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Major Article

Molecular characterization of Torque teno virus and SEN virus co-infection with HIV in patients from Southern Iran

Aliyar Pirouzi[1], *Mirzakhalil Bahmani*[1], *Mohammad Mehdi Feizabadi*[2] *and Rouhi Afkari*[1]

[1]. Cellular and Molecular Gerash Research Center, Shiraz University of Medical Sciences, Shiraz, Iran. [2]. Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

ABSTRACT

Introduction: Torque teno virus (TTV) and SEN virus are circular single-stranded DNA viruses that cause blood-borne infections. The SEN virus (SEN-V) was originally detected in the serum of an injection drug user infected with human immunodeficiency virus (HIV). Recently TTV was discovered as a potential causative agent of non-A-E hepatitis. The aim of this study was to investigate the prevalence of the SEN-V-D/H and TTV in HIV patients and healthy blood donors in Iran. **Methods:** One hundred and fifty HIV patients with a mean age of 50.46 ± 18.46 years and 150 healthy blood donors with a mean age of 48.16 ± 13.73 years were included in this study. TTV and SEN-V were detected by the PCR and were quantitatively assayed by competitive PCR (nested and semi-nested PCR). Restriction fragment length polymorphisms (RFLPs) were used to determine the heterogeneity of TTV. **Results:** TTV and SEN-V were detected 96 (64%) and 84 (56%) of 150 HIV patients respectively. These rates were 34% (n=51) and 37.33% (n=56) in healthy blood donors (significant, $p<0.05$). PCR detected SEN-V/TTV DNA from 32 of the healthy blood donors (21.33%), while 65 (43.33%) of HIV patients were positive for SEN-V/TTV DNA. Of 150 HIV patients, 32.66% and 23.33% were positive for SEN-V-H and SEN-V-D, respectively and 18.66% (n=28) were co-infected with SEN-V-D/H. **Conclusions:** The prevalence of SEN-VD/H and TTV is higher in HIV patients than in healthy blood donors in Southern Iran. Our results suggest that TTV and SEN-V might play a role in the development of liver disease in patients with immunodeficiency diseases.

Keywords: Torque teno virus. SEN virus. HIV. Genotype. Blood donors.

INTRODUCTION

Recently, a new virus, designated SEN virus (SEN-V), was isolated from serum of an human immunodeficiency virus (HIV)-positive patient who was an injection drug user[1]. Nine different SEN-V genotypes have been identified (A to I) which have at least 25% divergence in their nucleotide sequences[2]. The SEN-V-D and SEN-V-H genotypes are most frequently in patients with unknown hepatitis (non-A E hepatitis) but exhibit lower frequencies in the sera of healthy blood donors[3]. In addition Torque teno virus (TTV), also known as transfusion-transmitted virus was first identified in a Japanese patient in 1997[4,5]. Torque teno virus is a circular, single-stranded deoxyribonucleic acid (DNA) virus that chronically infects healthy individuals of all ages worldwide. Substantial data exists describing the prevalence and genetic heterogeneity of TTV in healthy populations and patients infected with HIV. The genome of SEN-V is similar to TTV and both are classified within the circovirus family[5]. Both SEN-V and TTV are single-stranded non-enveloped DNA viruses of 3,800 nucleotides[6,7]. Several studies have shown that in HIV-infected patients the prevalence of TTV is slightly higher compare to healthy blood donors[8]. Puig-Basagoiti et al. described a high TTV prevalence among a large HIV-infected group in Spain[9]. The TTV prevalence of 76% among HIV positive individual suggested that the presence of TTV is an independent factor determining patient survival[10].

The aims of this study were to determine the frequency of SEN-V/TTV among patients infected with HIV and health blood donors in Southern and to determine the frequency of the SEN-V-D and SEN-V-H genotypes.

METHODS

This study was performed in the Gerash Research Center affiliated with the Shiraz University of Medical Sciences in Southern Iran between October 2012 and July 2013. The study population consisted of 150 patients with HIV who were referred to the Gerash and Namazee Hospitals. In parallel, 150 blood samples donated by healthy individuals at Larestan Blood Transfusion Center between March 2010 and March 2012 were investigated. Five ml of blood was collected from all study participants (both HIV positive and healthy individuals), and sera were screened with anti-HIV antibody (Dia lab ®HIV Ab, Austria) in duplicate.

Serum samples from HIV patients with a variety of liver diseases including hepatitis C virus/hepatitis B virus (HCV/HBV)

Address to: Dr. Rouhi Afkari. Cellular and Molecular Gerash Research Center/ Shiraz University of Medical Sciences. Fars province, 7435144791 Gerash City, Shiraz, Iran.
Phone: 98 782 222-6633; **Fax:** 98 782 222-8104
e-mail: r.afkari77@yahoo.com; r.afkari@gmail.com
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co-infection and HIV alone were previously collected under the auspices of protocols approved by our Institutional Review Board. HIV-infected patients were matched against HIV-uninfected controls. Matching criteria included age, gender, history of transfusion and liver disease[6]. Blood samples were centrifuged and stored at -70°C within hours of collection. Total nucleic acids from 100µl of serum were isolated with the QIAamp Blood Kit (QIAGEN GmbH, Hilden, Germany), and the nucleic acids was deluted in 50µl of distilled water. Polymerase chain reaction (PCR) assays were performed in 25µl volumes containing 3µl of each sample, 1 × PCR buffer [10mM Tris-HCl (pH 9.0) 50mM KCl, 1mM $MgCl_2$, 0.01% gelatin, and 0.1% Triton X-100], 200µM of each triphosphates (dNTPs), and 100ng of each primer. Total DNA was amplified by semi-nested PCR using the primers NG059, NG061, and NG063[11].

The first round of PCR program consisted of 35 cycles of denaturation at 94°C for 30s, annealing at 60°C for 45s, and extension at 72°C for 45s, with the sense primer NG059 (5′-ACAGACAGAGGAGAAGGCAACATG-3′) and anti-sense primer NG06 (5′-CTGGCATTTTACCATTTCCAAAGTT-3′). The second round of PCR was performed with the sense primer NG061 (5′-GGCAACATGTTATGGATAGACTGG-3′) and the anti-sense primer NG063 for 25 cycles, under the same cycling conditions as used for the first round of PCR[11]. PCR was also used to detect SEN-V-D/H. DNA was amplified by nested PCR, with the forward primer AI-1F (5′-TWCYCMAAC GAC CAG CTA GAC CT-3′; W=A or T, Y=C or T, M=A or C) and the reverse primer AI-1R (5′-GTT TGT GGT GAG CAG AAC GGA-3′)for the first round for all the SEN-V genotypes[11]. The PCR conditions in the first round were: denaturation at 94°C for 45s, primer annealing at 56°C for 45s, and extension at 72°C for 45s, for 35 cycles followed by with a final extension step for 7min at 72°C. Second round PCR amplification was performed with specific primers.

Forward and reverse primers for SEN-V-D included D-1148F (5′-CTA AGC AGC CCT AAC ACTCATCCAG-3′) and D-1341R (5′-GCAGTTGACCGCAAAGTTACAAGA G-3′), while those for SEN-V-H included H-1020F (5′-TTT GGC TGC ACC TTC TGG TT-3′) and H-1138R (5′-AGA AAT GAT GGGTGAGTGTTAGGG-3′) (11).The amplified DNA products separated by agarose gel electrophoresis on 3% agarose gel, stained with ethidium bromide, and visualized under ultraviolet (UV) light.

Statistical analysis

Data were statistically analyzed using SPSS software version 15.0. The differences in SEN-V infection frequencies between HIV patients and healthy blood donors were examined.by *t*-test, Chi-square test, and one-way analysis of variance (ANOVA) statistical analysis. P values < 0.05 were considered statistically significant.

RESULTS

This study included 150 HIV patients with a mean age of 50.46 ± 18.46 years and 150 healthy blood donors with a mean age 48.16 ± 13.73 years. The HIV infection was most common in the age groups of 31-50 (58.06%) and 51-70 (42.11%) in men and women respectively. ANOVA results showed no significant association between age and gender of the patients under investigation (p=0.06). In addition, 86% and 64% of patients were men and women respectively. Statistical analysis, suggested a significant association between SEN-V infection and Liver diseases or history of transfusion in HIV patients in all cases (p=0.001). ANOVA testing demonstrated a significant association between history of transfusion and SEN-V-D and SEN-V-H (p=0.013: **Tables 1** and **2**).

The plasma sample of 150 HIV-1 patients and 150 blood donors were screened for TTV and SEN-V in PCR assays. TTV and SEN-V were detected 96 (64%) and 84 (56%) of 150 HIV patients respectively. In blood samples from healthy donors, the frequencies of TTV and SEN-V were 51 (34%) and 56 (37.33%) respectively (significant, p<0.05). Co SEN-V and TTV were detected in 32 healthy blood donors (21.33%) and in 43.33%. Among the 150 HIV patients, 32.66% and 23.33% were positive for SEN-V-H and SEN-V-D, respectively and 28 (18.66%) were co-infected with SEN-V-D/H. In this study, HIV-1 patients and control individuals were separated assay test results and human immunodeficiency virus-ribonucleic acid (HIV-RNA). In addition, TTV and SEN-V were quantitatively assayed by competitive PCR (nested and semi-nested PCR). The difference in TTV frequency between the 2 groups (healthy donors and patient group: **Tables 1, 2** and **3**) was statistically (χ^2) significant (p=0.0001). For further characterization, we evaluated clinical background including mean age, sex, and transfusion history in TTV-PCR positive and negative patients.

TABLE 1 - Frequency of SEN-virus in patients with HIV (n=150) as determined by PCR.

	SEN-V$^+$(n=84)				
Characterization	SEN-V (H^+)	SEN-V (D^+)	SEN-V (H^+/D^+)	SEN-V$^-$(n=66)	P value
Number(%)	49 (32.70)	35 (23.30)	28 (18.70)	66 (44)	—
Gender (M/F)	31/18	20/15	12/16	35/31	NS
Age	42.22 ± 18.22	43.69 ± 16.12	42.46 ± 18.40	38.90 ± 15.86	NS
History of transfusion M/F (%)	29/49 (59.18)	22/35 (62.85)	19/28 (67.85)	12/66 (36.36)	S
History of liver M/F (%)	32/49 (65.30)	30/35 (85.71)	22/28 (78.50)	24/66 (18.18)	S

HIV: human immunodeficiency virus; **PCR**: polymerase chain reaction; **SEN-V:** SEN virus; **M:** male; **F:** female **NS:** not significant; **S:** significant.

TABLE 2 - Frequency of Torque teno virus in patients with human immunodeficiency virus (n=150).

	HIV+			
Back ground characteristic	TTV+	TTV-	SEN-V +/TTV+	P value
Number (%)	96 (64.0)	54 (36.0)	65 (43.33)	—
Gender (M/F)	30/34	34/20	30/35	
NS				
Age (year)	41.90 ± 15.86	45.58 ± 16.14	39.46 ± 18.46	NS
History of transfusion n/N (%)	51/96 (53/12)	22/54 (40.74)	48/65 (73.85)	S
History of liver disease n/N (%)	59/96 (61.45)	28/54 (51.85)	52/65 (80.0)	S

HIV: human immunodeficiency virus**; TTV:** Torque teno vírus; **SEN-V:** SEN virus; **M**: male; **F**: female; **NS:** not significant; **S**: significant; **n**: number of patients; **N**: Number of total population.

TABLE 3 - Frequency of SEN-virus and Torque teno virus in voluntary blood donors (n=150).

	SEN^+(n=56)				
Background characteristic	SEN-V (D +)	SEN-V (H+)	TTV+ (n=51)	SEN-V+/TTV+ (N=32)	P value
Number n/N (%)	38/56 (67.86)	18/56 (32.14)	51/150 (34.0)	32/150 (21.33)	—
Gender (M/F)	21/17	12/6	21/30	19/13	NS
Age (year)	40.85 ± 12.36	35.21 ± 18.12	47.6 ±4 2.7	44.25 ± 1.36	NS
History of transfusion n/N (%)	18/38 (47.37)	4/18 (22.22)	19/51 (37.25)	15/32 (46.87)	S
History of liver disease n/N (%)	14/38 (36.84)	11/18 (16.11)	12/51 (23.53)	12/32 (37.50)	S

SEN-V: SEN virus; **TTV:** Torque teno virus; **M:** male; **F:** female; **NS**: not significant; **S**: significant. **n**: number of patients; **N**: Number of total population.

DISCUSSION

TTV was first detected in subjects with post-transfusion hepatitis and indicated as a possible etiological source of non A-non C hepatitis[12]. Although TTV DNA was present at high concentrations in liver tissue and in sera of patients with liver disease[13], several studies have reported a high endemicity of infection in subjects with no evidence of hepatitis[14]. Therefore, the role of TTV as a causative agent of liver disease is unclear. In this study, we evaluated the frequency of serum TTV DNA and their genotypes in relation to liver disease in HIV patients as well as in healthy control blood donors .The aim of this study was to investigate the prevalence of SEN-V-D/H and TTV co infection in HIV patients and healthy blood donors in southern Iran. Because of the therapeutic procedures that are frequently associated with bleeding and blood transfusions, HIV patients are at increased risk for infection with blood-borne viruses[7,15]. TTV and SEN-V were detected 34% and 37.33% of healthy blood donors respectively. The rates of TTV infection among blood donors are 1.9% in the United Kingdom[16], 3.2% in Germany[17],7.5-10% in the United States[18,19], 29.4% in Egypt[20], and 62% in Brazil[21]. These differences in frequency between countries could be due to the different geographical distribution of TTV infections, and the heterogeneity and variability of TTV isolates[11,16]. Variation could also arise due to differences in experimental methods used to determine TTV infection, such as the primers used, and the sensitivity of the PCR methods employed[8,22]. The primer used in our study were identical to that used in the aforementioned studies, suggesting that the discrepancies of TTV frequency between countries were not due to variation in the primer used.

In this study, we detected an overall frequency of TTV infection of 61.45%, which is higher than the previously reported frequency in patients with liver disease, in volunteers or commercial blood donors and in high-risk populations from western countries (1-13%)[16]. In contrast, our data are similar to those reported in patients with chronic hepatitis or cirrhosis in Japan, Taiwan and Thailand[17]. In this study, the proportion of SEN-V-D/H DNA was 18.66% (SEN-V-D 23.33% and SEN-V-H 32.66%) and that of TTV DNA was 64% among HIV patients. SEN-V DNA and TTV DNA were detected in 37.33% and 34% of healthy blood donors, respectively. SEN-V-D/H and TTV infections were found more frequently in HIV patients than in healthy blood donors. The high prevalence of these viruses in HIV patients may be due to contaminated equipment and blood products. In addition,patient histories of transfusion and liver diseases have more effects on SEN-V and TTV positivity.

The results of this study showed that the proportion of SEN-V and TTV in HIV patients is higher than in healthy blood donors, consistent with previous studies. The correlation between TTV infection and liver disease has been reported in Japan, the United States and the United Kingdom. TTV DNA was detected in 47% of Japanese[11] and 27% of American[23] patients with fulminant hepatitis and in 46% of Japanese and 21% of British[23] patients with chronic liver disease. The percentage of SEN-V infection in healthy blood donors in this study was 37.33% which is much lower than the rate reported from Isfahan (90.8% a central province in Iran)[24] and Japan (75%)[25]. The frequency of SEN-V-D, SEN-V (SEN-V-H or SEN-V-D) and co-infection (both SEN-V-D and SEN-V-H) viremia has been reported to be significantly higher among thalassemic patients than healthy individuals. Results fromprevious studies have shown that both SEN-V and TTV are associated with elevated serum transaminase in patients with hemophillia, thalassemia, aplastic anemia and hemodialysis after blood transfusion[26]. Nevertheless, chronic transfusion recipients, such as patients affected with homozygous beta-thalassemia, have high frequencies of liver disease[27] and levels in liver tissue are equal to or 10-100 times higher than those in the serum, suggesting that this viruses replicates in the liver[28]. These results suggest that TTV-DNA has been transmitted to recipients of blood and blood products. Therefore, blood transfusions are one of the most efficient way for TTV to be the transmitted.

Some clinical parameters such as, sex, age, history of liver disease and history of transfusion have been evaluated with TTV infection. The results of these studies-have indicated a significant association between TTV infection and history of liver disease and history of blood transfusion, but no association was observed between TTV infection , sex and age. In addition the proportion of TTV infection caused by blood transfusion also differs depending on the country or area. Using the PCR, epidemiological studies have indicated a worldwide distribution of TTV, with prevalence in the general population ranging from 12 to 19% in Japan[5].

In conclusion this study has demonstrated that TTV and SEN-V infection rates are higher in HIV patients than in healthy the blood donors suggesting that blood transfusion may be an important route for TTV and SEN-V transmission. However, the fact TTV is also detected in healthy population with no history of blood transfusion suggests that it can be transmitted by means other than blood and injection potential mechanisms of transmission worthy of consideration include contaminated foods, amniotic fluid from TTV-positive women, breast milk, feces, bile, saliva, throat swabs, fecal-oral route and other tissues not examined recently[29]. This issue need sadditional investigation to identify other factors that can influence the proportion of these viruses in HIV patients.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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