Prevalence of Hepatitis C Virus Antibodies among Beta-Thalassemia Major Patients in Kurdistan Province, Iran

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Abstract

Background: Blood transfusion is used to treat patients with beta-thalassemia major. Blood transfusion is associated with risk of infection, especially hepatitis C virus (HCV).

Objectives: The aim of this study was to determine the prevalence of anti-HCV antibodies in patients with beta-thalassemia major in Kurdistan, Iran.

Methods: In this cross-sectional study, serum samples were collected from 106 out of 129 registered patients with beta-thalassemia major, referred to hospitals in Kurdistan province, Iran in 2015. The sera were kept at -20°C until analysis. Antibodies to HCV (IgG and IgM) were determined using enzyme-linked immunosorbent assay (ELISA); ELISA-positive samples were verified by the recombinant immunoblot assay (RIBA).

Results: The prevalence of anti-HCV antibodies was 5.66% (4 males, 2 females), based on the ELISA assay. Four (3.77%) ELISA-positive patients were confirmed by RIBA assay (2 males, 2 females), while the results of 2 (1.88%) men were indeterminate. HCV antibodies showed a significant correlation with the patient’s age (P = 0.001) and duration of receiving blood transfusions (P = 0.003). However, there was no significant association between HCV antibodies and gender (P = 0.428).

Conclusions: The prevalence of HCV in patients with beta-thalassemia major was lower in Kurdistan, compared to other provinces of Iran. Although screening programs for blood donors in blood transfusion centers can be effective, it is recommended to use nucleic acid tests for screening blood donors.

Keywords: Prevalence, Hepatitis C Virus, Antibodies, Beta-Thalassemia, ELISA, RIBA

1. Background

Hepatitis C virus (HCV) is the main causative agent of posttransfusion hepatitis. HCV is distributed throughout the world with varying prevalence rates, affecting more than 3% of the world’s population. This virus can account for approximately 20% of all cases of acute hepatitis, 80% of cases of chronic hepatitis, 40% of cirrhosis cases, 70% of cases of hepatocellular carcinoma, and 30% of cases of liver transplantation (1-3).

HCV is transmitted through unsafe intravenous injections, sexual intercourse, blood transfusion, and blood products. Some groups, such as patients with hemophilia, thalassemia, renal diseases (under hemodialysis), and intravenous drug use are at a high risk of HCV infection. No vaccine is available against HCV infection, and the accessible antiviral drugs are long-acting, difficult to use, expensive, and ineffective for patients (1-3). Betathalassemia is an autosomal recessive hereditary disease, affecting the synthesis of beta globin chains in hemoglobin. Similar to countries in the Eastern Mediterranean region, Iran is located in the middle of the so-called thalassemia belt. According to estimations, there are 2 to 3 million carriers and 25 000 patients with beta-thalassemia in Iran (4). Therefore, thalassemia is an important health problem, particularly in northern and southern provinces of Iran (5).

Beta thalassemia major causes severe anemia, hepatomegaly, splenomegaly, and acromegaly. Regular blood transfusion is a standard treatment for this disease. Although blood transfusion can lead to patient survival in thalassemia, it can increase the risk of blood-borne infections, such as HCV, hepatitis B virus (HBV), and human immunodeficiency virus (HIV) (3), as well as complications such as iron overload (6).
The prevalence of HCV infection among blood donors is about 0.5% in Iran. Although the prevalence of HCV infection has been decreasing in recent years among Iranian blood donors due to blood supply tests (1), the frequency of HCV subtypes differs among HCV patients in various provinces of Iran and fluctuates with time (7). The prevalence of HCV is much higher among high-risk groups, such as beta-thalassemia patients (8).

In this regard, a systematic review has been carried out, based on the literature database. The seroepidemiology of HCV infection among beta-thalassemia patients has been estimated at 18%, 45%, 63%, and 69% in Iran, Pakistan, Saudi Arabia, and Egypt, respectively. Among Iranian beta-thalassemia patients, splenectomy, high frequency of blood transfusion, advanced age, and transfusion before 1996 were risk factors for HCV infection (8).

Recently, the prevalence of HCV infection in patients with beta-thalassemia major has been estimated at 13.6% in Guilan province, north of Iran. The risk of HCV infection among beta-thalassemia major patients was higher before screening the blood donors for HCV (before 1996). The seroprevalence of HCV has decreased dramatically among patients receiving blood donations after the implementation of donor screening (5). Therefore, study of HCV prevalence in thalassemia patients, who supportively receive blood transfusions, can reflect the safety of blood supplies (5). There is a scarcity of data regarding the prevalence of HCV infection in patients with beta-thalassemia major to help establish more preventive strategies in Kurdistan province, West of Iran.

2. Objectives

The aim of this study was to determine the prevalence of HCV antibodies in patients with beta-thalassemia major in Kurdistan province, West of Iran.

3. Methods

3.1. Study Population and Specimens

According to the last census conducted in 2011, Kurdistan province in west of Iran had a total population of 1,493,645. The sex ratio of the population was 1:1, and most patients were Kurds. According to the report of the treatment deputy of Kurdistan University of Medical Sciences, there were about 129 registered patients with beta-thalassemia major in Kurdistan province. These patients were referred to 6 general and 1 specialized governmental hospitals in 7 cities of the province (Sanandaj, Bijar, Saqqez, Baneh, Qorveh, Marivan, and Kamyaran) for supportive treatment.

In this cross sectional study, 106 serum samples were collected from patients with beta-thalassemia major, referred to the hospitals in 2015. The serum remnants of the blood samples for routine laboratory tests were gathered from the hospital laboratories; therefore, there was no need for additional venipuncture. The blood samples were centrifuged at 3000 rpm for 15 minutes, and the sera were separated. The serum samples were stored in a freezer at -20°C until analysis.

Demographic information, including age, gender, age at onset of blood transfusion, blood transfusion intervals, and duration of receiving blood transfusions, was collected from the available data at the hospitals. The patients’ names and their personal information remained confidential. The study was approved by the Faculty of Sciences, Science and Research Branch of Islamic Azad University of Sanandaj, Sanandaj, Iran (proposal code, 61530507931004).

3.2. Laboratory Tests for HCV Antibodies

Total antibodies for HCV (IgG and IgM) were measured by enzyme-linked immunosorbent assay (ELISA) kit (EIA-gen HCV Ab V.4, Adaltis, Italy), according to the manufacturer’s instructions, using an ELISA reader and washer (Tecan, Australia). ELISA-positive samples were confirmed by the recombinant immunoblot assay (RIBA) kit (MP Diagnostics, Germany). The tests were carried out at the Iranian blood transfusion organization (Sanandaj branch) in the presence of 1 observer. The laboratory equipments were calibrated by the Iranian blood transfusion organization.

3.3. Statistical Analysis

The laboratory test results and demographic data were entered in SPSS version 19. The data are presented as descriptive statistics and analyzed by T-Test and Chi square test. To estimate the odds ratio of gender, univariate logistic regression analysis was applied. To estimate the parameters and significance levels accurately, the bootstrap technique was used with a sample size of 100, including 1000 repeated samples. P value below 0.05 was considered statistically significant.

4. Results

Out of 106 patients with beta-thalassemia major, 54 (50.9%) were male and 52 (49.1%) were female. The age range of the patients was 2 - 39 years (mean, 18 ± 8.05). The mean age of the patients at the onset of blood transfusion was 21.79 months (range, 2 - 144 months). In addition, distribution of patients’ age at the onset of blood transfusion was as follows: < 12 months (n, 53; 50%), 12 - 24 months
Based on the ELISA results in 106 patients with beta-thalassemia major, 6 (5.66%) were positive for HCV antibodies (IgG and IgM), including 4 males and 2 females. Among ELISA-positive samples, 4 (3.77%) were confirmed by RIBA assay (2 males, 2 females), while 2 (1.88%) men showed intermediate results. HCV antibodies were positive only among patients above 27 years, while negative results were reported among younger patients. The T-Test results showed a significant difference in the presence of HCV antibodies with respect to the mean age of patients ($P = 0.001$) and duration of receiving blood transfusions ($P = 0.003$) (Table 1). In addition, Chi square test results showed a significant relationship between ELISA-positive findings and age of patients ($< 18$ and $\geq 18$ years; $X^2 = 5.56, P = 0.018$) (Table 2). However, there was no significant association between HCV antibodies and gender ($X^2 = 0.629; P = 0.428$).

According to T-Test results, there was no significant association between HCV antibodies and age at the onset of blood transfusion ($P = 0.095$) or blood transfusion intervals ($P = 0.995$). To determine the relationship between gender and ELISA results (HCV antibodies), simple logistic regression analysis was applied. Therefore, men had a 2-fold increased chance of positive ELISA compared to women (OR: 2; 95% CI: 0.35 - 11.42; $P = 0.435$). For presentation of logistic regression parameters, bootstrap sampling was conducted. This method showed probability of 0.435 with very low bias. It should be noted that the results of RIBA assay were not included in the statistical analysis.

5. Discussion

We detected anti-HCV antibodies in 6 (5.66%) patients with beta-thalassemia major (4 males, 2 females) in Kurdistan province, west of Iran. The seroepidemiology of HCV among beta-thalassemia patients has been estimated at 18%, 45%, 63%, and 69% in Iran, Pakistan, Saudi Arabia, and Egypt, respectively (8). In this regard, two studies have been conducted in north of Iran. The prevalence of HCV infection among patients with beta-thalassemia was 8% in Isfahan province in the center of Iran. History of surgery, dental procedures, frequency of blood transfusion per month, and duration of transfusion had significant associations with HCV seropositivity (10). The prevalence of HCV antibodies in patients with beta-thalassemia dropped from 22.8% to 2.6% after the implementation of the anti-HCV screening program since 1996 in Tehran, Kerman, Qazvin, Semnan, and Zanjan (11).

Additionally, two studies have been carried out in south of Iran. Patients with beta-thalassemia were screened by ELISA assay and confirmed by RT-PCR. Overall, 44.7% of beta-thalassemia patients were infected with HCV in Kerman, southeast of Iran. There was a significant relationship between HCV and frequency of blood transfusion (12). In addition, the prevalence of anti-HCV antibodies was 28.1% among patients with beta-thalassemia in Khuzestan province, southwest of Iran. Moreover, 79.3% of anti-HCV-positive patients were positive for HCV-RNA. HCV-positive patients were significantly older than their HCV-negative counterparts (13).

The present results are compatible with the results reported by Mirmomen and Akbari in Iran (11, 14). In comparison with the results of other studies in Iran, the prevalence of HCV infection was lower in our patients. In addition, in our study, HCV antibodies were positive only among patients above 27 years, while HCV-negative results were reported among younger patients. These results reflect that our patients had received blood after the implementation of the donor screening program in 1996 for the safety of blood supplies. In other words, the low prevalence of HCV in our study population may be attributed to the precise screening of donated blood and/or lower prevalence of HCV infection in Kurdistan province. Therefore, it can be concluded that HCV-positive patients in our study population were infected before the establishment of screening programs for blood donation by the Iranian blood transfusion organization.

In the present study, 4 out of 6 ELISA-positive samples were confirmed by RIBA assay, while 2 samples showed intermediate results. The current generations of HCV ELISA kits have sensitivity of about 97% and indicate the positive results within 1 to 2 months of infection. Overall, the specificity of ELISA assay is not 100%. Considering the antibody production lag, additional tests, such as RT-PCR for HCV-RNA, are required. In addition, clearance of HCV viremia (spontaneously or after successful treatment) is associated with decreasing antibody levels, sometimes below the level considered positive on ELISA. Currently, direct viral tests depend on the detection of viral RNA in the plasma or serum during or at the end of treatment to pre-
Table 1. The Correlation between HCV Antibodies (Positive ELISA Results) and Age of Patients, Age at the Onset of Blood Transfusion, Duration of Receiving Blood Transfusions, and Blood Transfusion Intervals According to T-Test Results

| Variables                                      | ELISA Results | Number | Mean     | Std. Deviation | P Value |
|------------------------------------------------|---------------|--------|----------|----------------|---------|
| Age (years)                                    | +             | 6      | 28.83    | 5.03           | 0.001   |
|                                                | -             | 99     | 17.97    | 7.79           |         |
| Age at the onset of blood transfusion (months) | +             | 6      | 36.00    | 30.35          | 0.095   |
|                                                | -             | 99     | 20.92    | 20.69          |         |
| Duration of receiving blood transfusions (years)| +             | 6      | 26.00    | 6.00           | 0.003   |
|                                                | -             | 99     | 16.18    | 7.84           |         |
| Blood transfusion interval (days)              | +             | 6      | 22.33    | 6.74           | 0.995   |
|                                                | -             | 99     | 22.28    | 11.90          |         |

*There was missing age-related information for one of the patients.

Table 2. The Association between HCV Antibodies (Positive ELISA Results) and Age According to Chi Square Test Results

| ELISA Results | Age, y*, P = 0.018 | Total |
|---------------|-------------------|-------|
|               | < 18              | ≥ 18  |
| -             |                   |       |
| Count         | 49                | 50    | 99    |
| Percentage on ELISA | 49.5%          | 50.5% | 100%  |
| Percentage within new age | 100%       | 89.6% | 94.3% |
| Total percentage | 46.7%            | 47.6% | 94.3% |
| +             |                   |       |
| Count         | 0                 | 6     | 6     |
| Percentage on ELISA | 0.0%            | 100%  | 100%  |
| Percentage within new age | 0.0%       | 10.7% | 5.7%  |
| Total percentage | 0.0%            | 5.7%  | 5.7%  |
| Total         |                   |       |
| Count         | 49                | 56    | 105   |
| Percentage on ELISA | 46.7%          | 53.3% | 100%  |
| Percentage within new age | 100%         | 100%  | 100%  |
| Total percentage | 46.7%            | 53.3% | 100%  |

*There was missing age-related information for one of the patients.

The main strength of this study was collection of new information about HCV prevalence among patients receiving blood transfusions in Kurdistan province. However, we could not detect viral RNA via RT-PCR and were unable to collect the patients’ clinical profiles, including the number of blood transfusions, medications, and complications.

5.1. Conclusion

The prevalence of anti-HCV antibodies in patients with beta-thalassemia major in Kurdistan province was lower than other provinces of Iran. Screening programs for blood donors in blood transfusion organizations can be effective. It is recommended to use nucleic acid tests to screen blood donors, since HCV has a preserological window period, where the donated blood may contain infectious viral particles (only detectible by a nucleic acid test) without any detectable antibodies.
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Footnotes

Authors’ Contribution: Silan Mohammadi gathered the specimens and patients’ data and performed the laboratory tests. Mazaher Khodabandehloo provided technical support, analyzed the data, and wrote the manuscript. Both authors read and approved the final version of the manuscript.

Conflict of Interests: The authors declare no conflicts of interest.

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