

Prevalence of HBV and HCV infection in beta-thalassemia major patients of Tabriz city, Iran

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Abstract

People with beta-thalassemia major are more likely to acquire blood-borne viral infections due to the need for frequent blood transfusions. Of these viruses, hepatitis B virus (HBV) and hepatitis C virus (HCV) are of particular importance. In this study, the prevalence of HBV, HCV and their risk factors in beta-thalassemia major patients in East Azerbaijan province was investigated. The study was descriptive cross-sectional, and 116 beta-thalassemia major patients who received blood in Shahid Ghazi hospital and Children's hospital in Tabriz city were studied. Data were collected by a questionnaire, and blood samples of patients in terms of serum markers HCV-Ab, HBsAg and HBs-Ab were analyzed by ELISA, and positive HCV-Ab results were confirmed by Real Time-PCR. Then using SPSS software version 22 and with the help of t-tests including Anova T-test, Man-Whitney U test, Independent sample t-test, chi-square and Fisher exact test, Statistical studies were performed. Of the 116 patients studied, no HBsAg positive cases were found. Four patients (3.4%) were positive for HCV-Ab, of which two patients (1.7%) became HCV-RNA positive after Real Time-PCR. There was a significant relationship between HCV-Ab positive and HCV-RNA positive ($P = 0.000$), blood transfusion intervals ($P = 0.043$), number of injected blood units ($P = 0.001$) and duration of blood transfusion ($P = 0.006$). The prevalence of HCV was lower in patients who started receiving blood after a blood donor screening program. HCV is less prevalent in thalassemia patients in East Azerbaijan province than in some studies in the country and various global statistics. After 1996, the prevalence of HCV in the thalassemia patient population has decreased significantly, and it seems that HCV infections since 1996 have been associated with various factors such as people's jobs, position, behaviour in society, etc. © 2021 The Author(s). Published by Elsevier Ltd.

Keywords: Beta-thalassemia, hepatitis B virus, hepatitis C virus

Original Submission: 5 April 2021; **Accepted:** 24 June 2021

Article published online: 6 July 2021

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Introduction

Beta-thalassemia is a group of inherited blood disorders characterized by reduced or no synthesis of the beta-globin chain, and if the synthesis of both genes is reduced or

absent, the person has beta-thalassemia major [1,2]. Patients with beta-thalassemia major are at risk for blood-borne viral infections such as Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV) due to frequent blood transfusions [3]. Frequent blood transfusions increase the life expectancy of these patients but are associated with an increased risk of blood-borne diseases. Prevention of viral infections is an important goal in the treatment of patients with beta-thalassemia major [4]. According to reports, before 1990, in Western countries and before 1995 in Iran, donated blood was not screened in blood transfusion organizations for hepatitis C virus. Therefore, patients in beta-

thalassemia major were more likely to become infected with viral infections due to the need for frequent blood transfusions [3]. Screening donors with serological tests and through interviews and medical examinations has reduced the risk of post-transfusion infections, especially hepatitis C. Today, due to the increasing use of hepatitis B vaccine and screening tests for all donated blood for hepatitis B, the prevalence of HBV in patients with beta-thalassemia major has decreased significantly [5]. The prevalence of HBV infection in the general population of Iran is estimated at 2.2% [6], and in beta-thalassemia major patients, it varies according to the type of province, which is usually less than one per cent in most provinces [7–9]. Therefore, according to reports, the prevalence of HBV infection in patients with beta-thalassemia major is lower than the normal population in the country. According to studies, the prevalence of HBV in blood donors in Iran has been reported to be 0.8% [8]. The prevalence of HCV in patients such as beta-thalassemia major patients who frequently receive blood has decreased due to screening of donated blood, but there are still cases of HCV in these patients [10–13]. In Iran, the HCV blood donation screening program has been performed since 1995, and many patients with beta-thalassemia major with HCV-Ab positive received blood before 1995 [14]. The prevalence of HCV in the community in the country is 0.3% [15]. According to a study conducted in 2018, the prevalence of HCV in beta-thalassemia patients in Iran is 19%. The prevalence of HCV in beta-thalassemia patients is higher than the general prevalence of HCV in the Iranian population. As a result, these patients are at risk for HCV [16]. According to studies, the prevalence of HCV in Iranian blood donors is reported to be 0.06% [17]. Today, cases of HBsAg and HCV-Ab positive in patients with beta-thalassemia major may be due to receiving blood that was in the window period, ie blood that was HCV-Ab and HBsAg negative but PCR positive. The aim of this study was to evaluate the frequency of HBV and HCV infection in thalassemia patients undergoing blood transfusion in East Azerbaijan province in 2020.

Materials and methods

This study was a descriptive cross-sectional study that was performed on all beta-thalassemia major patients referred to Shahid Ghazi hospital and Children's hospital in Tabriz city. It should be noted that most beta-thalassemia major patients in the cities of East Azerbaijan province go to Shahid Ghazi hospital and Children's Hospital to receive blood. The study population consisted of 116 beta-thalassemia major patients of East Azerbaijan province who were referred to the above-mentioned

hospitals for blood transfusion. Before sampling, a questionnaire and consent form were prepared and completed for all patients. The questionnaire form includes sampling date, centre name, patient code, name, date of birth, sex, marital status, ethnicity, level of education, approximate date of diagnosis of thalassemia, approximate date of first blood transfusion, distance travelled to blood transfusion (day), number of injected blood units, duration of blood transfusion, history of spleen resection, history of jaundice, history of diabetes, history of hepatitis B virus vaccination, serological tests such as HBsAg, HBs-Ab, HCV-Ab, and molecular tests such as HBV DNA, HCV-RNA and blood type of patients. After filling in the questionnaire and with complete satisfaction, blood samples were taken from all patients, and serological tests were performed on all samples. HBsAg test was performed by ELISA method with Siemens kit, HBs-Ab test by ELISA method was performed with Pishtaz Teb kit and HCV-Ab test by ELISA method was performed with Adaltis kit. All positive cases were re-sampled and repeated with the same kit and all positive results in the ELISA method were finalized using a Real Time-PCR kit called Geneproof Hepatitis. It should be noted that viral RNA extraction was performed using the MagCore extraction kit. The obtained data were statistically analyzed by SPSS software version 22. In this study, for descriptive statistics, mean, standard deviation for quantitative data, frequency and frequency percentage for qualitative data have been used. Also, from Chi-square test, and if necessary, Fisher's exact test to check the relationship between qualitative variables, and Anova t-test, Man-Whitney *U* test, Independent sample t-test to check the relationship between quantitative data, were used. Reliability was significant in all results 95% and $P < 0.05$.

Results

The study population in this study was 116 beta-thalassemia major patients, of which 63 were male (54.3%) and 53 were female (45.7%). The mean age of these patients was 20 ± 9.5 years, and their age range was 1–51 years. Table 1 shows the evaluated variables in beta-thalassemia major patients.

The results of hepatitis serology tests are given in Table 2. According to this table, all patients are HBsAg negative. Also, out of 116 beta-thalassemia major patients of East Azerbaijan province, four patients (3.4%) were HCV-Ab positive, of which two patients (1.7%) were HCV-Ab positive, HCV-RNA positive. Out of 116 patients with beta-thalassemia major, 101 patients (87.1%) were vaccinated against the hepatitis B virus, and 73 patients (62.9%) of these 101 patients were HBs-Ab positive. HBs-Ab not being positive in patients who have been vaccinated against the virus may be due to repeated blood transfusions, which reduces the effectiveness of the vaccine in these patients.

TABLE 1. Evaluated variables in beta-thalassemia major patients

No	Variables		Frequency and frequency percentage For qualitative variables	Mean and standard deviation (highest–lowest) For quantitative variables
1	Gender	Man	63 (54.3 %)	—
2		Female	53 (45.7 %)	—
3	Age (years)		—	20 ± 9.5
4	Marital status	Single	98 (84.5 %)	—
5		Married	18 (15.5 %)	—
6	Ethnicity	Azeri	106 (91.4 %)	—
7		Fars	1 (0.9 %)	—
8		Kurds	3 (2.6 %)	—
9		Non Iranian	6 (5.2 %)	—
10	level of education	illiterate	11 (9.5 %)	—
11		High school	63 (54.3 %)	—
12		Diploma or bachelor's degree in non-medical fields	39 (33.6 %)	—
13		2.6 %Bachelor of Medicine or higher	3 (2.6 %)	—
14	Date of diagnosis of thalassemia	Before 1996	48 (41.4 %)	—
15		After 1996	68 (58.6 %)	—
16	Date of first blood transfusion	Before 1996	44 (37.9 %)	—
17		After 1996	72 (62.1 %)	—
18	Blood transfusion intervals (days)	—	(Min = 7, Max = 120) 29.4 ± 13.4	—
19	Number of blood units injected	—	—	(Min = 3, Max = 1266) 443.3 ± 289.6
20	Duration of blood transfusion (years)	—	—	(Min = 1, Max = 36) 15.9 ± 8
21	Spleen resection history	Yes	29 (25 %)	—
22		No	87 (75 %)	—
23	History of jaundice	Yes	22 (19 %)	—
24		No	94 (81 %)	—
25	Hepatitis B virus vaccination history	Yes	101 (87.1 %)	—
26		No	15 (12.9 %)	—
27	History of diabetes	Yes	16 (13.8 %)	—
28		No	100 (86.2 %)	—
29	Type of blood groups	A ⁺	46 (39.7 %)	—
30		A ⁻	4 (3.4 %)	—
31		B ⁺	20 (17.2 %)	—
32		B ⁻	5 (4.3 %)	—
33		AB ⁺	8 (6.9 %)	—
34		O ⁺	31 (26.7 %)	—
35		O ⁻	2 (1.7 %)	—

Some of the variables listed in Table 1 are compared between HCV-Ab positive and HCV-Ab negative patients in Table 3. The relationship between HCV-Ab positive and HCV-RNA positive, blood transfusion intervals, number of transfused blood units and duration of blood transfusion was significant.

The date of first blood transfusion in 44 patients (37.8%) was before 1996 and in 72 patients (62%) after 1996. Classification of the date of the first blood transfusion into two groups before 1996 and after 1996 in patients with beta-thalassemia major was due to the fact that the country's blood transfusion organizations did not perform screening tests on donated blood before 1996. Therefore, patients with thalassemia who received blood transfusions were more likely to develop blood-borne infections,

especially hepatitis B, hepatitis C, and HIV. The prevalence of hepatitis C virus in patients with beta-thalassemia major who received blood after 1996 was significantly lower than in patients who received blood before 1996 before the screening program. Infection of thalassemia patients with hepatitis C virus after 1997 can depend on various reasons, such as type of job, place of residence and behaviours in the community, etc.

Discussion

The process of repeated transfusions of blood and blood products is one of the methods of transmitting hepatitis B, hepatitis C and HIV virus to patients in need of blood, especially patients with thalassemia [18]. Before the HCV-Ab and HBsAg tests became mandatory to screen donated blood, hepatitis B and C viruses were the main cause of hepatitis following blood transfusions in patients receiving long-term injections. Today, the hepatitis B virus is significantly controlled by vaccination. However, thalassemia patients still have some degree of risk of acquiring hepatitis C through blood collected during the window period. Factors that weaken the immune system in thalassemia patients should also be considered, such as frequent

TABLE 2. Results of hepatitis serology tests

Factors		Frequency (%)
HBs-Ag	Positive	0 (0 %)
	Negative	100 (100 %)
HBs-Ab	Positive	73 (62.9 %)
	Negative	43 (37.1 %)
HCV-Ab	Positive	4 (3.4 %)
	Negative	112 (96.6 %)
HCV-RNA	Positive	2 (1.7 %)
	Negative	2 (1.7 %)
	Not done	112 (96.5 %)

TABLE 3. Comparison of variables between HCV-Ab positive and HCV-Ab negative patients

No	متغیرها		HCV-Ab positive	HCV-Ab negative	Relationship between variables
1	Gender	Man Female	1 (0.86 %) 3 (2.58 %)	62 (53.4 %) 50 (43.1 %)	No significant <P = 0.05 <P = 0.2
2	Age (years)		28 ± 6.9	19.7 ± 9.4	No significant <P = 0.05 <P = 0.08
3	Marital status	Single Married	2 (1.7 %) 2 (1.7 %)	96 (82.7 %) 16 (13.7 %)	No significant <P = 0.05 <P = 1.1
4	Date of diagnosis of thalassemia	Before 1996 After 1996	3 (2.5 %) 1 (1.7 %)	45 (38.7 %) 67 (57.7 %)	No significant <P = 0.05 <P = 0.19
5	Date of first blood transfusion	Before 1996 After 1996	3 (2.5 %) 1 (0.8 %)	41 (35.3 %) 71 (61.2 %)	No significant <P = 0.05 <P = 0.15
6	Blood transfusion intervals (days)		(Min = 10, Max = 30) 18.7 ± 8.7	(Min = 7, Max = 120) 29.8 ± 13.4	Significant <P = 0.05 <P = 0.043
7	Number of blood units injected		(Min = 442, Max = 1216) 905.5 ± 339.7	(Min = 3, Max = 1266) 416.5 ± 284.4	Significant <P = 0.05 <P = 0.001
8	Duration of blood transfusion (years)		26.7 ± 6.3	15.5 ± 7.8	Significant <P = 0.05 <P = 0.006
9	Spleen resection history	Yes No	2 (1.7 %) 2 (1.7 %)	27 (23.2 %) 85 (73.2 %)	No significant <P = 0.05 <P = 0.22
10	History of jaundice	Yes No	1 (0.8 %) 3 (2.5 %)	21 (18.1 %) 91 (78.4 %)	No significant <P = 0.05 <P = 0.57
11	Hepatitis B virus vaccination history	Yes No	4 (3.4 %) 0 (0 %)	97 (83.6 %) 15 (12.9 %)	No significant <P = 0.05 <P = 0.57
12	History of diabetes	Yes	1 (0.8 %)	15 (12.9 %)	No significant <P = 0.05 <P = 0.45
13	HCV-RNA	No Not done Positive Negative	3 (2.5 %) 0 (0.0 %) 2 (1.7 %) 2 (1.7 %)	97 (83.6 %) 112 (96.5 %) 0 (0.0 %) 0 (0.0 %)	Significant <P = 0.05 <P = 0.00

blood transfusions, iron overload, spleen resection in some patients, and decreased zinc levels and the possibility of acquiring infection from hospital settings. According to the results of our study, all thalassemia patients are negative for HBsAg, which shows that the prevalence of hepatitis B virus among beta-thalassemia major patients in East Azerbaijan province is zero per cent. This condition is due to the effectiveness of HBsAg tests in blood screening and also the extensive hepatitis B vaccination program in the country. Out of 116 thalassemia patients, 101 patients (87%) were vaccinated, so that out of 101 vaccinated patients, 73 patients (62.9%) responded to vaccination and were HBs-Ab positive. These 73 patients vaccinated against hepatitis B virus and HBs-Ab positive have been vaccinated after 1995. This condition is due to the increase in the level of immunity of patients to the hepatitis B virus after 1995. Therefore, there is a significant relationship between vaccination history and HBs-Ab. According to a study conducted in 2009 on patients with thalassemia in South Khorasan-Birjand, the rate of HBs-Ab (57.9%) positive among patients is somewhat consistent with the present study [19]. In a study conducted in Bandar Abbas in 2018, the prevalence of HBV in beta-thalassemia patients was 3% [20]. In another study conducted in Lorestan in 2018, the prevalence

of HBV in these patients was 0% [21]. According to a study conducted in 2015 on thalassemia patients in the northwest of the country, the prevalence of HBsAg is zero per cent, which is consistent with the present study. The results of the study show that the prevalence of HCV-Ab is zero per cent, which is not consistent with the present study [22]. The prevalence of HCV-Ab in this study was 3.4% (4 out of 116 patients), of which four patients (1.7%) were HCV-RNA positive. The results of studies conducted in 2011 in Isfahan show that the prevalence of HCV-Ab is 8%, which is higher than the present study. There is also a significant relationship between HCV positive with the number of blood units and the duration of blood transfusion [23], which is consistent with the present study. In a 2017 study in Zabul, among 152 thalassemia patients, 13 (8.5%) were infected with HCV [24]. In another study conducted in Bandar Abbas in 2018, 17 % of patients with thalassemia major were infected with HCV [20]. The prevalence of HCV infection in thalassemia patients varies in different parts of Iran, although in all cases, the donor blood is screened for HCV, but again the prevalence of HCV is observed in these patients, which may be through HCV-infected blood collected in the Window period are infected with this virus. The results of a 2012 study in Greece show that the prevalence of HBsAg is 29%, which is not

consistent with the present study. Also, the prevalence of hepatitis C in thalassemia patients is 40.5%, which is higher than in the present study. Finally, there is a significant relationship between the high prevalence of HCV and increasing the number of blood transfusion units and in this regard is consistent with the present study [11]. The results of studies conducted by Khaled in 2014 in Iraq show that the prevalence of HBsAg is 0.04%, which is not consistent with the present study [25]. In a study conducted in India in 2020, the prevalence of HCV-RNA was 33.6%, and HBsAg was 1.5% [12]. According to a meta-analysis conducted in 2020, the overall prevalence of HCV in Pakistan is 36.21% [26]. Based on studies conducted around the world, various statistics have been presented in this field, which can be due to the different prevalence of infection in the target community, donor selection method, screening of donated blood, differences in the type of studies and age population of patients studied.

Conclusion

This study shows that the prevalence of HCV in thalassemia patients of East Azerbaijan province is lower than some studies conducted in the country and various global statistics. On the other hand, HBV vaccination and screening tests have been effective in this province. After 1996, due to screening tests, the prevalence of HCV in thalassemia patients has significantly decreased. HCV infection of patients after 1996 can be due to various reasons, including the type of job, place of residence, individual behaviours in the community, and etc. be related. However, due to the prevalence of these viruses in patients with beta thalassemia in other parts of the world, it seems that the measures taken in blood preparation and testing to prevent infections transmitted by blood transfusion in these areas are still not enough.

Author contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

Ethics committee approval

The study has been approved by the ethical committee of Ahar University of Sciences (Permission granted: 21.03.2015, Decision no: 22030507931015).

Informed consent

All patients signed the free and informed consent form.

Transparency declaration

The authors have no conflicts of interest to declare. The authors declared that this study had received no financial support.

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