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Molecular Prevalence and Clinical Importance of Torque Teno Virus Infection in Thalassemia Patients as High Risk Individuals

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Recently a novel DNA virus (Torque Teno Virus (TTV) has been identified in Japan and shown to be associated with elevated aminotransferase s levels after transfusion. However the exact role of TTV in pathogenesis of liver disease is yet to be established. The purpose of this study was to determine the prevalence of TTV in thalassemic patients and its relationship with elevated alanine-aminotransfrase (ALT) and aspartate-aminotransfrase (AST). This cross-sectional analysis study was conducted on 452 thallasemic patients. Serums were collected from all of the patients, first ALT and AST levels were determined. Then, after DNA extraction, TTV DNA was amplified and detected using semi-nested PCR, followed by gel electrophoresis. Demographic characteristics and clinical data were collected from each participant for statistical analysis. The findings showed that 160 of 452 (35.4%) samples had TTV DNA detected by PCR. From 160 TTV DNA positive, 98 (61.20%) were female and 62 (38.80%) of them were male (P=0.549). The mean ALT and AST values in TTV positive group were higher than in TTV negative group, and the difference was statistically significant (p<0.0001). The result showed that the prevalence of TTV in thalassemic patients in Jahrom is less than other studies in Iran and the mean ALT and AST values in TTV positive individuals were about 2 times more than in TTV negative individuals.

Key words: Transfusion Transmitted Virus (TTV), ALT, AST, Thalassemia, Jahrom

Thalassemia are among the most common genetic disorders of the red blood cells in the world and specially has a wide distribution from Europe to south Asia[1]. Because genetically hemolytic anemia in these patients must be supplied blood products to lifelong blood transfusions[2]. In these cases, frequently blood transfusions are associated with various adverse effects such as like iron overload, splenectomy, and risk of transfusion transmitted infections[3,4]. Other complications in thalassemic patients are prone to transfusion-related hepatitis, because transfusion-related iron overloads and exposure to viruses, which may cause hepatitis[5-7]. The screening of hepatitis agents such as hepatitis B virus (HBV), hepatitis C virus (HCV) always is performed, however a significant rate of thalassemic patients have increased levels of serum alanine aminotransferase (ALT) unknown origin[8].

In recent periods, a novel non-enveloped single-stranded DNA virus, transfusion-

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transmitted virus (TTV) which indicated to be associated with non A-G post-transfusion hepatitis[9]. TTV infection occurs in the general population and in patients at risk for parenteral exposure, such as those with thalassemia, hemophilia and liver disease[10-12]. TTV is hepatotropic, and has been indicated that TTV infection is related to increased serum transaminases as ALT[13]. Therefore coinfection with other hepatitis agents can increased tissue injury[14]. Previous study shown that individuals infected with hepatitis B virus or hepatitis C who are positive for TTV infection had evidence of greater liver damage and higher levels of ALT than those with single HBV and HCV[15, 16]. In these surveys, all of hepatitis patients who were positive for TTV DNA had relatively higher levels of ALT than those who were negative for TTV DNA. However, relationship between TTV infection and post transfusion hepatitis, acute hepatitis, and chronic liver disease with unknown etiology has also been unclear[17].

Different studies reported that TTV DNA was detectable in 25%-96% and 50.8%-84.9% of patients with chronic hepatitis of unknown cause[13, 18] and common origin (15, 19), in 27%-69% of hemophiliacs [20, 21], and in 9.33%-18% of healthy individuals[15, 22]. In other reports this rate are demonstrate 64.4% and 39.4% in thalassemic patients[1,23]. The prevalence of TTV in hemodialysis patients in different regions of world were 17%, 48.01%, 64.8%[22, 24, 25]. Also in Jahrom city, the prevalence of TTV in high risk groups such as hemodialysis and blood donors was 27.8% and 13.4% respectively[26, 27].

Because the significant prevalence of this virus among thalassemia patients and that thalassemic patients are prone to acquiring TTV infection and also its possibly potential role as a primary cause of post-transfusion hepatitis and increasing the severity of liver disease, we decide investigates the prevalence and clinical importance of TTV infection in multiply transfused thalassemia patients during the study period in Jahrom, southern of Iran.

MATERIALS AND METHODS

Study population

A cross-sectional study was carried out Coliz unit of Motahhari hospital related to the Jahrom University of Medical Science, Iran, during July from 2012 to December 2013. A total of 452 thalassemic patients were recruited for this study. The standard and primary screening tests, including human immunodeficiency virus (HIV), human T-cell leukemia (HTLV), and hepatitis B (HBV) and C (HCV) were performed in all the study participants and those with any positive result were excluded. Information related to demographic characteristics such as sex, age and splenectomy were collected. Informed consent was obtained from all participants and their parents if the patients were under 18 years of age. The study design was approved by the ethics committee of Jahrom University of Medical Sciences (JUMS).

Serology and Biochemical laboratory tests

Serum ALT and AST levels were measured using an automated analyzer and values higher than 50 and 40 IU/L, respectively, were considered to be abnormal. Anti-HCV, Anti-HIV and Anti-HTLV status was determined by a commercially available Enzyme-linked immunosorbent assay (ELISA) (DIA.PRO, Diagnostic Bioprobes Srl, Italy) according to the manufacturer instructions and hepatitis B surface antigen (HBsAg) was determined by ELISA (A DIA.PRO, Diagnostic Bioprobes Srl, Italy). HCV RNA was detected by RT-PCR as described previously [28].

Detection of TTV DNA by Semi-Nested-PCR method

About 3 mls of venous blood was collected from each subject. The blood was allowed to clot completely before centrifugation. The DNA genome of TTV was extracted from all serum samples the patients by DNP™ Kit (CinnaGen-Iran) according to manufacturer's protocol then were stored at "20 æ%C. Polymerase chain reaction (PCR) was also carried out that the specific primers against to TTV and semi-nested-PCR reaction protocol use as described in previous study[29].

Statistical tests

Data were entered and analyzed using SPSS software version 17.1. The Chi-square test or Fisher's exact test was used for categorical variables. Results were reported as the mean ± standard deviation (SD) for quantitative variables and percentages for qualitative variables. The significant relationships of molecular prevalence of TTV infection in thalassemic patients with

probable studied risk factors were analyzed by use of t-test. Statistical significance was at the P≤0.05 level.

RESULTS

The patient characterizes are shown in Table 1. The mean age of the patients was 6.7 ± 0.8 years (range 1-44 years). Females comprised the majority (59.1%) and most of the patients were between 2-4 years (51.1%). This distribu-tion reflects the current diversity in our population. Maximum serum levels ALT and AST of enrolled subjects were 277 IU/L and 143 IU/L respectively. Our finding shows that TTV-DNA was detected in 160 (35.4%) of patients. Table 1 shows the prevalence of TTV-DNA in the serum samples. The analysis of PCR products revealed a 271 bp fragment (Figure 1).

Table 2 indicated the demographic and some clinical data for the TTV-positive and TTV-negative thalassemia patients. Seventy-five percent of TTV patients were male and also 67.5% of TTV-positive thalassemia patients have not Splenectomy. There was not a significant difference in gender and splenectomy between the TTV-positive thalassemia patients and TTV-negative patients (P>0.05). The mean age of TTV-positive patients was higher than TTV-negative patients that this difference was statistically significant. The distribution of TTV infection based on age different groups in thalassemia patients in between 2-4 years age was higher than other groups that this different was statistically significant. In patients who were positive for TTV infection had higher levels of ALT and AST than TTV-negative patients. Also positive correlation was seen between ALT serum levels with the prevalence of TTV infection that was a significant correlation (r=+0.45 P <0.0001). Of course there are same

Table 1. Demographic and clinical data for the 452 thalassemia patients

Variables	N	%	Mean±SD
Age groups			6.7±0.8
≤2	92	20.4	
2-4	231	51.1	
4-6	32	7.1	
6-8	15	3.3	
e”8	82	18.1	
Gender			
Male	185	40.9	
Female	267	59.1	
Splenectomy			
Yes	169	37.9	
No	238	62.1	
ALT (IU/L)			34.5±3.3
AST (IU/L)			33.1±2.8
TTV-DNAPositive	160	35.4	
Negative	292	64.6	

Table 2. Comparison of the mean plasma levels of ALT, AST and demographic and clinical data between two groups patients with and without TTV infection

Demographic & baseline characteristics	TTV positive N (%)	TTV negative N (%)	Total N (%)	P-value
Gender				0.5
Male	62 (38.8%)	123 (78.7%)	185	
Female	98 (61.2%)	169 (79.3%)	267	
Age groups (Years)	3.6±0.38	1±1.2		0.0001
≤ 2	38 (23.8 %)	54 (58.9%)	92	
2-4	106 (66.2%)	125 (54.1%)	231	
4-6	6 (3.8%)	26 (81.2%)	32	
6-8	3 (1.9%)	12 (80%)	15	
≥8		7 (4.4%)	75 (91.5%)	82
Splenectomy				0.13
Yes	52 (32.5%)	117 (40.6%)	169	
No		108 (67.5%)	130 (44.5 %)	238
ALT(IU/L)	49.6±3.2	25.9±3		≤0.0001
AST(IU/L)	45.2±3.1	26.8±2.4		≤0.0001

correlation between AST serum levels with the prevalence of TTV infection that was statistically significant (r=+0.38 P <0.0001).

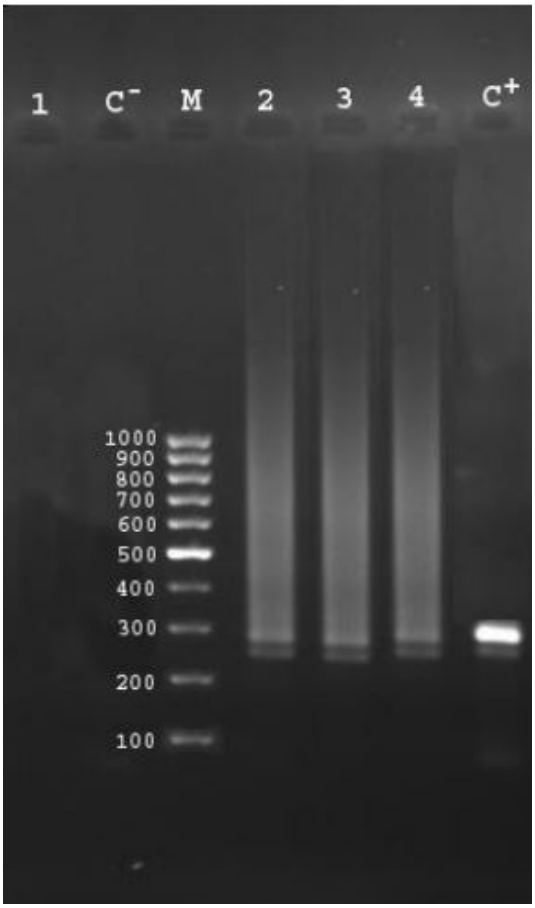

Fig. 1. Identification of TTV by semi nested PCR amplification. From left to right: 1= Negative sample, C- = Negative control, M= 100 bp DNA Ladder (Fermentas, Germany), 2, 3, 4= Positive samples, C+= Positive control respectively. The PCR products size was 271 bp.

DISCUSSION

The use of blood products by thalassemic patients may not always screened therefore infected they receive infectious transmissible agents. These patients may receive repeated transfusions in per month that this condition is able to directly transfer the risk of Transfusion-Transmitted Diseases (TTDs) such as microbial and viral infections[30]. Torque teno virus is observed in almost all the tissues and body fluids therefore the possibility of its transmission is high. Furthermore, the potential role and clinical importance of TTV infection in transfusion-dependent diseases such as hepatitis in thalassemic patients and other disease, has been important but yet to be established[17, 31]. Also subsequent studies raised evidences about the hypothesis that TTV infection leads to clinical manifestation in all infected patients[32].

In the present study, the prevalence of TTV–DNA was 35.4% polytransfused thalassemic Iranian patients. The prevalence of TTV varies among thalassemic patients from different regions that this difference in rate of infection is due to differences in diagnostic techniques, study sample size, and geographic distribution[33]. In previous surveys, 50.5% and 64.4% patients were TTV-DNA positive[1, 17]. Similarly, TTV infection was found to be highly prevalent 63.1%-73.4% in the β-thalassemic child and adult patients[32, 34]. This rate of TTV infection was lower than upper studies. These results with previous studies, suggest that TTV-DNA may has been transmitted in the recipients by blood and blood products. So, blood transfusion is one of the most way for the transmission of TTV in our study.on other hand, However, the fact that High level of TTV is also detected in healthy population with no history of blood transfusion suggests that it can be transmitted not only via blood and injection, but also by other ways[35]. On other hand this could also reflect the greater importance of the parenteral route for virus transmission that has been noted in our study.

In this study, gender and splenectomy did not differ significantly between the present study's TTV-positive and TTV-negative thalassemia patients but age in between two groups was significant. Indeed the prevalence of TTV infection in thalassemic patients based on other variables such as age is important because these patients need to frequent transfusion a long time. In this study the rates of TTV infection in 2-4 years was higher other groups. For example, the rate of TTV infection was increase with age such as 57% in individuals older than 50 years[36]. The infection may occur at a particular age group and the rates of prevalence of TTV differ among age whereas little children were infected by TTV in two age groups that the age of these patients was from 2-

40 years[37]. Other survey in Tehran and Ahwaz (a province in southern Iran) showed that there was a significant correlation between TTV infection and age[38]. Our findings indicate the infection rate of TTV increase in particular age that similar to other study[38, 39].

In our study, increased levels of ALT and AST were observed in a significant proportion of TTV+ patients and in negative-TTV patients. The reason for this is not clear but might be explained by liver disease associated with transfusion-related iron overload, the presence of undetectable TTV genotypes, or other blood-borne agents. This result was in accordant with previous studies (32, 40). On other hand, non significant raised liver enzymes in co-infection with TTV with hepatitis agents such as HCV in thalassemic patients does not alter the plasma level of biochemical markers when compared with TTV infection alone and also for TTV+HCV+ patients compared with those infected with HCV alone (40). Therefore evaluating the severity of liver disease solely by measurements of plasma transaminase levels is inadequate. Regardless of whether TTV is a cause of liver disease in thalassemic patients, pathogenic mechanisms of the virus need to be rapidly elucidated in order to develop new strategies to prevent transmission and for therapeutic intervention. On the basis of our study, it can be concluded that TTV appears to have a negligible role in increasing the severity of liver disease Thus; we plan to design studies in the future to assess the clinical importance and features of TTV in thalassemia patients.

In conclusion this study has demonstrated that TTV was high in thalasemic patients, which, strongly suggest that blood transfusion may be an important route for TTV transmission. Also, our data support the important role that the parentral route of transfusion plays in the spread of TTV infection. In addition to, our data indicated the effect of TTV infection on severity of liver using increased liver enzymes in between TTV+ patients and in negative-TTV patients.

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