

Prevalence and Molecular Evaluation of Hepatitis C Virus Infection among Multi-Transfused Thalassemia Patients in South of Iran

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Abstract

Objective: Information regarding the magnitude of hepatitis C virus (HCV) infection among thalassemia patients is of great importance for health care providers to assess blood safety and improve the quality of screening systems. Therefore, this study was designed to evaluate the prevalence, risk factors and genotypic pattern of HCV infection among β -thalassemia patients in South of Iran.

Methods: All patients with β -thalassemia major from Borazjan, Bushehr, Delvar, Kangan and Ahram cities participated in this study. Serum samples were tested for presence of anti-HCV antibodies by ELISA assay. The seropositive serum samples were tested for detection of HCV viremia and genotypes by semi-nested RT-PCR and sequencing.

Results: Of 125 thalassemia patients, 22 cases (17.6%) were positive for anti-HCV antibodies, and 2 cases (1.6%) had HCV viremia with genotype 3a. HCV seroprevalence increased with age, so that anti-HCV seropositive thalassemia patients had significantly higher mean age compared to anti-HCV seronegative patients. HCV seroprevalence was higher among female patients, residents of Kangan, patients with blood transfusion every two weeks, Fars patients, and thalassemia patients with ALT levels <20 IU/L and AST levels of >80 IU/L. Nevertheless, anti-

HCV seroprevalence among thalassemia patients was not statistically associated with these variables.

Conclusion: These results indicate ongoing HCV incidence among thalassemia population in this region. Transfusion of HCV-seronegative viremic blood units donated during infectious window period contributes to HCV infection in thalassemia patients. These findings highlight the need to include sensitive molecular assays in the screening process of donated blood for HCV infection in Iran.

Keywords: Hepatitis C virus; β -thalassemia; Prevalence; Genotype; Risk factor; Iran

Introduction

β -thalassemia, with a global carrier rate of 1.5%, equating to 80–90 million subjects, and an estimated 50,000–60,000 new cases annually, is considered to be one of the most prevalent genetic diseases around the world.¹ β -thalassemia is characterized by inherited disorders in β -globin chain synthesis, which result in anemia due to hemolysis of red blood cells and erythroblasts as well as ineffective erythropoiesis.²⁻⁴ In view of recent progress in the management of thalassemia patients, the survival and quality of life of this group of patients have increased significantly.⁵⁻⁷ However, hepatocellular carcinoma (HCC) has become the leading cause of mortality in thalassemia patients. In addition to hepatic iron overload and organ damage, a high prevalence of hepatitis C virus (HCV) infection among thalassemia patients is the major risk factor for the development of HCC.^{2, 8}

HCV is a small virus in the family *Flaviviridae*, with a single-stranded RNA genome and an enveloped icosahedral capsid. The genome encodes seven non-structural proteins (NS1, NS2, NS3, NS4A, NS4B, NS5A, and NS5B), a core protein, and two envelope glycoproteins (E1 and E2).^{9, 10} Based on sequence analysis, there are seven genotypes and 67 subtypes of HCV.⁹ This virus is mainly transmitted through transfusion of blood and blood products, intravenous drug

use, surgery and tattooing.^{11, 12} HCV infection is an asymptomatic or acute hepatitis, which might clear or lead to chronic infection in approximately 75% to 85% of infected cases. Chronic HCV infection induces liver cirrhosis and HCC in a proportion of those chronically infected over time.^{13, 14}

Frequent blood transfusions increase the vulnerability of thalassemia patients to transfusion-mediated HCV infection. The high prevalence of HCV infection among thalassemia patients in different regions confirms this vulnerability.^{15, 16} The prevalence of HCV among thalassemia patients depends on the levels of transfusion safety measures in the blood transfusion organizations.¹⁷ In Iran, despite the implementation of safe blood transfusion practices, the prevalence of HCV among the thalassemia population is considerably high.¹² This high prevalence indicates deficiencies in the blood screening strategies. Therefore, information regarding the magnitude of HCV infection among thalassemia patients is of great importance for health care providers to assess blood safety and improve the quality of screening systems. However, despite this importance, no report is available on the epidemiology of HCV infection among thalassemia patients in the South of Iran. Therefore, the present study was designed to evaluate the prevalence, risk factors and genotypic pattern of HCV infection among β -thalassemia patients in this region.

Subjects and Methods

Sample collection and laboratory diagnosis

This descriptive-analytical cross-sectional study was conducted from March to June 2019 and included all patients with β -thalassemia major attending the transfusion center of the Bushehr University of Medical Sciences located in southern Iran. As a control group, 125 volunteers from

out-patient subjects attending the hospitals of the university for blood tests were included in the study. The controls were matched in sex, age (± 3 years), and date of admission with the thalassemia patients and did not have a history of blood transfusion. All participants or the legal guardians of the minor subjects were requested to give written informed consent to screen their leftover serum samples for HCV infection and analysis. The socio-demographic characteristics and clinical information were obtained from the medical record of each patient at the transfusion centers. The Ethics Committee of the Bushehr University of Medical Sciences approved this study with reference number IR.BPUMS.REC.1395.183, and the Deputy Research and Affairs of the University funded this study with grant number 3225.

Serum samples of participants were tested for the presence of anti-HCV antibodies using commercially available ELISA kits (HCV Ab ELISA kit, DIA.PRO, Milan, Italy). Furthermore, the seropositive serum samples were tested for the detection of HCV viremia and genotypes by semi-nested RT-PCR, targeting the 5'UTR and core region of the genome, and sequencing as described previously.^{18, 19} Following the extraction of HCV RNA from the samples using High Pure Viral Nucleic Acid kit (Roche, Mannheim, Germany) and reverse-transcription into cDNA using the SuperScript III cDNA synthesis kit (Invitrogen, Carlsbad, CA, USA), the 680 bp length fragment of HCV genome was amplified in the first round PCR using outer primers AGCGTCTAGCCATGGCGT (-268 to -251) and ATGTACCCCATGAGGTCGGC (+410 to +391). The second-round PCR was performed using inner primers AGCGTCTAGCCATGGCGT (-268 to -251) and CACGTTAGGGTATCGATGAC (+383 to +364). The 580 bp length fragments from the second-round PCR were sequenced to determine HCV genotypes (Macrogen Co., Korea).

Statistical analysis

The data were analyzed by SPSS 17 package program (SPSS Inc., Chicago, IL, USA), and P values < 0.05 were considered significant. The Student's t-test was used to compare quantitative data between HCV-positive and HCV-negative thalassemia patients. Categorical data were analyzed by the χ^2 test or the Fisher's exact test. Logistic regression analysis was used to determine the variables associated with the prevalence of HCV infection among thalassemia patients, and odds ratio with 95% confidence intervals was determined.

Results

Serum samples were obtained from 125 patients with β -thalassemia major, including 91 patients from Bushehr, 15 patients from Borazjan, 10 patients from Delvar, 6 patients from Ahram city and 3 patients from Kangan, ages ranging from 1 to 48 years (25.44 ± 10.24). The majority of thalassemia patients were in the age groups 20-29 years (39.2%) and 30-39 years (28.8%), respectively. Of 125 thalassemia patients, 22 cases (17.6%, 95% CI: 11.9%-25.2%) were positive for anti-HCV antibodies. The controls were negative for anti-HCV antibodies. The highest rate of anti-HCV seropositivity was observed in the age group 30-39 years (33.3%) followed by the age group 20-29 years (18.4%), whereas the age groups <10 years and >39 did not show anti-HCV seropositivity. Overall, anti-HCV seroprevalence increased with age, so that anti-HCV seropositive thalassemia patients had significantly higher mean age (29.18 ± 7.06) compared to anti-HCV seronegative thalassemia patients (24.64 ± 10.66) ($P = 0.049$). Anti-HCV seroprevalence was higher among female thalassemia patients (18.1%), residents of Kangan (33.3%), Fars thalassemia patients (18.8%), single patients (18.1%), patients with blood transfusion every two weeks (22.2%), diploma patients (27.9%), and thalassemia patients with

ALT levels <20 IU/L (21.3%) and AST levels of >80 IU/L (42.9%). Nevertheless, anti-HCV seroprevalence among thalassemia patients was not statistically associated with gender distribution, place of residency, ethnicity, marital status, frequency of blood transfusion, and serum levels of ALT and AST (Table 1). Notably, all of the anti-HCV seropositive thalassemia patients were negative for HBV and HIV.

Table 1. Prevalence of anti-HCV antibodies according to socio-demographic and quantitative variables among thalassemia patients in South of Iran

	No. of all participants (%): 125 (100%)	No. of HCV Ab negative subjects (%): 103 (82.4%)	No. of HCV Ab positive subjects (%): 22 (17.6%)	P-Value
Age groups (years)				0.014
<10	7 (5.6%)	7 (100%)	0 (0.0%)	
10-19	23 (18.4%)	22 (95.7%)	1 (4.3%)	
20-29	49 (39.2%)	40 (81.6%)	9 (18.4%)	
30-39	36 (28.8%)	24 (66.7%)	12 (33.3%)	
>39	10 (8.0%)	10 (100%)	0 (0.0%)	
Gender				0.876
Female	72 (57.6%)	59 (81.9%)	13 (18.1%)	
Male	53 (42.4%)	44 (83.0%)	9 (17.0%)	
Place of residence (city)				0.598
Bushehr	91 (72.8%)	76 (83.5%)	15 (16.5%)	
Borazjan	15 (12.0%)	11 (73.3%)	4 (26.7%)	
Delvar	10 (8.0%)	8 (80.0%)	2 (20.0%)	
Ahram	6 (4.8%)	6 (100.0%)	0 (0.0%)	
Kangan	3 (2.4%)	2 (66.7%)	1 (33.3%)	
Ethnicity				0.565
Fars	112 (89.6%)	91 (81.3%)	21 (18.8%)	
Afghan	3 (2.4%)	3 (100.0%)	0 (0.0%)	
Arab	10 (8.0%)	9 (90.0%)	1 (10.0%)	
Marital Status				0.596
single	116 (92.8%)	95 (81.9%)	21 (18.1%)	
Married	9 (7.2%)	8 (88.9%)	1 (11.1%)	
Frequency of Blood Transfusion				0.834
Once every two weeks	9 (7.2%)	7 (77.8%)	2 (22.2%)	
Once every three weeks	21 (16.8%)	17 (81.0%)	4 (19.0%)	
Once a month	62 (49.6%)	51 (82.3%)	11 (17.7%)	
As needed (every 35-50 days)	6 (4.8%)	6 (100.0%)	0 (0.0%)	
Unknown	27 (21.6%)	22 (81.5%)	5 (18.5%)	
Education				0.029
Under diploma	66 (52.8%)	60 (90.9%)	6 (9.1%)	
Diploma	43 (34.4%)	31 (72.1%)	12 (27.9%)	

Upper diploma	16 (12.8%)	12 (75.0%)	4 (25.0%)	
ALT level (IU/L)				0.846
<20	47 (37.6%)	37 (78.7%)	10 (21.3%)	
20-40	19 (15.2%)	17 (89.5%)	2 (10.5%)	
41-80	21 (16.8%)	17 (81.0%)	4 (19.0%)	
>80	9 (7.2%)	8 (88.9%)	1 (11.1%)	
Unknown	29 (23.2%)	24 (82.8%)	5 (17.2%)	
AST level (IU/L)				0.359
<20	36 (28.8%)	29 (80.6%)	7 (19.4%)	
20-40	23 (18.4%)	19 (82.6%)	4 (17.4%)	
41-80	30 (24.0%)	27 (90.0%)	3 (10.0%)	
>80	7 (5.6%)	4 (57.1%)	3 (42.9%)	
Unknown	29 (23.2%)	24 (82.8%)	5 (17.2%)	

Of 22 anti-HCV seropositive thalassemia patients, 2 cases (9.1%) had HCV viremia with genotype 3a. One of these cases was a 24-year-old woman with elevated levels of ALT and AST who had blood transfusion every 4 weeks. This case was found to be positive in the first round of PCR. The second case was a 33-year-old man with normal levels of liver enzymes and blood transfusion every four weeks, which was positive in the second round of PCR. Overall, the prevalence of HCV viremia among thalassemia patients was 1.6% (95% CI: 0.5% –5.6%). Electrophoresis of RT-PCR products of 5'UTR and core regions of HCV genome extracted from serum samples of thalassemia patients on 2% agarose gel is shown in figure 1.



Figure 1. Electrophoresis of RT-PCR products of 5'UTR and core regions of HCV genome extracted from serum samples of thalassemia patients on 2% agarose gel. (L: 100-bp DNA ladder; P: Positive control; N: Negative control). Out of 22 anti-HCV seropositive thalassemia patients, 2 cases (Lanes: 3 and 5) were positive for HCV (~580 bp). Lanes: 1,2,4,6 and 7 are of negative patients for HCV.

Discussion

HCV infection, with over 40% mortality in thalassemia patients, is considered to be a challenging transfusion-transmitted infection in Iran.²⁰ In the absence of a prophylactic vaccine, the best way to control this disease is to improve the safety of blood transfusion. HCV prevalence rate in thalassemia patients can be used as an index for evaluating the blood safety filters in different regions.¹⁷ Moreover, diagnosis and subsequent treatment of HCV-infected thalassemia patients can reduce the burden of HCV infection in the thalassemia population. Despite this importance, the epidemiology of HCV infection among thalassemia patients remains unknown in the South of Iran. Therefore, we evaluated the prevalence and genotypic distribution of HCV infection among patients with β -thalassemia major in this region and found HCV prevalence of 17.6% for anti-HCV antibodies and 1.6% for HCV viremia with genotype 3a.

The anti-HCV seroprevalence of 17.6% reported in the thalassemia patients is considerably higher than those reported among the general population (0.6%) and the blood donors (0.5%) of Iran.^{21, 22} The anti-HCV seroprevalence observed in this study is higher than the anti-HCV seroprevalence of 0.1% reported in the blood donors of this region.²³ Moreover, the controls were negative for anti-HCV antibodies. The control group was used to compare the prevalence of HCV infection between the multi-transfused thalassemia patients and the patients receiving no blood transfusion and evaluate the effect of blood transfusion on the prevalence of HCV infection. The results of this study indicate that multi transfused patients, such as thalassemia patients, are at high risk for HCV infection. The high prevalence of HCV in this population is related to the transfusion of HCV-seronegative viremic blood units donated during the window period.¹² Although screening of blood units based on detection of anti-HCV antibodies has significantly decreased the rate of HCV infection in patients with thalassemia in Iran,^{15, 20} the current blood screening policy is not adequate to eliminate transfusion-transmitted HCV infection. Therefore, the blood screening strategies should be thoroughly reappraised and sensitive molecular assays should be included in the screening process of donated blood for HCV infection in Iran.

The anti-HCV seroprevalence reported in this study is higher than those observed among thalassemia patients in the other parts of Iran, 3.4% in Tabriz (North-West of Iran),²⁴ 5.66% in Kurdistan (West of Iran),²⁵ 8% in Isfahan (Center of Iran)²⁶ and 10.2 % in Bandar Abbas (South-East of Iran)²⁷ but lower than 18.8% in Gilan and Mazandaran (North of Iran),²⁸ 27.5% in Tehran (North-Center of Iran)²⁹ and 28.1% in Khuzestan (South-West of Iran).³⁰ According to a meta-analysis study, the overall seroprevalence of HCV among patients with thalassemia in Iran is 19%.¹⁵ High seroprevalence of HCV has been detected among thalassemia patients in Iraq

(27.5%),³¹ Pakistan (29.4%),³² and Egypt (40.5%).³³ While low seroprevalence of HCV has been reported in India (3.4%)³⁴ and Bangladesh (13.51%).³⁵ These studies show a wide range of variations in the geographical distribution of HCV infection, reflecting differences in the transfusion safety measures, the predominant risk factors and the rates of HCV infection endemicity in different regions. As it is necessary to study the prevalence of HCV infection among blood donors to assess the current most common risk factors, the study of the epidemiology of HCV infection among thalassemia patients as blood recipients is also essential to evaluate the effectiveness of the transfusion safety measures employed in the blood transfusion organizations of the country. These studies not only clarify the burden of HCV infection among thalassemia patients but are also essential for improving the current blood screening strategies to minimize the risk of transmitting hepatitis C through blood transfusion.

In this study, anti-HCV seroprevalence was higher among female thalassemia patients and those patients with the highest frequency of blood transfusion. Moreover, those patients with abnormal levels of AST had higher seroprevalence of HCV than thalassemia patients with normal levels of AST. However, neither gender nor frequency of blood transfusion and level of liver enzymes was associated with the seroprevalence of HCV among thalassemia patients. In addition, no significant association was found between HCV seroprevalence and place of residency, ethnicity, marital status, and level of education. In contrast, HCV seropositivity among thalassemia patients was statistically associated with age, so that the highest rate of anti-HCV seroprevalence was observed in the age group 30-39 years compared to the other age groups ($P = 0.014$). Moreover, the age group >39 did not show anti-HCV seropositivity. The probable reason for the absence of anti-HCV antibodies among the thalassemia patients aged >39 years could be that the older thalassemia patients were infected years ago and after recovery, their anti-HCV antibody

titer decreased and reached an undetectable level over time. Notably, except for the age distribution, no risk factor was reported for HCV seroprevalence among thalassemia patients. In previous studies from Iran, HCV seroprevalence among thalassemia patients was significantly associated with the duration of blood transfusion.^{24-26, 30} Similarly, some studies from Egypt and Iran have reported a significant association between the number of transfusions per month and HCV seropositivity.^{26, 33} In contrast, some studies in Pakistan and Iraq demonstrated a higher seroprevalence of HCV among male thalassemia patients.^{32, 36} In studies from Indonesia and Egypt, HCV seropositive thalassemia patients had higher levels of ALT and AST compared to HCV seronegative patients.^{37, 38}

In this study, 9.1% of anti-HCV seropositive thalassemia patients (2/22) had HCV viremia. Twenty anti-HCV seropositive thalassemia patients with negative HCV RNA results had past HCV infection and were recovered as a result of antiviral therapy. Overall, 1.6% of the thalassemia patients (2/125) had HCV viremia, which is higher than that observed in the general population of Iran (0.4%).²² According to the previous studies from Iran, it can be concluded that from 1998 to 2017, the prevalence of HCV in thalassemia patients has decreased significantly due to the implementation of donor screening programs and the effective antiviral treatment by direct-acting antivirals (DAAs).^{15, 20, 39, 40} So that, the prevalence of HCV viremia among thalassemia patients in a study conducted in 2006-2007 was 22.3%³⁰ compared to HCV viremia of 1.6% in the present study in 2020.

According to the molecular evaluation, HCV genotype 3a was detected among thalassemia patients in this study. HCV genotype 3a reported in the present study follows the genotypic distribution of HCV in this region, since the same genotypic pattern has been observed in previous studies from this region.^{18, 19} Infection with HCV genotype 3 is prevalent in intravenous

drug users and is associated with advanced liver disease and progression of cirrhosis and hepatocellular carcinoma.¹⁰ Therefore, prompt treatment of HCV-infected patients can prevent worse clinical outcomes. In addition to pathogenicity and clinical outcome of the infection, response rate to antiviral therapy, type and duration of treatment are associated with HCV genotypes.^{10, 12} Patients with HCV genotype 1 and 4 show lower response rates to interferon and ribavirin combination therapy and need higher treatment duration as compared to patients with HCV genotype 2 and 3.^{10, 41} With the development of DAAs-based therapy, treatment decision based on HCV genotype is less pronounced. Since DAAs often show pan-genotypic antiviral activity and use in combination therapy. Nevertheless, access to DAAs-based therapy is limited in low- and middle-income countries due to the high price and the restricted availability of DAAs.^{10, 12, 41}

This study is the first report in the South of Iran, which has determined the prevalence and genotype distribution of hepatitis C infection in β -thalassemia patients. In addition, participation of all β -thalassemia major patients resident in Bushehr, Borazjan, Delvar, Ahram and Kangan cities increases the generalizability of the results of this study. However, we did not investigate the effects of hepatitis C on the survival rates of HCV-infected thalassemia patients. As another limitation, other risk factors of HCV infection such as history of surgery, dentistry or tattoo as well as injecting drug abuse and other high-risk behaviors were not evaluated in this study due to the lack of information in records of the thalassemia patients.

Conclusion

This study reports the HCV prevalence of 17.6% for anti-HCV antibodies and 1.6% for HCV viremia with genotype 3a among β -thalassemia patients. These results reveal a high prevalence

of HCV infection among β -thalassemia patients compared to the normal population, indicating ongoing HCV incidence among the thalassemia population in the South of Iran. Transfusion of HCV-seronegative viremic blood units donated during the seronegative window period contributes to HCV infection in thalassemia patients. Therefore, the determination of HCV infection in blood donors and recipients based on the detection of HCV-RNA by PCR assay should be considered. The findings of this study highlight the need to include sensitive molecular assays in the screening process of donated blood for HCV infection in Iran.

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Competing Interests

The authors declared that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Author contributions

Farshadpour F and Taherkhani R designed and performed the study. Taherkhani R drafted and edited the manuscript. All authors approved the final draft of the manuscript.

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Availability of data and materials

All relevant data are within the paper.

Ethical approval

This study was approved by the Ethical Committee of Bushehr University of Medical Sciences with reference number IR.BPUMS.REC.1395.183.

Consent to Participate

All participants or the legal guardians of the minor subjects gave written informed consent to screen their leftover serum samples for HCV infection and analysis.

Consent for Publication

The participants or the legal guardians of the minor subjects gave written informed consent to publish their data in a journal article.

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