

# Study of the Associations Between TT Virus Single and Mixed Infections With Leukemia

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**Background:** The pathogenic association of Transfusion Transmitted Virus or Torque teno Virus (TT Virus) single and mixed infections with leukemia was under evaluation in these years but confront with controversies. This hypothesis is based on the higher prevalence of TT Virus infection in patients with leukemia compared with controls.

**Objectives:** The aim of this study was to determine the frequency of TT Virus, Cytomegalovirus (CMV), Hepatitis B Virus (HBV), and Hepatitis C Virus (HCV) infections in patients with leukemia and healthy controls.

**Patients and Methods:** In this cross sectional study, 95 patients with leukemia and 100 healthy controls who were admitted to the Namazi Hospital affiliated to the Shiraz University of Medical Sciences, Shiraz, Iran, were enrolled between years 2012 and 2013. Blood samples treated with EDTA were collected from each patient with leukemia and controls. The existence of TT Virus infection was analyzed using the semi-nested PCR method. The immunological prevalence of HBV and HCV infections were evaluated using HBs-Ag and HCV-Ab ELISA based protocols, respectively. Active CMV infection was also evaluated using an immunofluorescence method. Also risk factors of leukemia and viral infections were statistically analyzed in patients with leukemia.

**Results:** The TT Virus infection was significantly found in 40 of 95 (42.1%) and 12 of 100 (12%) patients with leukemia and controls, respectively. The HBs-Ag and HCV-Ab were detected in 27 of 95 (28.4%) and 18 of 69 (26.1%) patients with leukemia but were not found in the controls. Active CMV infection was also found in 11 of 69 (16%) patients and none of the controls. Significant co-infection of TT Virus was found with HBV (15 of 40; 37.5%), HCV (14 of 40; 35%) and CMV (7 of 40; 17.5%) in patients with leukemia.

**Conclusions:** Confirmation of the significantly higher frequency of TT Virus, HBV, HCV and CMV single infection and their co-infection in patients with leukemia compared with healthy controls, emphasizes the determinative role of TT Virus pathogenesis in clinical outcomes observed in patients with leukemia, which requires extensive evaluation by further studies.

**Keywords:** Cytomegalovirus; Hepatitis B Virus; Hepatitis C Virus; Torque teno Virus

## 1. Background

Since the discovery of Transfusion Transmitted Virus or Torque teno Virus (TT Virus), in 1997, as a non-enveloped, single-stranded, circular DNA Virus of 3.8 kilobases (kb) belonging to the genus Anellovirus, its biological nature and defined link to human disease has been under evaluation with much controversy (1-3). The TT Virus is Transmitted parenterally, through blood and blood products and also via fecal-oral route (4, 5). The TT Virus infection is common in the normal population and has a worldwide distribution (6-10). This Virus has been found in multiple organs including: liver, kidneys, prostate, mammary glands, brain, bone hematopoietic cells, peripheral blood mononuclear cells (PBMCs), and polymorphonuclear cells (PMNs) (8, 11). Furthermore, TT Virus infection has been found in 1 - 12% of blood donors reported from different geographical regions. This viral infection was also

detected in 18% of patients exposed to blood products and in 4% of patients without parenteral risk factors (12).

However, a higher prevalence of TT Virus infection has been reported in high-risk patients including: 68% of patients with hemophilia, 46% of patients on hemodialysis, 40% of intravenous drug abusers, and 17.8% of patients with hematological malignancies (12, 13). Among these high-risk hematopoietic disorders, associations may exist between TT Virus infection and pathogenesis of different acute and chronic hematologic malignancies (2, 13, 14). This hypothesis is based on diagnosis of higher elevated titer of TT Virus genomic DNA in PBMCs of patients with cancers compared with controls (15, 16). On the other hand, mixed infection of TT Virus with human Viruses may cause more clinical complications in patients with of leukemia (13).

Information about single and co-infection of other blood born Viruses with TT Virus is limited (17, 18). Earlier reports have emphasized on mixed infection of TT Virus, with reactivation of Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) (19-31). The increasing risk of these blood born hepatitis Viruses were found in leukemia patients (32). Occult HBV infected carriers with chronic lymphocytic leukemia are at higher risk of chemotherapy-induced HBV reactivation (33). Immunosuppressive-related HBV reactivation is a problem in chronic carriers with leukemia (33, 34). Herpes Viruses like Cytomegalovirus (CMV), due to their worldwide distribution and frequent reactivations with periods of viremia, have also emerged in patients with leukemia, especially chronic lymphocytic leukemia as a result of receiving potent immunosuppressive therapies (35, 36).

## 2. Objectives

Based on these reports, the prevalence of TT Virus, CMV, HBV and HCV infections were evaluated in patients with leukemia.

## 3. Patients and Methods

### 3.1. Patients and samples

A total of 95 patients with leukemia and 100 healthy controls who were admitted to Namazi Hospital affiliated to Shiraz University of Medical Sciences, Shiraz, Iran, were enrolled in this study, conducted during years 2012 and 2013. Healthy controls were selected from people without any hematological abnormalities and malignancies as indicated by clinical and laboratory findings. Blood samples were collected from patients with leukemia and controls and stored in EDTA at the sample bank of the Transplant Research Center. All studied patients with leukemia transfused by blood and blood products need in treatment protocols. Also risk factors of leukemia and viral infections were statistically analyzed in patients with leukemia. The history of TT Virus infection was analyzed using a PCR based molecular method. The history of the immunologic prevalence of HBV and HCV infections were evaluated using HBs-Ag and HCV-Ab ELISA based protocols, respectively. Active CMV infection was also evaluated using an immunofluorescence-based method.

### 3.2. Transfusion Transmitted Virus Polymerase Chain Reaction Protocol

The genomic DNA of TT Virus was extracted from plasma of EDTA-treated blood samples collected from patients with leukemia and controls by the DNP kit (CinnaGen-Tehran-Iran) according to manufacturer's instructions. An in-house semi nested-PCR protocol was used to detect the TT Virus genomic DNA in the studied population. The primer pair sequences used in simple and semi-nested PCR steps were NG059, NG061 and NG063 (CinnaGen-

Tehran-Iran) that amplified the 286 bp and 271 bp fragments of the N22 open reading frame 1 (ORF1) of TT Virus genome, respected to length of amplified products. The details of simple and semi-nested PCR mix and thermocycling conditions were as previously described (13).

### 3.3. Immunologic Analysis of Hepatitis B Virus and Hepatitis C Virus Infections

The HBV (HBs-Ag) and HCV (HCV-Ab) immunological markers were evaluated in patients with leukemia and controls using a third generation ELISA kits (DIAPRO-Italy) according to the manufacturer's instructions. A total of 1 mL of plasma sample was needed for evaluation of the HBs-Ag and HCV-Ab in patients and controls. Some limitations occurred due to the available volume of 30 of the plasma samples collected from patients with leukemia and the presence of HCV-Ab was analyzed in 69 patients with leukemia.

### 3.4. Cytomegalovirus Antigenemia Method

Cytomegalovirus active infection was evaluated in EDTA-treated whole blood samples collected from kidney transplant recipients and donor's pre and post-transplantation by the CMV antigenemia method using the CMV Brite Turbo kit (IQ Products, Groningen, Netherlands) according to the manufactures' instruction, as previously described (37).

### 3.5. Statistics

Significant differences between the molecular prevalence of TT Virus, HBV, HCV and CMV infections in patients with leukemia and controls were analyzed using the SPSS software for Windows (version 16, Chicago, IL, USA). These statistical methods included descriptive analysis and parametric and non-parametric tests. P values of  $\leq 0.05$  were considered statistically significant.

## 4. Results

Sixty-three of 95 (66.3%) patients with leukemia were males and the remainders were females (32 of 95; 33.7%). The male/female ratio was 1.9 (63/32) for the studied patients. The most frequent blood group was B- in patients with leukemia. The age range was 1 - 58 years with a mean of  $19.95 \pm 16.26$  years in patients with leukemia. Thirty-nine of 100 (39%) controls were male and the remainders were female (61 of 100; 61%). The age range was 17 - 68 years with a mean of  $36.5 \pm SD = 12.88$  years. The female/male ratio was 1.56 (61/39) for the studied patients.

### 4.1. Transfusion Transmitted Virus Infection in Patients with Leukemia and Controls

Infection by TT Virus was found in 40 of 95 (42.1%) patients with leukemia, where 29 of 40 (72.5%) were male and 11 of 40 (24.5%) female. The genomic DNA of TT Virus

**Table 1.** Associations Between Transfusion Transmitted Virus, Hepatitis C Virus, Hepatitis B Virus and Cytomegalovirus Infections, and Risk Factors<sup>a</sup>

Viral Infections	Age	Gender	Blood-Group	Underlying Diseases	Chemotherapy
TT Virus	0.007	0.19	0.002	0.19	0.003
CMV	0.23	0.45	0.45	0.04	0.05
HCV	0.30	0.28	0.33	0.15	0.12
HBV	0.01	0.22	0.23	0.001	0.04

<sup>a</sup> Abbreviations: TT Virus, Transfusion Transmitted Virus; CMV, Cytomegalovirus; HCV, Hepatitis C Virus; HBV, Hepatitis B Virus.

was found in 12 of 100 (12%) controls. Ten of these 12 cases (83.3%) were males and two (16.7%) were females. Significant associations were found between age, diet, and blood grouping with TT Virus infection in patients with leukemia ( $P = 0.007$ ,  $P = 0.003$  and  $P = 0.002$ , respectively) (Table 1). However, no significant association was found between the studied risk factors and TT viral infection in the controls.

#### 4.2. Hepatitis B Virus Infection in Patients with Leukemia and Controls

The HBsAg was detected in 27 of 95 (28.4%) patients with leukemia, where 20 of 27 (74.1%) were males and remainders (7 of 27; 25.9%) were female. However, HBV infection was not found in any of the controls. A significant difference was found in presentation of HBV infection between patients and controls ( $P = 0.05$ ). On the other hand, significant associations were found between age and diet with HBV infection in patients with leukemia ( $P = 0.001$  and  $P = 0.04$ , respectively), (Table 1). Significant co-infection of TT Virus with HBV was found in 15 of 40 (37.5%) patients with leukemia ( $P = 0.03$ ), (Table 2).

**Table 2.** Hepatitis C Virus, Hepatitis B Virus and Cytomegalovirus Co-Infections With Transfusion Transmitted Virus in Patients With Leukemia<sup>a</sup>

TT Virus Viruses	TT Virus <sup>b</sup>		P Value
	Positive	Negative	
HBV	15 of 40 (37.5)	12 of 55 (21.8)	0.07
HCV	14 of 40 (35.0)	4 of 55 (7.2)	0.003
CMV	7 of 40 (17.5)	4 of 55 (7.2)	0.20

<sup>a</sup> Abbreviations: TT Virus, Transfusion Transmitted Virus; CMV, Cytomegalovirus; HCV, Hepatitis C Virus; HBV, Hepatitis B Virus.

<sup>b</sup> Data are presented as No. (%).

#### 4.3. Hepatitis C Virus Infection in Patients with Leukemia and Controls

The HCV-Ab was detected in 18 of 69 (26.1%) patients with leukemia where 15 of 18 (83.3%) were male and remainders (3 of 18; 16.7%) were female. However, HCV-Ab was not found in any of the controls. A significant difference was found in the history of HCV infection between patients with leukemia and controls ( $P = 0.05$ ). No significant as-

sociation was found between HCV infection and any of the risk factors in patients with leukemia (Table 1). Significant co-infection of TT Virus with HCV was found in 14 of 40 (35%) patients with leukemia (Table 2).

#### 4.4. Active Cytomegalovirus Infection in Patients with Leukemia and Controls

Active CMV infection was found in 11 of 69 (16%) patients with leukemia, where 9 of 11 (81.8%) were male and remainders were female (2 of 11; 18.2%). However, PP65 antigen positive polymorph nuclear cells were not found in any of the controls. A significant difference was found in the presentation of active CMV infection between patients with leukemia and controls ( $P = 0.05$ ), (Table 1). A significant association was found between CMV infection and diet in patients with leukemia (Table 1). Transfusion Transmitted Virus and CMV co-infection was found in seven of 40 (17.5%) patients with leukemia (Table 2).

### 5. Discussion

Pathogenic abilities of TT Virus and other blood born Viruses with the potential of producing chronic or latent infections, which may be involved in complicating clinical outcomes in patients with hematological malignancies especially leukemia, have been underestimated by many researchers while there has been much controversy in this field (13, 15, 32). Therefore, in this study the prevalence of TT Virus, CMV, HBV and HCV infections were evaluated in patients with leukemia compared with healthy controls. TT Virus genomic DNA has been detected in different neoplastic tissues (38). The replicative intermediate of TT Virus genome was also detected in hematopoietic cells with more tropism to hyper proliferated PBMCs of cancer patients compared with normal controls (1, 15, 39, 40).

Finding of higher amplification of TT Virus in patients with leukemia may relate to impairments of cellular immunity or a specific relationship with cancer or both (41). Some earlier reports supported this hypothesis: in our earlier study, TT Virus infection was diagnosed with a significantly higher frequency in patients with leukemia compared with controls (17.8 and 2%, respectively) (13). Similarly, in other reports a higher load of TT Virus genomic DNA was detected in PBMCs of patients with cancer compared with healthy controls (41). In another

study a higher prevalence of TT Virus was found in multi-transfused pediatric patients with malignancies (12, 40). Similarly, in this study, a significantly higher frequency of TT Virus molecular infection was found in patients with leukemia compared with controls (42.1% and 12%, respectively). Significant associations were also found between age, diet, and blood grouping with TT Virus infection in patients with leukemia. On the other hand, infectivity of HBV, HCV and CMV as other prevalent blood born Viruses was also reported in patients with hematological malignancies.

Hepatitis B Virus and HCV infections were solely associated with a greater risk for hematological malignancies due to repeated Transfusions of blood and blood products (31, 42). Co-infection by HBV and HCV for synergistic interplay was also strongly related to malignant lymphoma (42). Several previous studies have suggested an association between single and co-infection of HBV and HCV with leukemia. The hematopoietic progenitor cells can infect by HBV and HCV in leukemia patients (29). In one study the association of HBV and HCV infection was reported with acute myelocytic leukemia (29, 42, 43). Higher frequency of HBV and HCV infections in patients with leukemia and lymphoma may be due to reactivation of these hepatitis Viruses following chemotherapy (25-27, 42). In another study HBV infection was associated with a greater risk for hematological malignancy when compared to HCV infection (32, 42, 44, 45). Occult HBV infection can be more activate in patients with chronic myelogenous leukemia (CML) and chronic lymphocytic leukemia (CLL), after receiving chemotherapy (33, 34, 46). Similar to earlier reports, in this study HBV and HCV infections were significantly found in 28.4% and 26.1% of patients with leukemia yet not in any of the controls, respectively.

Cytomegalovirus is another blood born Virus that has been associated with leukemia (35, 47-49). Active CMV infection was found with higher frequency in children with leukemia (range, 27%-46%) (35). However, limited data exists regarding active CMV infection in adult patients with leukemia. Also life-threatening CMV disease has been uncommon in these patients (35). Furthermore, CMV pneumonia has been found by autopsy in 17% - 75% of patients with T cell leukemia (35, 46). In another study, the overall frequency of CMV pneumonia in adults with leukemia increased by two folds, especially in patients with CLL (47). Cytomegalovirus genomic DNA was detected in CLL patients who expressed stereotyped IGHV4-34 B-cell receptors (48). On the other hand, Vd2neg gd T cells expansion after CMV reactivation is capable of responding to both CMV-infected and primary leukemic blasts cells. This phenomenon explains the reduction of the relapse rate of CMV reactivation and complication of clinical outcomes in patients with leukemia (49). Similarly, in this study active CMV infection was significantly found in 16% of patients with leukemia while no subject with CMV infection was found in the control group.

These studied blood born Viruses can produce co-infections in patients with leukemia. The TT Virus genomic DNA was reported in 12.5% - 27% of European and North American patients with chronic HCV infection (50). In another study TT Virus genomic DNA was also found in 17.6% of French HCV-infected patients (51). TT Virus and HCV co-infection may increase the endothelial cell alterations, leading to more frequent mixed cryoglobulinemia-vasculitis (51). A higher replication of TT Virus was found in patients with chronic HCV infection who had lower platelet counts, and patients with controversies in HCV treated with alpha interferon (2, 16, 19, 52). Co-infection of TT Virus with HCV and Hepatitis G Virus (HGV) was also reported in patients with leukemia (12, 40). In line with other studies, significant co-infection of TT Virus with HBV and HCV was found in 37.5% and 35% of patients with leukemia, respectively. However unlike earlier reports, in this study TT Virus and CMV co-infection was found in 17.5% of patients with leukemia.

In conclusion, a significantly higher frequency of TT Virus, HBV, HCV, and CMV single infections and also co-infectivity of TT Virus with each of the evaluated viral infections were found in the studied patients with leukemia compared with healthy controls. Administration of immunosuppressive and chemotherapeutic regimens leading to impairment of immunological based viral clearance pathways in patients with leukemia accelerates the introduction and colonization of the systemic infection of these Viruses especially TT Virus and enhances Virus-related clinical outcomes in patients with leukemia. Therefore, in line with earlier studies, evaluation of the role of TT Virus and other blood born Viruses in outcomes of patients with leukemia is needed to deeply evaluate this concept. Future studies should include a larger group of patients and longer follow up durations.

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## Authors' Contributions

Study concept and design: Ramin Yaghobi, Marjan Shaheli and Mani Ramzi. Acquisition of data: Ramin Yaghobi and Mahdiyar Irvani Saadi. Analysis and interpretation of data: Ramin Yaghobi, Marjan Shaheli and Abbasali Rezaeian. Drafting of the manuscript: Ramin Yaghobi, Shaheli, Abbasali Rezaeian and Mahdiyar Irvani Saadi. Critical revision of the manuscript for important intellectual content: Ramin Yaghobi, Marjan Shaheli and Mani Ramzi. Statistical analysis: Ramin Yaghobi and Mahdiyar

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