

Transfusion Transmitted virus (TTV) infection in Thalassemic patients

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Abstract

TTV was first isolated from the serum of a Japanese patient with post transfusion hepatitis of unknown etiology in 1977. TTV has been visualized by electron microscopy and was found to be an unenveloped, small, spherical particle with a diameter of 30-32 nm, and is a member of family related to Circoviridae family. The exact role of TTV in the pathogenesis of liver disease is yet to be established. Our aim was to determine the prevalence of TTV in thalassemic patients in Ahwaz. Viral DNA was studied in 250 thalassemic patients. The results were compared with those of 250 blood donor controls. DNA was extracted from plasma and amplified by semi nested polymerase chain reaction with reported primer sets from a conserved region of the TTV genome. 57.2% (143/250) samples obtained from patients and 20% (54/250) of blood donors were positive for TTV-DNA detected by PCR. The difference in TTV prevalence between the two groups was statistically (χ^2) significant ($P= 0.0001$). The prevalence of TTV-DNA in Iranian thalassemic patients is high, which is the same as other countries.

Keywords: *Transfusion transmitted virus (TTV), Thalassemia, PCR, infection, Iran*

Introduction

The incidence of transfusion-associated hepatitis has been substantially reduced after the implementation of screening for antibodies to hepatitis C virus (HCV) and HBsAg in blood donors, but the etiology of some cases of acute or chronic hepatitis has not yet been elucidated. Hepatitis G virus (HGV), a member of the Flaviviridae family, was initially suggested as a causative agent of transfusion-associated non-A-C hepatitis has been hypothesized (1- 3). TTV was first isolated from the serum of a Japanese patient with post-transfusion hepatitis of unknown etiology in 1977 (4-6). TTV has been visualized by electron microscopy and was found to be a nonenveloped, small, spherical particle with a diameter of 30-32 nm, and is a member of family related to Circoviridae family

(7, 8). Some investigators have proposed that TTV belongs to a new virus family tentatively designated Circinoviridea (9). TTV has an extremely wide range of sequence divergence and divided to 5 large groups (G1-G5) and 29 genotypes (10, 11). TTV has been shown to temporally associated with elevated serum transaminase levels in some patients as hemophilia, thalassemia, aplastic anemia and hemodialysis after blood transfusion (12).

Nevertheless, chronic transfusion recipients, such as patients affected with homozygous β -thalassemia, still have high frequency of liver disease due to transfusion-related iron overload infection with blood borne agents, either known or undiscovered (13). Preliminary data from the United Kingdom and Japan indicate that TTV sequences are detectable in 25% to 45% of pa-

tients with fulminant or choronic hepatitis of unkown origin, in 27% to 68% of hemophiliacs, and in 1.9% to 12% of apparently healthy blood donors. However, more epidemiological and clinical information from both blood donors and recipients is required to establish if TTV is transfusion-transmissible and to clarify its role in the pathogenesis of non-A-C hepatitis. This study provides data on the prevalence of TTV in Iranian thalassemic patients and our aim was to determine the prevalence of TTV infection and to investigate relation of viremia with sex, age, number of blood receiving in Thalassemic patients reffered to Shafa Hospital in Ahwaz.

Materials and Methods

This work was a case-control study. We tested a group of thalassemic patients (n= 250) aged 1-32 yr who attended in Shafa hospital, Ahvaz, south of Iran. Five ml blood was taken from each patient and then centrifuged at 3500 g at room temprature. Plasma were separated in and transferred into sterile tubes.

Nucleic Acid Extraction Plasma sample was frozen at -70° C before starting nucleic acid extraction procedure. DNA was extracted by high pure viral nucleic acid kit (Roche Diagnostics High Pure Viral nucleic Germany) according manufacture procedure and DNA was stored in -70° C.

Determination of TTV-DNA by PCR TTV-DNA was determined by seminested PCR described by Okamoto et al. (11). In brief, the first round of PCR was preformed with NG059 primer (sense: 5'-ACA GAC AGA GGA GAA GGC AAC ATG-3') and NG063 (antisense: 5'-CTG GCA TTT TAC CAT TTC CAA AGT T-3') for 1 cycles (95° C, 9 min) and 30 cycles {94° C, 30s; 58° C 30s; 72° C, 45s; (additional 7 min 72° C for the last cycle)}. The second round PCR with NGo61 (5'-GGC AAC ATG TTA TGG ATA GAC TGG 3`antisense) for 25 cycles with the same conditions .The size of the first round PCR product was 286 bp, and that of the second round PCR was 271 bp. All assays

were carried out in an amplicon- free work area. Positive and negative controls were included in each PCR assay, and PCR products were analyzed in a 2% agarose gel electrophoresis with ethidium bromide staining and were evaluated.

Results

TTV-DNA was detected in the plasma of 250 thalassemic patients and 250 blood donors .As shown in table and Fig.1 of 250 patient plasma tested for TTV-DNA, 57.2% (143/250) were positive and 42.8% (107/250) were negative.

As shown in Table 2 of the 250 donor plasma tested for TTV-DNA, 20% (54/250) were positive and 80% (196/250) were negative. The difference in TTV prevalence between the two groups (Fig. 2) was statistically (χ^2) significant ($P= 0.000 1$).

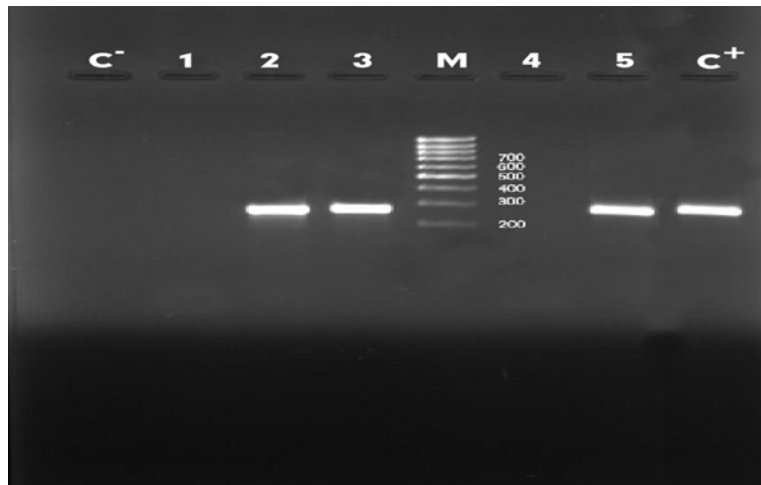
For further characterization we evaluated clinical background including mean age, sex, and transfusion history of TTV-PCR positive and negative patients. There is statistical differ between TTV-DNA positive and negative with age ($P= 0.0$) and transfusion history ($P= 0.04$), but was not differ between TTV-DNA positive and negative and sex.

Table 1: Frequency of TTV-DNA in the plasma of 250 transfusion-dependent β -thalasemic patients

TTV-PCR	Number	Percent
Positive	143	57.2
Negative	107	42.8
Total number	250	100

Table 2: Frequency of TTV-DNA in the plasma of 250 blood donors

TTV-PCR	Number	percent
Positive	54	20
Negative	196	80
Total number	250	100



C+= Positive control, C- = Negative control, 1= Negative patient, 2 & 3 & 4 & 5= Positive patient, M= Size marker

Fig. 1: Result of TTV-PCR in thalassemic patient plus Positive and negative control

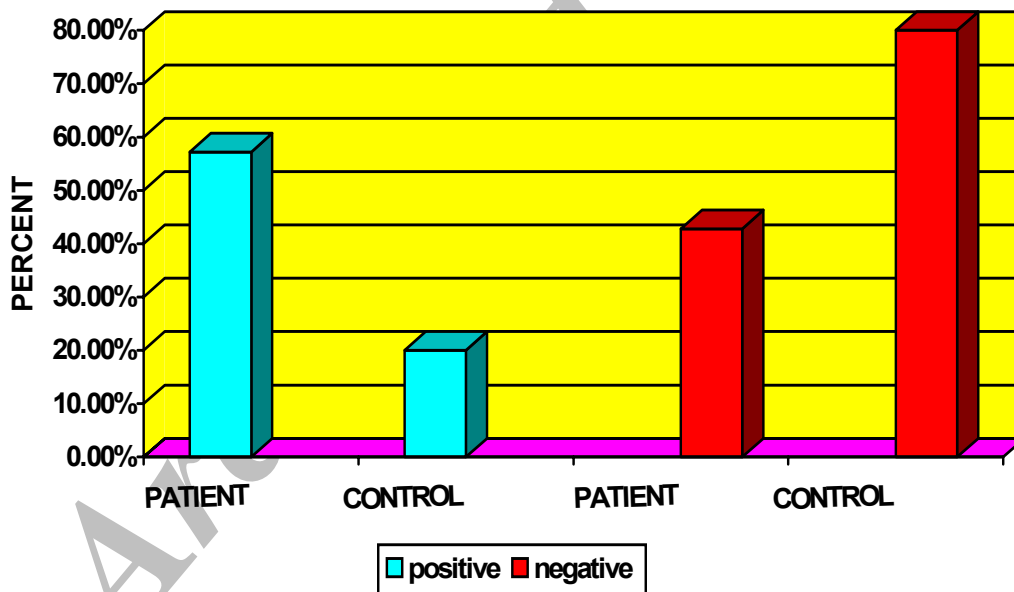


Fig. 2: Percentage of TTV in 2 groups of thalassemic patients and control

Discussion

TTV was initially identified as a transfusion-transmissible agent present in a large number of patients with acute and chronic hepatitis of non-A to G etiology (14). This virus was also reported in thalassemic and hemophilic patients in most of countries. Specific TTV sequence was detected in a vast majority of transfusion

dependent thalassemic patients in Italy, supporting the hypothesis that TTV is a transfusion-transmissible agent (15). For the patients evaluated in this study, the rate of TTV infection at the baseline evaluation was higher than (93.5%) that previously found for HCV and HGV in the same population of β -thalassemic patients (85% and 33%, respectively). Purpose of the present

investigation was to examine the prevalence of TTV in Iranian thalassemic patients and relation of viremia with sex, age, number of blood receiving in thalassemic patients referred to Shafa Hospital in Ahwaz. Cases included 250 β -thalassemic patients plus 250 blood donor as control. In our study the prevalence of TTV was significantly greater (57.2 %) in thalassemic patients than in blood donors (20%). Several studies, have shown that TTV is highly endemic in healthy control and recipient patients in word wide. This virus was reported in Turkish by Erensoy and co-workers in thalassemic patients. Sixty-one patients were TTV positive in this study (16). Similarly, TTV infection was found to be highly prevalent (73%) in the Italian β -thalassemic child by Kondili, et al (17) and it was also reported to be 69.1% in adult Italian β -thalassemic (18). These results with previous studies, suggest that TTV-DNA 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. However, prevalence of TTV in our control population is 20%, but other country has shown different prevalence with semi-nested methods and same primers. A prevalence study showed 10% in Uroupian population (19), 34% in Japanese (20), 10-62% in south American (21), 22% in Italian population (15), 18.5% in Hungary (22), and 1% in north American (20). Prevalence of TTV-DNA in our population is similar to result of Italian and Hungary, but was significantly higher than in Uroupian, North American and Japanese.

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 contaminated foods, amniotic fluid from all TTV-positive women, breast milk of the mothers, feces, bile, saliva, throat swabs, fecal-oral route and other tissues not examined recently (11).

Thus, TTV infection may possibly spread through routes other than overall parenteal.

At present studies, some clinical parameters such as, sex, age, history of transfusion was evaluated with TTV infection. The results, indicated significant correlation between TTV infection and age ($P<0.001$), and history of blood transfusion ($P< 0.004$), but no correlation was observed between TTV infection and sex.

In conclusion this study has demonstrated that TTV is higher in thalassemic patients than control which, strongly suggest that blood transfusion may be an important route for TTV transmission. Constrant, our data support the important role that the parentral route of transfusion plays in the spread of TTV infection.

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