

Association Between TT Virus Infection and Cirrhosis in Liver Transplant Patients

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Background: Cirrhosis is one of the most severe liver complications, with multiple etiologies. The torque teno virus (TTV), also known as transfusion transmitted virus, which has a high incidence in the world population, is one of the possible increasing risk factors in patients with idiopathic fulminant hepatitis and cryptogenic cirrhosis.

Objectives: The aim of this study was to evaluate solitary and co-infection with TTV, in patients with cryptogenic and determined cause of cirrhosis.

Patients and Methods: In this cross-sectional study, 200 liver transplant patients were consecutively recruited between years 2007 and 2011. Patients were classified, based on recognition of the etiology of cirrhosis to determined (n = 81) and cryptogenic (n = 119) patient groups. The existence of TTV infection was analyzed, using a semi-nested polymerase chain reaction method. The presence of hepatitis B virus (HBV) infective markers, including HBV DNA, hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), hepatitis B core antibody (HBcAb), and hepatitis B e antibody (HBeAb), was evaluated using qualitative polymerase chain reaction and enzyme linked immunosorbent assay protocols, respectively.

Results: The TTV infection was found in 37 of 200 (18.5%) and 53 of 200 (26.5%) plasma and tissue samples of studied liver transplanted patients, respectively. The TTV genomic DNA was found in 32 (26.9%) and 28 (23.5%) of 119 liver tissue and plasma samples of transplanted patients with cryptogenic cirrhosis, respectively. The genomic DNA of TTV was also diagnosed in 21 (25.9%) and nine (11.1%) of the 81 liver tissue and plasma samples of patients with determined cirrhosis, respectively. Significant associations were found between TTV infection with HBV molecular and immunologic infective markers, in liver transplanted patients, with determined and cryptogenic cirrhosis.

Conclusions: The diagnosis of the high frequency of solitary TTV and co-infection with HBV, in both liver transplanted patients with cryptogenic and determined cirrhosis, emphasized on the importance of TTV infection in the development of cirrhosis, especially in the cases of cryptogenic ones, prompting for further studies the confirm this agent in the etiology of determined cirrhosis.

Keywords: Liver Cirrhosis; Transplantation; Torque Teno Virus; Hepatitis B Virus

1. Background

Transfusion transmitted virus or torque teno virus (TTV) belongs to the Alphatorquevirus genus in the family of Anelloviridae (1). The TTV lacks an accurate definition of the biological nature and relationship to any human disease (2-4). Many epidemiological studies clearly point out the global distribution of the TTV in different populations, with various modes of propagation (5). This viral infection has been found in 1 - 18% of blood donors and patients, who received blood and blood products (3-7).

The TTV can infect hepatocytes and most of its replication takes place in the liver (8, 9). Nevertheless, it has several pathogenic effects that specifically promote liver damage (10, 11). The irregular regeneration of hepatocytes was significantly higher in TTV infected patients (12, 13). However, the association between liver diseases

and TTV infection seems conflicted (14-18). Most of earlier reports focused on the role of TTV infection in liver disorders and reported a possible association with cryptogenic liver disease and cirrhosis (14-17). Cryptogenic cirrhosis is a type of cirrhosis with unknown etiology and no history of alcoholism, or previous acute hepatitis, which cannot be explained by conventional clinical, laboratory and histological findings (14). Diagnostic requisites of cryptogenic cirrhosis have improved with progression of novel basic and technical knowledge. Cryptogenic cirrhosis is a heterogeneous advanced liver complication that needs to be defined clearly, like other defined liver cirrhosis (17, 19). Moreover, to determine the association between hepatitis B virus (HBV) and especially hepatitis C virus (HCV) especially, unde-

finer or nonstandard viral infections, like HIV, the TTV and SEN virus may have a role in the pathogenesis of cryptogenic cirrhosis (19-31). The genomic DNA of TTV has been detected in near 15% of patients with cryptogenic cirrhosis, while in other studies the percent of detection remains controversial (32). The pathogenic role of nonstandard or occult hepatotropic viruses, like TTV, in cryptogenic cirrhosis, is an important domain in the field of hepatitis and its complications, which needs to be evaluated in the vast majority of patients awaiting liver transplantation (33).

2. Objectives

The objective of this study was to evaluate the frequency of TTV infection in patients with cryptogenic cirrhosis, compared with cirrhosis of determined cases.

3. Patients and Methods

In this historical cohort study, 200 liver transplanted patients, who admitted to The Transplant Center at Namazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran, were consecutively recruited between years: 2007 and 2011. The etiology of cirrhosis was identified by an expert gastroenterologist team. Patients were classified based on recognition of the etiology of cirrhosis to determined (n = 81) and cryptogenic (n = 119) cirrhotic patients. Pathology based diagnosis of cryptogenic cirrhosis was also confirmed in 81 of 119 (68.1%) liver transplanted patients, with clinical follow-up.

The EDTA treated blood and paraffin-embedded liver tissue samples that were already collected and saved in the Pathology Laboratory and Sample Bank of Shiraz Transplant Research Center, Shiraz, Iran, were used in this study. Local Shiraz University of Medical Sciences, Shiraz, Iran, Research Ethics Board Committee approved the study. The history of TTV infection was analyzed using the polymerase chain reaction (PCR) based molecular method. The history of the molecular and immunologic prevalence of HBV infective markers, including HBV DNA, hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), hepatitis B core antibody (HBcAb), and hepatitis B e antibody (HBeAb), were analyzed using PCR and enzyme linked immunosorbent assay (ELISA) based protocols.

3.1. Molecular Analysis of Torque Teno Virus Infection

The TTV genomic DNA was extracted from blood and paraffin-embedded liver tissue samples were collected from patients with cryptogenic and determined cirrhosis, using dinitrophenol (DNP) kit (Cinna Gen Inc., Tehran, Iran) according to manufacturer instruction. The presentation of TTV genomic DNA was analyzed in liver transplanted patient samples, using an in-house semi nested-PCR protocol, as previously described (34). The primer sequences used in simple and semi-nested PCR steps were included as: NG059, NG061 and NG063 that amplified 286 bp and 271 fragments

of the N22 open reading frame 1 of TTV genome, respectively.

3.2. Molecular Analysis of Hepatitis B Virus Infection

The HBV genomic DNA was also diagnosed in liver transplanted patient samples, using a qualitative hepatitis B virus PCR detection kit (Cinna Gen Inc., Tehran, Iran), as previously described (35).

3.3. Hepatitis B Virus Immunological Markers

The Hepatitis B Virus immunological markers, including HBsAg, HBcAb, HBeAg and HBeAb, were evaluated in liver recipients with determined or cryptogenic cirrhosis, using third generation ELISA kits (Diapro Diagnostic Bioprobes Srl., Milan, Italy), according to manufacturer's instructions.

3.4. Statistics

Statistical analysis of the serum and tissue distribution of TTV in liver transplanted patients, with determined or cryptogenic cirrhosis, were performed using parametric and non-parametric methods, with SPSS software v.16 for Windows (SPSS Inc., Chicago, IL, USA). The $P \leq 0.05$ was accepted as statistically significant.

4. Results

4.1. Patient's Profile

Details of demographic data of studied liver transplanted patients, with determined or cryptogenic cirrhosis, are shown in Table 1. The age range was 4 - 69, with mean of 38.48 ± 14.43 years old. A total of 139 (69.5%) of the 200 patients were male and the rest of them 61 of them were female (Table 1).

4.2. Torque Teno Virus and Risk Factors

Significant associations were found between several demographic, laboratory and clinical indices, with an increased risk of TTV infection in clinical samples of studied liver transplanted patients. The age ($P = 0.02$), blood grouping ($P = 0.04$), alanine transferase ($P = 0.01$), asparagine transferase ($P = 0.05$), blood urea nitrogen level ($P = 0.04$), and underlying disease ($P = 0.01$) were significantly associated with the presence of TTV infection, in plasma and liver tissue samples of liver transplanted patients, with determined and/or cryptogenic cirrhosis.

4.3. Torque Teno Virus Infection

The TTV infection was found in 37 (18.5%) of 200 liver transplanted plasma samples. Based on gender, 27 (19.4%) of 139 them were male and rest (10 of 61) were female. The TTV infection was also diagnosed in 53 (26.5%) liver tissue samples, in which 39 (28.1%) of 139 were male and 14 (23.0%) of 61 were female. The TTV genomic DNA was found in 32 (26.9%) and 28 (23.5%) of 119 liver tissue and

plasma samples. of liver transplanted patients with cryptogenic cirrhosis, respectively. The genomic DNA of TTV was also diagnosed in 21 (25.9%) and nine (11.1%) of 81 liver tissue and plasma samples, of patients with determines cirrhosis, respectively. The TTV genomic DNA was found in nine (13.2%) of 68 patients, with determined cirrhosis who received liver transplant for HBV infection, as the underlying disease. However, TTV infection was not found in any transplant recipients with HCV infection, as underlying disease.

4.4. Torque Teno Virus and Hepatitis B Virus Infection

The association of TTV infection and diagnosis of HBV molecular and immunologic markers are presented in Table 2.

4.5. Torque Teno Virus and Hepatitis B Virus Genomic DNA

The HBV genomic DNA was found in 65 (52.0%) of 125 patients, comprising of 48 (54.5%) of 88 males and 17 (45.9%) of 37 females. Also, the HBV genomic DNA was found in 23 (67.65%) of 34 of all TTV infected liver transplanted plasma samples. The HBV DNA was found in 15 (57.6%) of 26 and eight (100%) of eight plasma samples of TTV infected liver transplanted patients with cryptogenic and determined cirrhosis, respectively. The HBV genomic DNA was found in 18 (54.5%) of 33 of all TTV infected liver transplanted tissue samples, while it was also found in 13 (65%) of 20 and five (38.4%) of 13 tissue samples of TTV infected liver transplanted patients, with cryptogenic and determined cirrhosis, respectively. Significant associations were found between TTV infection and presence of HBV DNA in plasma samples of all liver transplanted patients ($P = 0.02$) and patients with determined cirrhosis ($P = 0.002$), respectively (Table 2).

4.6. Torque Teno Virus and Hepatitis B Surface Antigen

The HBsAg was detected in 46 (37.7%) of 122 patients, comprising of 32 (37.6%) of 85 males and 14 (37.8%) of 37 females. Of all the 34 TTV infected plasma samples of liver transplant patients, HBsAg was found in 21 (61.7%). The HBsAg was found in 10 (38.4%) of 26 and three (37.5%) of eight plasma samples of TTV infected liver transplant patients, with cryptogenic and determined cirrhosis, respectively (Table 2). The HBsAg was found in 15 (45.5%) of 33 of all TTV infected tissue samples of liver transplant patients. The 13 (65%) of 20 and five (38.4%) of 13 of the tissue samples of TTV infected liver transplanted patients, with cryptogenic and determined cirrhosis, were simultaneously co-infected with HBsAg positive, respectively (Table 2). The TTV infection and HBsAg were significantly concomitantly diagnosed in plasma samples of liver transplanted patients, with determined cirrhosis ($P = 0.02$) (Table 2).

4.7. Torque Teno Virus and Hepatitis B e Antigen

The HBeAg was detected in 61 (50%) of 122 patients, corresponding to 32 (37.6%) of 85 males and 14 (37.8%) of 37 females. The HBeAg was diagnosed in 19 (55.8%) of 34 of all TTV infected plasma samples of liver transplant patients. The HBeAg was found in 13 (50%) of 26 and six (75%) of eight of the plasma samples of TTV infected liver transplanted patients, with cryptogenic and determined cirrhosis, respectively (Table 2). The HBeAg was found in 14 (42.4%) of 33 of all TTV infected tissue samples of liver transplant patients. Nine (45%) of 20 and five (38.4%) of 13 of the tissue samples of TTV infected liver transplanted patients, with cryptogenic and determined cirrhosis, were simultaneously co-infected with HBsAg, respectively (Table 2). No significant association was found between TTV infection and diagnosis of HBeAg in plasma and tissue samples of liver transplanted patients, with cryptogenic and determined cirrhosis (Table 2).

4.8. Torque Teno Virus and Hepatitis B Core Antibody

The HBcAb was detected in 38 (31.4%) of 121 liver transplant patients, corresponding to 30 (35.3%) of 85 males and eight (22.2%) of 36 female. The HBcAb was diagnosed in 16 (47%) of 34 of all TTV infected plasma samples. The HBcAb was detected in nine (34.6%) of 26 and seven (87.5%) of eight plasma samples of TTV infected patients, with cryptogenic and determined cirrhosis, respectively (Table 2). The HBcAb was found in six (18.1%) of 33 tissue samples of all TTV infected patients. The HBcAb was diagnosed in one (5%) of 20 and five (38.4%) of 13 tissue samples of TTV infected recipients with cryptogenic and determined cirrhosis, respectively (Table 2). Significant associations were found between TTV infection and HBcAb in plasma and tissue samples of liver transplanted patients, with cryptogenic and determined cirrhosis (Table 2).

4.9. Torque Teno Virus and Hepatitis B e Antibody

The HBeAb was detected in 21 (18.9%) of 111 patients, representing 15 (71.43%) of 21 males and six (18.2%) of 21 females. The HBeAb was found in eight (23.5%) of 34 plasma samples of all TTV infected patients. The HBeAb was detected in four (15.3%) of 26 and four (50%) of eight plasma samples of TTV infected patients, with cryptogenic and determined cirrhosis, respectively (Table 2). The HBeAb was found in six (18.1%) of 33 tissue samples of all TTV infected patients. The HBeAb was diagnosed in only five (38.4%) of 13 tissue samples of TTV infected recipients, with determined cirrhosis (Table 2). Also, HBeAb was significantly found in plasma samples of TTV infected liver transplanted patients, with cryptogenic cirrhosis ($P = 0.04$) (Table 2).

Table 1. Demographic Parameters of Liver Transplanted Patients With Determined or Cryptogenic Cirrhosis^a

Parameter	Cryptogenic Cirrhosis No. (%)	Determined Cirrhosis No. (%)
Number of Patients	119	81
HBV as Underlying Disease	Not Found	68 (84.0)
HCV as Underlying Disease	Not Found	13 (16.0)
Age of Donors		
Age Range	4 - 73	14 - 59
Mean Age	30 ± 13.08	35.99 ± 13.67
Age of Recipients		
Age Range	4 - 69	17 - 63
Mean Age	33.66 ± 15.2	45.54 ± 9.42
Sex of Recipients		
Male	76 (63.8)	63 (77.8)
Female	43 (36.1)	18 (22.2)
Male/Female	1.7 (76/43)	3.5 (63/18)

^a Abbreviations: HBV, Hepatitis B Virus; HCV, Hepatitis C Virus.

Table 2. Torque Teno Virus and Hepatitis B Virus Co-infection in Liver Transplanted Patients With Cryptogenic and Determined Cirrhosis^a

TTV Co-infection	Cryptogenic Cirrhosis				Determined Cirrhosis				Total Co-infection With TTV			
	TTV-DNA in Plasma (n = 26)		TTV-DNA in Liver (n = 20)		TTV-DNA in Plasma (n = 8)		TTV-DNA in Liver (n = 13)		TTV-DNA in Plasma (n = 34)		TTV-DNA in Liver (n = 33)	
	No. (%)	P	No. (%)	P	No. (%)	P	No. (%)	P	No. (%)	P	No. (%)	P
HBV DNA in Plasma	15 (57.6)	0.33	13 (65.0)	0.15	8 (100.0)	0.002 ^b	5 (38.4)	0.21	23 (67.6)	0.02 ^b	18 (54.5)	0.44
HBsAg	10 (38.4)	0.39	5 (25.0)	0.06	3 (37.5)	0.31	10 (76.9)	0.02 ^b	21 (61.7)	0.54	15 (45.5)	0.12
HBcAb	9 (34.6)	0.02 ^b	1 (5.0)	0.05 ^b	7 (87.5)	0.05 ^b	5 (38.4)	0.10	16 (47.0)	0.01 ^b	6 (18.1)	0.04 ^b
HBeAg	13 (50.0)	0.5	9 (45.0)	0.47	6 (75.0)	0.5	5 (38.4)	0.26	19 (55.8)	0.44	14 (42.4)	0.43
HBeAb	4 (15.3)	0.04 ^b	0 (0.0)	0.16	4 (50.0)	0.3	5 (38.4)	0.64	8 (23.5)	0.11	5 (15.1)	0.43

^a Abbreviations: HBcAb, Hepatitis B Core Antibody; HBeAb, Hepatitis B e Antibody; HBeAg, Hepatitis B e Antigen; HBsAg, Hepatitis B Surface Antigen; HBV, Hepatitis B Virus; TTV, Torque Teno Virus.

^b $P \leq 0.05$ was accepted as statistically significant.

5. Discussion

Knowledge about TTV is growing fast, although several fundamental aspects remain unclear. The worldwide prevalence and clinical significance of TTV are being evaluated. A higher frequency of TTV infection was found in patients with idiopathic fulminant hepatitis and with cryptogenic cirrhosis (36). The cause and effect relationship of TTV infection with liver disease, especially the determinative role on introducing or promoting cirrhosis, remains controversial (37-39). This study was enrolled based on limited information exist about the role of TTV infection in pathogenesis of cryptogenic versus determined cirrhosis in liver transplant patients. In this report, TTV infection was found in 18.5% and 26.5% of liver transplanted plasma and tissue samples, respectively. The TTV genomic DNA was found separately in 26.9% vs.

23.5% and 25.9% vs. 11.1% of liver tissue and plasma samples of transplant patients with cryptogenic and determined cirrhosis, respectively. Similarly, in other previous reports, the importance of this viral infection in promoting liver damage was also studied. The TTV infection was found in Japanese patients with cirrhosis, in which the etiologic role of other defined hepatitis viruses was ruled out (40). Multiple speculations suggested that TTV can induce liver damage. The potential of TTV infection to cause hepatitis was backed-up by the association of TT viremia, with liver enzyme elevation (41-43). Although an elevated load of TTV genomic DNA was found in serum and liver tissue of patients with hepatitis, several reports have tracked this viral infection in patients without hepatitis (8). Earlier studies have revealed TTV infection in chil-

dren with cryptogenic hepatitis, chronic HBV hepatitis, and also, in children without hepatitis (44, 45). Variable frequencies of TTV infection that were reported in patients with leukemia have revealed a different spectrum of liver involvement, ranging from minimal elevation of liver enzymes to severe hepatic failure (8). Two characteristics emphasize on the potential role of TTV in the pathogenesis of liver disease. The first is the observation of the higher load of TTV genome in liver disordered patients, without presence of non-A-G hepatitis viruses (11). The second regards the fact that TTV becomes undetectable in viral infected patients, with normalized levels of liver enzymes (24, 46). Also, this study analyzed the possible associations between different HBV infective markers with TTV infection. The HBV DNA was found in 57.6% vs. 100% and 65% vs. 38.4% of plasma and tissue samples of TTV infected liver transplant patients, with cryptogenic and determined cirrhosis, respectively. Significant associations were found between TTV infection and presence of HBV DNA in plasma samples of liver transplanted patients, with determined cirrhosis. The HBsAg was found in 38.4% vs. 37.5% and 65% vs. 38.4% of plasma and tissue samples of TTV infected liver transplant patients, with cryptogenic and determined cirrhosis, respectively. The TTV infection and HBsAg were significantly correlated in plasma samples of liver transplanted patients, with determined cirrhosis. The HBeAg was also found in 50% vs. 75% and 45% vs. 38.4% of plasma and tissue samples of TTV infected liver transplant patients with cryptogenic and determined cirrhosis, respectively. The HBeAb was detected in 15.3% vs. 50% and none vs. 38.4% of plasma and tissue samples of TTV infected patients, with cryptogenic and determines cirrhosis, respectively. The HBeAb was significantly found in plasma samples of TTV infected liver transplant patients, with cryptogenic cirrhosis.

The HBCAb was detected in 34.6% vs. 87.5% and 5.0% vs. 38.4% of plasma and tissue samples of TTV infected patients, with cryptogenic and determined cirrhosis, respectively. Significant associations were found between TTV infection and HBCAb in plasma and tissue samples of all liver transplanted patients, with cryptogenic and determined cirrhosis. The co-infection of TTV with other important hepatitis viruses was also reported earlier. This co-infectivity was found in different reports, as follows: 73% with Hepatitis C Virus (HCV) and 91% with HBV infections (47), 53.8% with HCV and 47.3% with HBV infections, respectively (48). Also 34% and 6% of patients with chronic HBV infection were diagnosed with TTV infection, using two different primers sets (49). In other earlier reports, the importance of the relationships between co-infectivity of TTV and HCV with increased severity of liver diseases (50, 51). Moreover, high TTV load is associated with occurrence of hepatocellular carcinoma in HCV infected patients (39).

The diagnosis of high frequency of solitary TTV infection and, also, significant association of TTV with different infective markers of HBV were confirmed in both

liver transplanted patients with cryptogenic and determined cirrhosis. These results suggest the increasing importance of TTV infection in the development of cirrhosis and a particular interest should be given to cryptogenic forms, which should be performed in further studies.

Authors' Contributions

Study concept and design: Mohammad Javad Kazemi, Ramin Yaghobi, Bita Geramizadeh. Analysis and interpretation of data: Mohammad Javad Kazemi, Ramin Yaghobi, Mahdiyar Irvani Saadi, Javad Moayed. Drafting of the manuscript: Mohammad Javad Kazemi, Ramin Yaghobi, Bita Geramizadeh, Mahdiyar Irvani Saadi. Critical revision of the manuscript for important intellectual content: Mohammad Javad Kazemi, Ramin Yaghobi, Bita Geramizadeh, Mahdiyar Irvani Saadi. Statistical analysis: Ramin Yaghobi, Mahdiyar Irvani Saadi.

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