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Original Article

Epidemiology of Hepatitis B, Hepatitis C, and Human Immunodeficiency Virus Infecions in Patients with Beta-Thalassemia in Iran: A Multicenter Study

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Background: **Though regular blood transfusion improves the overall survival of patients with β-thalassemia, it carries a definite risk of infection with blood-borne viruses. We carried out this multicenter study to provide epidemiologic data on hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) infection among Iranian β-thalassemic patients. Moreover, HCV infection-associated risk factors were investigated in this population.**

Methods: **Seven hundred and thirty-two patients with β-thalassemia major or β-thalassemia intermedia, selected from five provinces of Iran including Tehran (n = 410), Kerman (n = 100), Qazvin (n = 95), Semnan (n = 81), and Zanjan (n = 46), were enrolled in this study. Using ELISA, their sera were tested for HBsAg, HBcAb, HBsAb, HCVAb, and HIVAb. The positive HCVAb results were confirmed by RIBA-2nd generation.**

Results: **The study sample consisted of 413 males and 319 females, with a mean ± SD age of 17.9 ± 9.0 years. One hundred forty-one (19.3%) patients were HCVAb positive; 11 (1.5%) were HBsAg positive. No one was HIVAb positive. Univariate analysis showed that β-thalassemia major (*P* = 0.01), older age (*P* = 0.001), longer transfusion duration (*P* = 0.000), HBsAg seropositivity (*P* = 0.03), and higher serum ferritin level (*P* = 0.002) were significantly associated with a higher prevalence of HCV. Furthermore, the prevalence of HCV infection dropped significantly after the implementation of blood donors screening (22.8% *vs.* 2.6%; *P* = 0.000). Using multivariate analysis, β-thalassemia major (*P* = 0.002), age (*P* < 0.001), serum ferritin level (*P* < 0.001), as well as consumption of unscreened blood (*P* = 0.003), were independent factors associated with HCV infection.**

Conclusion: **The prevalence of HCV infection is much higher among Iranian β-thalassemic patients as compared with HBV and HIV infections. Routine screening of donated blood for HCV is highly recommended.**

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Keywords: Blood transfusion • human immunodeficiency virus • hepatitis B • hepatitis C • thalassemia

Introduction

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. Nowadays, vaccination against hepatitis B has efficiently been able to restrict the transmission of hepatitis B virus (HBV)

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infection. However, posttransfusion transmission of hepatitis C virus (HCV) has still remained a major health concern in thalassemic patients. In addition, since marked liver iron overload, which is often inevitable in patients on regular blood transfusion, and HCV infection have been shown to have a potentiating effect on hepatic fibrogenesis in thalassemic patients.[1] Chronic hepatitis C has been indicated as a progressive disease that dramatically increases the morbidity and mortality rates among these patients due to liver failure or hepatocellular carcinoma.[2]

HCV is responsible for 80 – 90% of post-transfusion hepatitis in patients who received blood transfusion prior to the introduction of routine blood products screening in 1990.[3] The prevalence of HCV infection among thalassemic patients has been reported to be up to 60% in Italy,[4] although the compulsory screening of donated bloods has decreased the incidence of both posttransfusion HBV and HCV infections.[5]

Due to the lack of sufficient reported data from Iran, we conducted this large multicenter study to provide a comprehensive data bank on the epidemiology of HBV, HCV, and human immunodeficiency virus (HIV) infections in patients with β-thalassemia in Iran. Furthermore, we tried to analyze HCV transmission-associated risk factors which allow the development of an effective policy to reduce the incidence of HCV infection in our thalassemic patients.

Patients and Methods

Seven hundred and thirty-two consecutive patients with β-thalassemia from five provinces of Iran including Tehran (n = 410), Kerman (n = 100), Qazvin (n = 95), Semnan (n = 81), and Zanjan (n = 46), who referred to the main Blood Transfusion Centers for blood transfusion were enrolled in this study between March and August 2002. Blood transfusion centers in each province are the branches of Iranian Blood Transfusion Organization (IBTO), which is responsible for providing blood and blood products from volunteer donors under a unified policy in the country. Initial data including demographic data, transfusion history, and previous medical history were obtained by reviewing medical records and interviews. Thereafter, venous blood samples were obtained from each patient before blood transfusion. All sera were tested by the second or third generation commercially available enzyme-linked immunosorbent assay (ELISA) kits to detect anti-HCV antibodies (HCVAb) (HCV 3.0 ELISA; ORTHO®, Raritan, NJ, USA), hepatitis B surface antigen (HBsAg) (Hepanostika HBsAg Uni-Form II; Organon Teknika®, Boxtel, Netherlands), and anti-HIV antibodies (HIVAb) (Genscreen HIV; Bio Rad®, France). All sera positive for HCVAb were retested by the second generation of recombinant immunoblot assay (RIBA) kits (HCV blot 3.0; Genelabs Diagnostics®, Singapore) as a complementary test. Patients found positive with both ELISA and RIBA HCVAb were considered to be infected with HCV. The required facilities for further testing of anti-hepatitis B surface antibodies (HBsAb) (DiaSorin; Saluggia (Vercelli)®, Italy) were provided in four provinces of Kerman, Qazvin, Semnan, and Zanjan (n = 322); two provinces of Tehran and Qazvin (n = 505) were equipped for testing of anti-hepatitis B core antibodies (HBcAb) (DiaSorin; Saluggia (Vercelli)®, Italy). A second sample was tested to measure hemoglobin and serum ferritin levels.

The study protocol was approved by our institutional review board. Informed written consent was taken from each patient involved.

Results are expressed as mean ± standard deviation (SD). Comparison between two groups was made using the Student's *t* test for continuous variables and Chi-square or Fisher's exact test, when appropriate, for categorical variables. In univariate analysis, the possible factors associated with HCV infection were evaluated. A multivariate analysis, based on a stepwise backward logistic regression model, was used to assess the independent variables. A P value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS software for Windows (version 10.0; SPSS Inc. Chicago, IL, USA).

Results

The study group comprised 732 β-thalassemic patients (413 [56.4%] males and 319 [43.6%] females), including 569 (77.7%) with β-thalassemia major and 163 (22.3%) with β-thalassemia intermedia. β-thalassemia intermedia is defined as a milder phenotype due to two alleles with at least one β^+. The mean ± SD age was 17.9 ± 9.0 (range: 1 – 56) years. Five hundred and fifty-six (76.0%) patients received complete vaccination for hepatitis B.

Table 1. Prevalence of viral seromarkers in the study population.

Viral markers	Total No.	No. of positive cases	Valid percentage	95%Confidence interval
HCVAb	732	141	19.6	16.9 – 22.3
HBsAg	732	11	1.5	0.8 – 2.2
HBsAb	322	160	55.2	49.8 – 60.6
HBcAb	505	68	13.6	10.7 – 16.5
HIVAb	732	0	0	–

The results of viral serologic markers are summarized in Table 1 and categorized by each province in Table 2. An overall prevalence rate of 19.6% was found for HCV, which varied widely depending on the provinces involved (from 2.2% in Zanjan to 25.3% in Qazvin). Eleven (1.5%) patients were found to be HBsAg seropositive; the rate was zero in Zanjan and Semnan to 6.0% in Kerman. None of our patients was HIV positive.

Using the univariate analysis, a number of factors, which could be potentially associated with HCV infection, were compared between HCV-positive and HCV-negative patients (Table 3). HCV seropositivity was significantly associated with an older age ($P = 0.001$), history of splenectomy ($P < 0.05$), longer history of transfusion ($P < 0.001$), HBsAg seropositivity ($P = 0.03$), and higher serum ferritin level ($P = 0.002$). Moreover, patients with β-thalassemia major were more likely to be HCV infected than those with β-thalassemia intermedia ($P = 0.01$). Patients who had received their first blood transfusion before implementation of compulsory blood donors screening in Iran in 1995, had a significantly higher rate of HCV infection as compared to those who were transfused after then ($P < 0.001$).

Based on multivariate analysis, older age ($P < 0.001$), having β-thalassemia major vs. β-thalassemia intermedia ($P = 0.002$), having the first transfusion before or in 1995 vs. after 1995 ($P = 0.003$), and a higher serum ferritin level ($P < 0.001$) were independent predictors of HCV infection (Table 4).

Table 2. Prevalence of viral seromarkers in each province.

Viral markers	Tehran	Kerman	Qazvin	Semnan	Zanjan
HCVAb pos.	80 (19.6%)	18 (18.8%)	23 (25.3%)	19 (24.4%)	1 (2.2%)
HBsAg pos.	4 (1.0%)	6 (6.0%)	1 (1.1%)	0	0
HBsAb pos.	ND*	45 (45.9%)	57 (62.6%)	31 (56.4%)	27 (58.7%)
HBcAb pos.	59 (14.4%)	ND	9 (9.9%)	ND	ND

*ND = not done; pos. = positive.

Discussion

This study is the first multicenter survey, which

Table 3. Comparison of factors between HCV-positive and HCV-negative patients.

Factors	HCV positive	HCV negative	*P* value
Sex			NS *
Female	60 (42.6%)	252 (43.6%)	
Male	81 (57.4%)	326 (56.4%)	
Age (yr)**	20.2 ± 8.2	17.5 ± 9.1	<0.001
Hx of splenectomy			<0.05
Yes	77 (55.4%)	263 (46.2%)	
Length of transfusion (months)	205.9 ± 76.8	165.4 ± 89.0	<0.001
First transfusion			<0.001
Before or in 1995	136 (97.8%)	460 (80.3%)	
After 1995	3 (2.2%)	113 (19.7%)	
Hemolytic anemia			0.01
β-thalassemia major	120 (85.1%)	437 (75.6%)	
β-thalassemia intermedia	21 (14.9%)	141 (24.4%)	
Hx of HBV vaccination			NS
Yes	102 (83.6%)	448 (80.9%)	
No	20 (16.4%)	106 (19.1%)	
HBsAg			0.03
Positive	5 (3.5%)	5 (0.9%)	
Hemoglobin level (g/dL)*	9.7 ± 1.2	9.6 ± 1.2	NS
Serum ferritin level (ng/mL)*	3292.9 ± 2496.8	2571.0 ± 1902.5	0.002

*NS= not significant; ** = mean ± SD; Hx = history.

Table 4. Logistic regression analysis of factors associated with HCV infection.

	OR (95% CI)	*P* value
Age	1.05 (1.02–1.08)	<0.001
Hx of splenectomy		
No	1.00	
Yes	1.09 (0.70 – 1.68)	NS*
Length of transfusion	1.00 (0.99 – 1.00)	NS
First transfusion		
Before or in 1995	1.00	
After 1995	6.10 (1.84 – 20.16)	0.003
Hemolytic anemia		
β-thalassemia intermedia	1.00	
β-thalassemia major	2.64 (1.43 – 4.85)	0.002
HBsAg		
Negative	1.00	
Positive	2.88 (0.74 – 11.14)	NS
Serum ferritin level	1.00 (1.00 – 1.00)	<0.001

OR = odd ratio, NS = not significant; Hx = history.

screened a large number of patients with β-thalassemia from various provinces of Iran and provides a comprehensive epidemiologic data on HBV, HCV, and HIV infections among Iranian thalassemic population.

Previous single-center studies on Iranian β-thalassemics revealed a wide range of 16 – 64% for prevalence of HCV infection.[6 – 8] In this study, we revealed that 19.6% of β-thalassemic patients were HCV infected. The other studies from some neighboring Arabic countries reported an HCV infection rate of 33% in Kuwait[9] and 40% in Bahrain[10] and Jordan[11]. Therefore, the rate we found in this study does not appear to be very high, when compared with the HCV infection prevalence among the Iranian blood donors (0.12%).[12] HCV infection is, nevertheless, considered as a major health concern in the thalassemic population of Iran.

This study showed that the prevalence of HCV infection dropped significantly from 22.8% to 2.6%, after the implementation of blood donor screening in Iran in 1995. In addition, patients who received unscreened blood were exposed to HCV infection more than six times as much as those transfused after starting the screening program. It is probable that the meticulous screening of blood donors will eventually eliminate the incidence of HCV infection among our thalassemics in Iran in view of the fact that we have had only three new HCV-infected cases in our population during the past seven years. Since our investigation was conducted as a single point prevalence study, we cannot exclude nosocomial transmission of the virus over time. 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 mitigation of viral transmission. The cohort studies from Italy[5] and the USA[13] have also documented the effectiveness of blood donor screening. The higher rate of HCV infection in older patients, as well as patients with β-thalassemia major 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 thalassemic population.

One and a half percent of our patients were HBsAg positive, which is nearly equal or even lower than the value reported from the general Iranian population (2.5 – 3.5%).[14]

The prevalence of HIV infection in thalassemic patients was reported to be 8.9% in India,[15] while it was 1.6% in multitransfused subjects in Bahrain.[10] However, we found no HIV-seropositive cases in our patients. This fact revealed that fortunately, for the time being, HIV infection is not a health concern for thalassemic population in Iran. This may be due to the relatively low prevalence of HIV infection in Iran, as well as the fact that all donors are screened for HIV.

There are more than 25,000 patients with β-thalassemia major in Iran. Therefore, it is possible that there could be around 5,000 HCV-infected thalassemics in Iran who are at risk of progression to liver failure and hepatocellular carcinoma. The present blood donor screening protocol has been shown to be effective and it seems that applying refined methods, more sensitive laboratory screening techniques, and assured quality control measures, in addition to appropriate and timely protocols for management of infected patients are

likely to be the most useful strategies.

References

1 Ardalan FA, Osquei MRF, Toosi MN, Irvanloo G. Synergic effect of chronic hepatitis C infection and beta-thalassemia major with marked hepatic iron overload on liver fibrosis: a retrospective cross-sectional study. *BMC Gastroenterology*. 2004; **4:** 17.

2 Tong MJ, El-Farra NS, Reikes AR, Co RL. Clinical outcomes after transfusion-associated hepatitis C. *N Engl J Med.* 1995; **332:** 1463 – 1466.

3 Costello M, Yungbluth M. Viral infections. In: Henry J B, ed. *Clinical Diagnosis and Management by Laboratory Methods*. 20th ed. Philadelphia: WB Saunders; 2001: 1064 – 1066.

4 Angelucci E. Antibodies to hepatitis C virus in thalassemia. *Haematologica*. 1994; **79:** 353 – 355.

5 Mele A, Stroffolini T, Catapano R, Palumbo F, Moiraghi A, Novaco F. Incidence of transfusion-associated B and non-A, and non-B hepatitis in Italy. *Br Med J*. 1995; **311:** 846 – 847.

6 Karimi M, Ghavanini AA. Seroprevalence of hepatitis B, hepatitis C, and human immunodeficiency virus antibodies among multitransfused thalassemic children in Shiraz, Iran. *J Paediatr Child Health.* 2001; **37:** 564 – 566.

7 Ansar MM, Kooloobandi A. Prevalence of hepatitis C virus infection in thalassemic and hemodialysis patients in North Iran-Rasht. *J Viral Hepat*. 2002; **9:** 390 – 392.

8 Alavian SM, Kafaee J, Yektaparast B, Hajarizadeh B, Doroudi T. The efficacy of blood donor screening in reducing the incidence of hepatitis C virus infection among thalassemic patients in Iran. *Transfusion Today*. 2002; **53:** 3 – 4.

9 Al-Fuzae L, Aboolbacker KC, Al-Saleh Q. Beta-thalassemia major in Kuwait. *J Trop Pediatr*. 1998; **44:** 311 – 312.

10 Al-Mahroos FT, Ebrahim A. Prevalence of hepatitis B, hepatitis C, and human immune deficiency virus markers among patients with hereditary hemolytic anaemias. *Ann Trop Paediatr*. 1995; **15:** 121 – 128.

11 Al-Sheyyab M, Batieha A, El-Khateeb M. The prevalence of hepatitis B, hepatitis C, and human immune deficiency virus markers in multitransfused patients. *J Trop Pediatr*. 2001; **47:** 239 – 242.

12 Alavian SM, Gholami B, Massarat S. Hepatitis C risk factors in Iranian volunteer blood donors: a case control study. *J Gastroenterol Hepatol*. 2002; **17:** 1092 – 1097.

13 Donahue JG, Munoz A, Ness PM, Brown DE, Yawn DH, McAllister HA, et al. The declining risk of post-transfusion hepatitis C virus infection. *N Engl J Med*. 1992; **327:** 369 – 373.

14 Zali MR. Epidemiology of hepatitis B in the Islamic Republic of Iran. *East Mediterr Health J*. 1998; **2:** 290 – 297.

15 Sen S, Mishra NM, Giri T, Pande I, Khare SD, Kumar A, et al. Acquired immunodeficiency syndrome (AIDS) in multitransfused children with thalassemia. *Indian Pediatr*. 1993; **30:** 455 – 460.