion			0.003		0.001
Before or in 1996	136	47 (34.6)		41 (30.1)	
After 1996	70	11 (15.7)		5 (7.1)	
No. of units transfused			0.2		0.01
<100	40	7 (17.5)		4 (10)	
100-200	67	19 (28.4)		12 (17.9)	
>200	99	32 (32.3)		30 (30.3)	

The countries with a higher HCV prevalence in general population had a higher prevalence rate among thalassemia patients, too. For instance, a study in Egypt reported 75% of HCV prevalence among thalassemia patients, considering the fact that the prevalence in their blood donor population was 14.5%.[14] However, in India with a low HCV prevalence among blood donors (1.78%), the prevalence in thalassemics was reported relatively low (25.5%).[15] It should be noted that the Iran-Iraq War of 1980-1988, has had a devastating impact on public health. Moreover, during a period of 18 years, due to poor security and living conditions, many Iraqi refugees have crossed over the Iraqi border to Iran, mainly to the southwestern regions.[16] This geographical situation, mass immigration from Iraq, where a significantly higher prevalence of anti-HCV has been found among different populations[13,17,18] and frequent travels between Khuzestan province to neighboring Arabic countries, all could affect prevalence of HCV in our region.

When serologic tests for HCV became available, blood donor screening began to be performed in most countries. In Iran, mandatory anti-HCV screening was introduced to blood banks in 1996. The results of our study showed that anti- HCV seropositivity decreased significantly from 34.6% to 15.7%, after the implementation of screening blood components. It is important to consider that, in spite of the systematic screening of blood donors, testing blood donation recipients for HCV is still important. This finding demonstrates that more efforts should be made to improve blood transfusion safety. Serological tests are used worldwide for the screening of HCV infection. Some of the serological tests may have controversial results. The occurrence of HCV RNA detectable in patients with negative anti-HCV can be a consequence of immunosupression with decreased production of antibodies, or the window period of a recent infection.[19]

In this study, mean age ±SD was significantly ($p<0.001$) higher in patients with positive HCV antibody (18.98 ± 6.65) compared to negative subjects (15.47 ± 6.06). The higher rate of HCV infection in older patients, reflecting more frequency of transfusion ($p<0.01$) and revealed the importance of providing safe blood to reduce the incidence of HCV infection in thalassemic population.

The results of our study showed HCV RNA-positive patients had a significantly longer duration and frequency of transfusion compared with HCV RNA-negative cases ($p<0.001$ and $p<0.01$).

However, these observations strongly indicated blood transfusion as the main risk factor for HCV infection acquisition among thalassemic patients, and confirmed the marked efficacy of donor screening in the prevention or viral transmission. The higher rate of HCV infection in older patients with thalassemia and in the subjects who had higher serum ferritin level- all reflecting transfusion of more units of blood- revealed the importance of providing safe blood to reduce the incidence of HCV infection in thalassemia patients.

Forty six of the anti-HCV positive patients (46/58) were also HCV RNA positive. The agreement between anti-HCV and HCV RNA detection was 79.3%, a finding similar to those reported for other populations, i.e., rates ranging from 65 to 86%.[20-22] Failure to detect HCV RNA in serum may be due to various factors such as inactivation of viral RNA during serum collection and storage, fluctuating viremia levels, resolved infection, or false-positive anti-HCV results.[20,23]

In conclusion, although anti-HCV prevalence in thalassemia patients in Khuzestan province is lessr than those found in some other Iranian provinces and neighboring countries, they are still high. The fact that the risk of HCV infection diminished considerably after 1996 demonstrates the value of blood donor screening programs. However, simple measures such as enforced general asepsis rules, careful disinfection and equipment sterilization, routine testing of patients, serial determination of hepatic enzymes should be the common practice in dialysis centers in Iran.

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