

Full Length Research Paper

Study the prevalence of torque teno (TT) virus infection in patients with hematological malignancies

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Torque Teno (TT) virus infection may increase the risk of different types of leukemia. In this study the infective markers of TT virus, hepatitis B virus (HBV), and hepatitis C virus (HCV) were evaluated in patients with different types of leukemia in comparison with controls. In this cross sectional study Ethylenediaminetetraacetic acid (EDTA)-treated blood samples were collected from 163 patients with leukemia and 100 control between years: 2007-2011. Molecular prevalence of TT virus infection was evaluated in studied populations using in-house semi-nested polymerase chain reaction (PCR) protocol. HBsAg and HCVAb were analyzed in studied groups by third generation ELISA kits according to manufacturer's instructions. The genomic DNA of TT virus was found in 29 of 163 (17.8%) of leukemia patients and 2 of 100 (2%) of controls. HBsAg was diagnosed in 21 of 147 (14.3%) patients with leukemia and in 2 of 100 (2%) controls. HCVAb was diagnosed in 4 of 147 (2.7%) patients with leukemia and in none of controls. The co-infection of TT virus with HBV and with HCV was found in 4 of 147 (2.7%) and in 1 of 147 (0.7%) of patients with leukemia, respectively. Identification of high prevalence of TT virus infection in patients with leukemia compared with controls emphasize on possible association between TT virus infections with leukemia. Therefore evaluation of the prevalence of TT viral infection in larger groups of patients with long lasting follow up is suggested.

Key words: Torque Teno (TT) virus, hepatitis B virus (HBV), hepatitis C virus (HCV), leukemia, hematological malignancies.

INTRODUCTION

Human viruses may participate in initiation or complication of leukemia outcomes (Paul et al., 1999; Locasciulli et al., 1997). The knowledge about the relationships between viral infections and pathogenesis of hematological malignancies are certainly limited (Paul et al., 1999; Locasciulli et al., 1997). Direct contact, using invasive procedure and immunosuppressive chemotherapy increase the risk of viral infections

especially TT virus in patients with leukemia (De Rosa et al., 1997; Silvestri et al., 1996; Allander et al., 1995; Dibenedetto et al., 1996). The viremia of TT virus was found in volunteer blood donor for transmission through transfusion of blood components and therefore prevention of TT virus infection need précis monitoring of blood donation using specific and sensitive molecular procedures. Also the lack of significant liver damage was found with TT virus infection. These phenomena hypothesis the association of the TT virus replication with hematology disorders like leukemia (Charlton et al., 1998; Naoumov et al., 1998; Simmonds et al., 1998). TT virus infection spread in patients with leukemia silently. TT

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Table 1. Frequencies and average values of Indices in patients with leukemia and control.

Indices population of study	Patients with leukemia (n=163)	Controls (n=100)
Mean Age \pm SD (years)	45.0 \pm 25.1	36.5 \pm 12.8
Gender		
Male n (%)	100 (61.3)	39 (39)
Female n (%)	63 (38.7)	61 (61)
TT Virus-DNA n (%)	29 (17.8)	2 (2)
HBs-Ag n (%)	21 (14.3)	2 (2)
HCV-Ab n (%)	4 (2.7)	NoD

TT virus=Torque Teno virus; HBs-Ag=hepatitis B surface antigen; HCV-Ab =hepatitis C virus antibody; ND=Not Detected.

virus can infect haemopoietic progenitor or bone marrow cells and also whole blood leukocytes with measurable amounts of DNA, mRNA and proteins of TT virus (Okamoto et al., 2000 a, b, 2001; Maggi et al., 2001; Mariscal et al., 2001, 2002; Blanchard et al., 2001; Chu et al., 2011). Monitoring of the TT virus replication in peripheral blood leukocytes is consistent with the hypothesis that TT virus needs a cellular S phase (Hino and Miyata, 2007; Mariscal et al., 2002). A contrary was excited between earlier studies on the possible role of TT virus infection with cancer including leukemia. In a sari of studies enrolled in patients with various hematological cancers the elevated titer of TT virus genomic DNA was confirmed in patients with leukemia in comparing with healthy controls (Zhong et al., 2002; Chu et al., 2011). Measurement of the genomic TT virus DNA in peripheral blood may have the potential to be used as a biomarker in screening for hematological cancers. However, cannot exclude the possibility that the high titer of TT virus genome DNA in patients with leukemia may relate to the impairment of the cellular immune responses rather than having any specific relation to cancer (Maggi et al., 2010). On the other hand, in other studies disconnection of TT virus infection with hematological cancers was presented especially in patients with leukemia and lymphoma (Shiramizu et al., 2002; Cacoub et al., 2003). Additionally, the TT virus DNA was not detectable in blood leukocytes from patients with leukemia (Chu et al., 2011). Thus TT virus infection spread in these patients silently (Shimamura, 2009). Therefore in this study the prevalence of TT virus infection was evaluated in patients with hematological malignancy especially with leukemia.

MATERIALS AND METHODS

Patients and samples

In a cross sectional study, EDTA treated blood samples were collected from 163 patients with leukemia and 100 healthy control persons clinically and laboratory rule out any hematological abnormalities and malignancies. These two studied groups were both admitted in Hematology-Oncology Unit of Namazi Hospital affiliated to Shiraz University of Medical Sciences, Shiraz, Iran,

between years: 2009-2011. The ethical issue of this university was mentioned and approved for both leukemia and controls. The plasma of collected blood samples were isolated and preserved in -70°C till laboratory tests performed. Also some possible risk factors of leukemia and TT virus infection were statistically analyzed for studied patients with leukemia.

Molecular analysis of TT virus infection

DNA extraction protocol

The genomic DNA of TT virus was extracted from EDTA-treated blood samples collected from patients with leukemia and controls by DNP kit (CinnaGen-Iran) according to manufacturer instruction.

Semi nested PCR protocol

The presentation of TT virus genomic DNA was analyzed using in-house semi nested-PCR protocol. The primer pair sequences used in the first simple PCR step is: NG059 (5'-ACA GAC AGA GGA GAA GGC AAC ATG-3') and NG063 (5'-CTG GCA TTT TAC CAT TTC CAA AGT T-3') that amplifying a 286 bp fragment of TT virus DNA located in the N22 on open reading frame 1 (ORF1), (Table 1). In the simple PCR step, the total volume of PCR mix containing: 5 μ l of template DNA, 10 pmol/ μ l of primers, 0.25 mMol of dNTPs, 1U of Taq DNA polymerase, Tris-HCL 10 mM, KCl 30 mM, 1.5 mMol of MgCl₂, and 13 μ l of distilled water. The primer pair sequences used in the second semi nested PCR step is: NG061 (5'-GGC AAC ATG TTA TGG ATA GAC TGG-3') and NG063 (5'-CTG GCA TTT TAC CAT TTC CAA AGT T-3') that amplifying a 271 bp fragment of TT virus DNA located in the N22 on ORF1 (Table 1). The 5 μ l of simple PCR product was used in second round of semi nested-PCR step with the same condition as simple PCR mix. The total volume per reaction in the two rounds of simple and nested PCR steps was 20 μ l. The thermocycling condition of simple PCR step was initiated with a first round at 94°C for 10 min followed by a second round of 35 cycles at 94°C for 30 s, 60°C for 45 s, and 72°C for 45 s, and finalized with extension at 72°C for 7 min. The semi nested PCR protocol was initiated with a first round at 94°C for 10 min followed by a second round of 30 cycles at 94°C for 30 s, 59°C for 45 s, and 72°C for 45 s, and finalized with extension at 72°C for 7 min.

Serological analysis of HBV and HCV infections

The presence of HBsAg and HCVAb were evaluated in both patients with leukemia and controls using third generation ELISA kits (DIAPRO-Spain) according to manufacturer's instructions.

Table 2. Viral Hepatitis Infections and Types of leukemia.

Types of leukemia viral Hepatitis infections	ALL N (%)	AML N (%)	CLL N (%)	CML N (%)	HCL N (%)	Undifferentiated
TT Virus	10 (16.3)	16 (9.3)	ND	3 (1.8)	ND	ND
HBV	8 (5.4)	11 (7.5)	1(0.7)	ND	1 (0.7)	ND
HCV	2 (1.4)	2 (1.4)	ND	ND	ND	ND

TT Virus=Torque Teno virus; HBV=hepatitis B virus; HCV=hepatitis C virus; ALL= Acute lymphocytic leukemia; AML= Acute myelogenous leukemia; CLL= Chronic lymphocytic leukemia; CML= Chronic myelogenous leukemia; HCL= Hairy cell leukemia; ND=Not Detected.

The 300 µl plasma need for evaluation of the HBsAg and HCVAb. Therefore some limitations occurred for volume of the 16 plasma samples collected from studied patients with leukemia, the presence of HBsAg and HCVAb were analyzed in 147 patients with leukemia.

Statistics

Significant differences of the molecular prevalence of TT virus infection between patients and controls were analyzed using parametric and non parametric logistic regression methods with SPSS for Windows (version 12, Chicago, IL, USA). These statistical methods including: Descriptive analysis (Frequencies and Cross tabs). Parametric and non parametric tests including: chi-square, two tailed and fisher's exact test. A level of $P \leq 0.05$ was accepted as statistically significant.

RESULTS

The 100 of 163 (61.3%) of leukemia patients were male and 63 of 163 (38.7%) of them were female with mean age of $45 \pm SD=25.15$ years old. The 39 of 100 (39%) of controls were male and 61(61%) of them were female with mean age of $36.5 \pm SD=12.88$ years old (Table 1). The mean of the age for males and females in both studied groups are observed the mean age presented totally and separately for patients with leukemia and controls. The most prevalent age group in patients with leukemia (46 of 162; 28.4%) and controls (43 of 100; 43%) were 30-50 years old. The prevalence of different types of leukemia in studied patients were as follow: 50 of 163 (30.7%) patients with acute lymphocytic leukemia (ALL), 12 of 163 (7.4%) patients with chronic lymphocytic leukemia, (CLL), 86 of 163 (52.8%) patients with acute myelogenous leukemia (AML), 7 of 163 (4.3%) patients with chronic myelogenous leukemia, (CML), 2 of 163 (1.2%) patients with hairy cell leukemia (HCL), and 6 of 163 (3.7%) patients with other undifferentiated types of leukemia. Distribution of viral hepatitis infections in different types of leukemia are presented in Table 2.

Molecular presentation of TT virus infection

TT virus genomic DNA was found in 29 of 163 (17.8%) of leukemia patients and 134 of 163 (82.2%) of them was

not infected with TT virus DNA. The viremia of TT virus was detected in 2 of 100 (2%) of controls and 98 of 100 (98%) of studied controls did not shown TT virus DNA in their plasma samples. Significant difference was found in the prevalence of TT virus genomic DNA between patients with leukemia and controls ($P=0.0001$; $OR=.094$; $95\%CI=0.022-0.405$), (Table 3).

Antigenic and serologic presentation of HBV and HCV infections

The prevalence of HBsAg and HCVAb in patients with leukemia and controls and also significant differences between them were presented in Table 2. The presence of HBsAg and HCVAb were analyzed in 147 and 100 plasma samples of patients with leukemia and controls, respectively.

HBsAg was diagnosed in 21 of 147 (14.3%) and was not found in 126 of 147 (85.7%) of patients with leukemia. HBsAg was diagnosed in 2 of 100 (2%) and was not found in 98 of 100 (98%) of controls. Significant difference was found in the prevalence of HBsAg between two studied populations ($P=0.001$; $OR=0.122$; $95\%CI=0.028-0.535$), (Table 3). HCVAb was diagnosed in 4 of 147 (2.7%) and was not found in 143 of 147 (97.3%) of patients with leukemia. HCVAb was not found in any of controls. Significant difference was not found in the prevalence of HCVAb between two studied groups (Table 3).

Multiple infections of HBV, HCV and HDV

The co-infection of TT virus with HBV was found in 4 of 147 (2.7%) of patients with leukemia. TT virus and HCV co-infection was diagnosed in 1 of 147 (0.7%) of patients with leukemia. Also the co-infection of TT virus with HBV and also with HCV was not found in any studied controls.

Risk factors of leukemia and viral hepatitis infections

Significant relationships was found between gender with TT virus infection ($P=0.04$). Significant associations were not found between other risk factor with infectivity of TT

Table 3. Prevalence of TT virus, HBV and HCV infective markers in patients with leukemia and controls.

Viral markers	Patients with leukemia No. (%)	Controls No. (%)	P value	OR	95% CI
TT virus					
-Ve	134 (82.2)	98 (98)	0.0001	0.094	0.022-0.405
+Ve	29 (17.8)	2 (2)			
HBs-Ag					
-Ve	126(85.7)	98 (98)	0.001	0.122	0.028-0.535
+Ve	21(14.3)	2 (2)			
HCV-Ab					
-Ve	143 (97.3)	100 (100)	0.097	0.973	0.947-0.999
+Ve	4 (2.7)	0 (0)			

NHL=Non-Hodgkin's lymphoma; TT Virus=Torque Teno virus; HBs-Ag=hepatitis B surface antigen; HCV-Ab =hepatitis C virus antibody; +Ve: Positive;-Ve: Negative; NV: Not Valid.

virus, HBV, and HCV in patients with leukemia and in controls.

DISCUSSION

The pathogenesis of TT virus is firstly related to idiopathic hepatitis for related diagnosis with increasing in liver enzymes (Leosco et al., 2002). But shortly after presentation of this hypothesis a controversy raised about etiology of TT virus infection in liver diseases (Paul et al., 1999; Locasciulli et al., 1997; Mirzaee et al., 2012). Earlier studies reported variable prevalence of TT virus infection in patients with leukemia, in association with a different spectrum of liver involvement, ranging from minimal liver enzyme elevation to the life threatening hepatic failure (Zhong et al., 2002). Most of leukemia patients shown persistent elevation in transaminase levels with no significant liver dysfunction (Locasciulli et al., 1997; Mirzaee et al., 2012). Therefore, after diagnosis of TT virus DNA in the serum of 66 of 499 (13.4%) Iranian volunteer blood donors with TT viremia in comparing with uninfected population (Azinfar et al., 2012), in this study the prevalence of TT virus infection was evaluated in patients with hematological malignancy especially with leukemia. In this study TT virus infection was found in 17.8 vs. 2% of patients with leukemia and controls. Significant difference was found in the prevalence of TT virus DNA between patients with leukemia and controls ($P=0.0001$).

In this report at least one of different molecular and immunological markers of TT virus, HBV, and HCV was found in 17.8%, 22 of 163 (22%), and 1 of 163 (1%) patients with leukemia. Co-infection of TT virus with HBV and HCV was diagnosed in 16 of 100 (16%) of patients with leukemia. Similarly, in other studies the importance of TT virus infection in patients with cancers was emphasized: In a study the higher titer of TT virus

genomic DNA was found in the peripheral blood mononuclear cells of patients with cancer than healthy controls (Zhong et al., 2002). In other study TT virus infection was found with higher prevalence in multi-transfused pediatric patients with malignancies and hematological disorders. The genomic DNA of TT virus was diagnosed in 38 of 75 patients (51%). Co- infection of TT virus with HCV and with HGV was found in 6 and 12 patients, respectively (Maeda et al., 2002; Garbuglia et al., 2003). Another report hypothesized that maternal infection with TT virus leads to increased risk of childhood leukemia or lymphoma (Zur Hausen and Villiers , 2005).

Controversy to these reports, other studies emphasized on unimportant role of TT virus infection in pathogenesis and outcome of leukemia. In a study TT virus DNA was not detectable in peripheral blood mononuclear cells of patients with CLL. Thus, the presence of TT virus genomic DNA in plasma and in peripheral blood mononuclear cells was not correlate with CLL (Chu et al., 2011). Also in other study TT virus was not diagnosed in pediatric patients with ALL.

In this study 79 peripheral blood mononuclear cell specimens were negative for TT virus infection (Shiramizu et al., 2002; Maeda et al., 2002). Finally, diagnosis of significant higher prevalence of single and co-infection of TT virus with HBV and HCV in patients with leukemia in comparing with controls proposed a strong association between TT virus infections with leukemia outcomes. These results suggest that the previous immunosuppression may promote more rapid TT virus replication or impaired host viral clearance. Therefore, careful efforts should be established in evaluation of the prevalence of TT virus infection in larger group of patients with leukemia with long lasting follow up and also recommend the introducing of diagnostic and controlling measures of TT virus infection in these patients.

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