

Original Article

Prevalence of Torque Teno Virus in Thalassemia Patients in Yazd City

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A B S T R A C T

Article history

Received: 27 Oct 2024

Accepted: 4 Mar 2025

Available online: 17 Jun 2025

Keywords

Frequency

Thalassemia

Torque teno virus

Yazd

Introduction: The Torque Teno Virus (TTV) can be transmitted through blood and blood products such as coagulation factors. People with haematological disorders, such as thalassemia patients, may be exposed to the pathological consequences of this virus. This study aims to determine how prevalent this viral pathogen is in individuals with thalassemia.

Materials and Methods: In this cross-sectional study, serum samples were collected from 99 patients with thalassemia from a special disease center in Yazd city between August and September 2015. A Roche (Germany) DNA extraction kit extracted the genome from the patient's serum. Finally, the presence of the TTV genome was evaluated by a Nested polymerase chain reaction test.

Results: The data obtained from the molecular method revealed that the serum of 43 people with thalassemia was infected with TTV, among which 22 cases were observed in women and 21 cases in men ($p = 0.525$). The patients were significantly younger than 30. The frequency of TTV in thalassemia patients was not significantly affected by other parameters, such as gender, length of blood transfusion, and history of hepatitis C infection.

Conclusion: The data obtained from the present study confirmed the significant presence of the TTV virus in thalassemia patients, which can impact preventive measures and health programs in patients with blood disorders receiving blood factors.



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Introduction

The Torque Teno Virus (TTV) was discovered by Nishizawa et al. (1997) in Japan among individuals suffering from non-A-E transfusion-induced hepatitis. Due to the extensive occurrence of TTV in blood donors, patients with thalassemia commonly contract different genotypes of this virus via therapeutic blood transfusions [1]. The TTV has a genome composed of single-stranded circular DNA. It is crucial to note that this virus belongs to the Anelloviridae family rather than the previously speculated Circoviridae family [2]. In terms of its composition, this virus possesses roughly 3900 nucleotides and a diameter ranging from 30 to 50 nm [3].

Although saliva and oral faecal droplets can transmit this virus, blood transfusion is the main method of transmission. The presence of the virus in the bloodstream has been reported in a range of 1-10 blood donors in America, 2% in England, 12-14% in Japan, 14% in Korea, 13% in Germany, 62% in Brazil, 11% in Spain, and 10% in Colombia. These statistics highlight the global prevalence of the virus [4]. The close relationship between the transmission of this virus through blood and blood products has made its role in blood transfusion-related diseases to be investigated [1].

The TTV has been linked to a variety of medical conditions, such as liver diseases, kidney transplantation, and allograft rejection. Scientific investigation has demonstrated that the TTV viral load holds significant significance in understanding TTV infection, particularly in the context of liver and kidney transplantation. In

recipients of liver transplants, the TTV viral load demonstrates a positive correlation with the intensity of immunosuppression. It is connected to acute rejection and infection during the initial year after transplantation [5, 6].

Similarly, in kidney transplant recipients, TTV load kinetics predict allograft rejection, and there is an association between TTV and subclinical graft rejection [7]. Although TTV infection did not change the aspartate transaminase (AST) and alanine transaminase (ALT) enzyme levels in biliary tract patients, liver involvement may still exist in these patients. In summary, the investigation emphasizes the possible hazard of TTV contamination in individuals with thalassemia, specifically those receiving repeated blood transfusion therapy and haemodialysis [8]. Further research is needed to understand the clinical implications of TTV infection in thalassemia patients and to develop strategies for prevention and treatment.

Materials and Methods

Study population and patient information

In this cross-sectional study, the number of 99 thalassemia patients who visited the special disease center in Yazd from the beginning of August 2015 to the end of September 2015 was included based on the inclusion and exclusion criteria. The inclusion criteria were: 1) patients residing in Yazd city and 2) patients who provided written informed consent to participate in the study. Demographic information of all patients, including age, gender, duration of blood transfusion, and history of hepatitis B and C

infections, was obtained from all patients through a questionnaire.

Collecting and preparing samples

Sampling was performed using standard blood transfusion methods and in special gel tubes (Austria-Greiner bio-one-Gel clot activator vacuumed tube). After transportation to the laboratory, the serum was separated from the blood samples using a centrifuge with a speed of 4000 g, and the separated serum was kept in a -70-degree freezer until the tests were performed. Genome extraction and DNA identification of TTV virus. DNA was extracted using a DNA extraction kit (Roche, Germany) according to the kit protocol, which was stored at -20 °C until the experiment. All samples underwent β -globin amplification (110 bp) using PCO3/PC04 primers (Table 1), to assess DNA quality, and those yielding positive results were subjected to subsequent analysis (Fig. 1).

To identify the TTV genome in thalassemia patients, the Nested polymerase chain reaction (PCR) technique was employed to enhance the detection of low viral loads. In the first round, primers NG054 (sense, positions 3–22) and NG147 (antisense, positions 211–233) were used, followed by NG133 (sense, positions 91–115) and NG132 (antisense, positions 204–222)

in the second round, based on previous studies [9] (Table 1). These nested primers amplify conserved region at TTV 5'UTR. Both positive and negative control samples were included in each PCR run. All PCR products were then run on 1.5% agarose gel at 90 V and 45 minutes. When a band of 60 bp was present, the TTV gene was considered present (Fig. 2). **Statistical analysis**

Frequency and percentage were used to categorize qualitative variables. The Chi-Square Pearson test was used to examine the relationship between different parameters. SPSS software version 22 was used for data analysis. P-value < 0.05 was considered as statistically significant.

Results

Clinical parameters

Of the 99 patients participating in the study, 52 were men and 47 were women. The patients' age range was from 10 to 50 years, with the average age being 50 years, and most were less than 30 years old. The duration of the injection was divided into 5-year intervals, and most people were over 15 years old. The history of hepatitis B (HBV) and C (HCV) infections is briefly shown in Table 2.

Table 1. Sequence primers used in Nested PCR

	Primer name	Primer Sequences (5'-3')	Primer size (bp)
β-globin	F: PCO3	ACACAACCTGTGTTCACTAGC	110
	R: PC04	CAACTTCATCCACGTTCCACC	
The first round of PCR	F: NG054	5' TTTGCTACGTCACTAACCAC 3'	151
	R: NG147	5'GCGAGTCCCCGAGCCCGAATTGCC 3'	
The second round of PCR	F: NG133	5'GTAAGTGCACTTCCGAATGGCTGA3'	60
	R: NG132	5'AGCCCGAATTGCCCTTGAC3'	

Clinical findings and molecular test results

The data obtained from the molecular test revealed that the serum of 43 people with thalassemia was infected with TTV, among which 22 cases were observed in women and 21 cases in men ($p = 0.525$). Comparing the relationship between age and the virus showed that most patients were significantly less than 30 years old, so 29 positive samples were observed among these people ($p = 0.016$).

Discussion

The impact of TTV virus infection on haematological diseases has been extensively studied worldwide. Significant differences have been observed in the prevalence of TTV among people with thalassemia in different communities. Therefore, this study aimed to investigate the frequency of TTV infection in thalassemia patients and its relationship with age, sex, injection duration, and history of hepatitis B and C viral infections in Yazd city.

Table 2. Clinical parameters of 99 patients according to age, Gender, duration of blood transfusion and history of HCV and HBV

Parameters		TTV positive group N (%)	TTV negative group N (%)	P-value
Age	≤ 30	29 (67.4)	49 (87.5)	0.016
	> 30	14 (32.6)	7 (12.5)	
Gender	Male	21 (49)	31 (55)	0.520
	Female	22 (51)	25 (45)	
Duration of blood transfusion	0-5 years	4 (9.4)	8 (14.2)	0.656
	5-10 years	5 (11.6)	10 (17.9)	
	10-15 years	8 (18.6)	10 (17.9)	
	>15 years	26 (60.4)	28 (50)	
History of HCV		1 (2.3)	3 (5.3)	0.448
History of HBV		0	0	NA

HBV= Hepatitis B virus; HCV= Hepatitis C virus; TTV= Torque teno virus

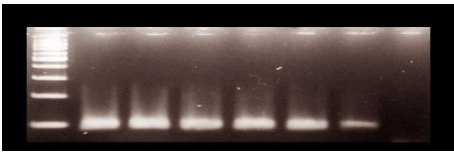


Fig. 1. Agarose gel electrophoresis of β - globin. Products: Lane 1-5: positive results, Lane 6: negative result

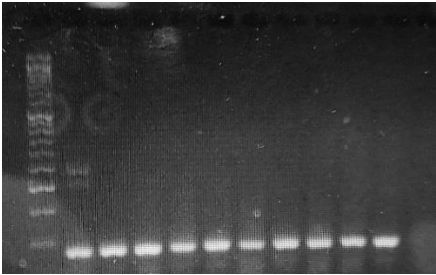


Fig. 2. TTV detection by electrophoresis on agarose gel. Products: Lanes 1-10: positive results, Lane 11: negative result

The molecular test revealed that the TTV was present in 43% of the serum samples collected from these patients.

In the Bouzari study in Isfahan (2007), TTV-DNA was detected in 17.9% of HBsAg carriers, 14% of anti-HCV-positive patients, 22.2% of non-A–non-E hepatitis patients, 22.8% of intravenous drug users, and 57.1% of fulminant hepatitis patients. TTV-DNA was also found in 20.4% of healthy subjects. There was no statistical difference in the prevalence between the different subgroups [13]. In Masia's study in Italy (2001), TTV DNA was detected with the PCR method in 10 (11.1%) patients with autoimmune hepatitis versus four (4.4%) in the control group, and no significant difference was found between the two groups [14].

In our study, the TTV was detected in 2.3% of HCV-positive patients and in none of HBV patients. In a study conducted in Ahwaz, Zandieh showed that 57.2% of samples obtained from patients and 20% of blood donors (control group) were positive for TTV-DNA detected by PCR. The two groups had a statistically significant difference in TTV prevalence [15]. Studies from Turkey and Italy have reported that TTV frequency among thalassemia patients is 61% and 73%, respectively. Takács's study among Italian multiply transfused patients detected TTV DNA in 47 (69.1%) adult beta-thalassemia major patients. TTV DNA was detected by semi-nested PCR (using N22 primers) in 18.5% of persons and 50.4% of patients with hepatitis of unknown origin in Hungary [17].

In another study in Turkish multitransfused patients with thalassemia, TTV was detected in 63% of the thalassemia patients and 54% of the control patients [18]. Our results are consistent with those of other countries. The frequency of this virus in different regions may be due to geographical differences. Also, The significant variation in viremia rate and TTV frequency in different studies can be attributed to various factors, such as sample size, diagnostic tests used, and selection of different target primers.

Sequenced by next-generation sequencing, the virome of 30 patients with beta-thalassemia major was compared in Brazil. The transfusion profile of beta-thalassemic patients received an average of 34 blood units (the average volume of transfusion events for the group). TTV 11 (1145 reads) was the most representative of the thalassemic group [19]. The frequency of TTV infections in transfusion-dependent beta-thalassemia by real-time SYBR Green PCR (evaluated the expression of MiR-222 and MiR-15a) in Shiraz was 21.1% and infection was approximately less than the reported prevalence in other parts of the world [20].

These results and previous studies suggest that TTV-DNA has been transmitted to recipients through blood and blood products. Blood transfusion is one of the most effective ways to transmit TTV [8, 15]. Because immune responses resulting from the TTV virus have not been detected so far, and no method has been reported to identify viral antigens in plasma, no serological methods can be used to identify the TTV virus. The PCR method is

commonly employed in studies to identify this virus in serum samples of thalassemia patients [10].

The extensive genetic diversity of TTV means that choosing the right primer significantly impacts PCR sensitivity. The N22 fragment of ORF1 has been widely used in PCR target studies. Although the designed primer fragments have become more advanced over time, they may still not be able to amplify the TTV isolate, especially when its amount is low in the sample, so the 5'-UTR of the TTV genome for Primer design is much better [11].

The current research confirms that TTV is transmitted through blood and blood products, based on its strong presence in the serum of thalassemia patients. The association between age and viral frequency suggests a potential link to increased infection risk. However, further studies are warranted to clarify this relationship due to limitations such as the relatively small sample size. Although the current study did not find a statistically significant relationship between viral frequency and other parameters, it was observed that individuals in the TTV-positive group generally had a longer history of injections [12].

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Conclusion

In the present study, we demonstrated a high frequency of TTV infection among thalassemia patients in Yazd city. This notable prevalence may serve as an indicator of the epidemiological status of the virus in this population. The findings highlight the need for preventive measures and the development of appropriate health and treatment policies to ensure the provision of safe blood for patients in need.

Ethical Considerations

This study was approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran under the Ethic code (IR.SSU.MEDICINE.REC.1396.1).

Funding

This study was supported financially by Shahid Sadoughi University of medical science

Conflict of Interest

The authors declared no conflict of interest.

Acknowledgment

We would like to thank Dr Mohsen Nakhaei for Cooperation in analyzing data.

Authors' Contributions

A.A, J.Ch: Study concept and design; J. Ch, M. A: Performing experiments; F. AT: Sample collection, N. T: Analysis; J. Ch, A. A: Interpretation of data; H. Sh: Drafting of the manuscript.

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