The high prevalence of Torque teno virus DNA in blood donors and haemodialysis patients in southern Brazil

Aline Massaú[1], Cristiana Martins[2], Gilca Costa Nachtigal[3], Anelise Bergmann Araújo[3], Maria Lucia Rossetti[4,6], Christian Niel[5], Cláudia Maria Dornelles da Silva[1,4,6]/+

[1]Programa de Pós-graduação em Diagnóstico Genético e Molecular [2]Faculdade de Farmácia [4]Programa de Pós-graduação em Genética e Toxicologia Aplicada, Universidade Luterana do Brasil, Av. Farroupilha 8001, 92425-900 Canoas, RS, Brasil [3]Hemocentro Regional de Pelotas, Pelotas, RS, Brasil [5]Laboratório de Virologia Molecular, Instituto Oswaldo Cruz-Fiocruz, Rio de Janeiro, RJ, Brasil [6]Centro de Desenvolvimento Científico e Tecnológico, Fundação Estadual de Produção e Pesquisa em Saúde, Porto Alegre, RS, Brasil

This study investigates the frequency of Torque teno virus (TTV) infection in 150 blood donors and 77 patients requiring haemodialysis in southern Brazil. Plasma samples were screened for TTV DNA using polymerase chain reaction (PCR). The prevalences of TTV among blood donors and patients requiring haemodialysis were 73.3% and 68.8%, respectively. The presence of TTV was correlated with age in the blood donors (p = 0.024). In haemodialysis patients, no association was found between TTV infection and the demographic parameters (age, sex and education), the duration of haemodialysis or a history of blood transfusion. This study is the first to evaluate the prevalence of TTV infection in Brazilian patients requiring haemodialysis.

Key words: TTV - blood donors - haemodialysis patients - southern Brazil

Torque teno virus is a human non-enveloped single-stranded circular DNA virus with icosahedral symmetry and a particle size of approximately 30 nm. TTV was first characterised as a blood-borne virus and was initially referred to as transfusion-transmitted virus (Nishizawa et al. 1997). Its negative-sense genome is 3.6-3.9 kb in size (Mushahwar et al. 1999). Recently, it was renamed Torque teno virus (TTV) and classified as a member of the genus Alphatorquevirus in the family Anelloviridae (ICTV Virus Taxonomy 2009) (ictvonline.org/).

Although TTV infection has been suggested to be associated with a number of diseases based on epidemiological data, there is no direct causal evidence linking TTV infection to specific clinical manifestations. This virus is distributed around the world and the prevalence of TTV infection is similarly high in all groups tested, including patients with liver (Asim et al. 2010), acute respiratory (Maggi et al. 2003) and renal diseases (Irshad et al. 2010), as well as in human immunodeficiency virus (HIV)-positive subjects (Devalle & Niel 2004), drug users (Alzahrani et al. 2009) and healthy individuals (Vasilyev et al. 2009).The objective of the present study was to determine the prevalence of TTV DNA in blood donors and patients requiring haemodialysis in the southern state of Rio Grande do Sul (RS), Brazil. A cross-sectional study was conducted from May-August 2010. Plasma samples from 150 consecutive blood donor candidates and all 77 patients receiving regular haemodialysis treatment at one dialysis unit in the city of Pelotas (in the southern region of RS) were tested for the presence of TTV DNA. This study was approved by the Research Committee of the Lutheran University of Brazil (2009-440H). All participants signed an informed consent form and filled out an epidemiological questionnaire.

Hepatitis B surface antigen (HBsAg), anti-hepatitis C virus (HCV) and anti-HIV I/II antibodies were detected using standard commercially available assays. The alanine aminotransferase (ALT) serum levels were measured in the patients requiring haemodialysis. Blood was collected and viral DNA was extracted according to the method of Niel et al. (1994). TTV DNA was amplified in a sensitive, single-step polymerase chain reaction (PCR) assay. Oligonucleotide primers were designed to hybridise to the untranslated region, which is the most conserved region of the TTV genome. PCR amplification was then conducted with the TTV1 (sense, 5'-TGCACTTCCGAATGGCTGAGTT-3', designed for this study; nt 94-115) and TTV NG0147 (antisense, 5'-GCCAGTCCCGAGCCCGAATTGCC-3'; nt 209-231) primers (Okamoto et al. 1999). The resulting amplicon was 136 bp in length. The indicated base positions of the primers were based on the nucleotide sequences described by Niel et al. (2005), which are available in GenBank (accession AY823989). The sensitivity of the primer pair was determined by testing the amplification of 10-fold serial dilutions of plasmid 3h (Niel et al. 2005), which carries the entire TTV genome, yielding a detection limit of 10 femtograms. These experiments were repeated three times. This plasmid was also used as a positive control in the PCR assays. The selected primer sequences were examined for similarities to sequences from other organisms using NCBI BLAST and a high level of specificity was found. Water was used as a negative control.

The amplification was performed using 3 µL of DNA and 2.5 units of Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA) in a final volume of 50 µL. Af-

Financial support: ULBRA/RS
+ Corresponding author: cmdornelles@gmail.com
Received 2 October 2011
Accepted 11 January 2012

ter an initial incubation at 94ºC for 2 min, 55 cycles of PCR were run using the following settings: 94ºC for 40 s, 60ºC for 40 s and 72ºC for 40 s. These cycles were followed by a final elongation at 72ºC for 7 min. Water was used as a negative control. Statistical comparisons were performed using SPSS 13.0 (Pearson chi-Square test or Fisher's exact test, as applicable). Differences were considered significant when the p value was less than 0.05.

The prevalence of TTV DNA in blood donors and patients requiring haemodialysis were 73.3% and 68.8%, respectively. No significant difference in TTV prevalence was observed between these groups. Table shows the demographic, educational, biochemical and serological data for all participants in the study, as well as the duration of treatment for the haemodialysis patients. The TTV prevalence increased with age among blood donors (p = 0.024). The mean ages of the blood donors and the patients requiring haemodialysis were 32.0 (range of 18-64) and 54.2 (range of 22-84) years, respectively. No significant correlation was found between TTV infection and demographic parameters (sex and level of education) in either of the two groups studied. Of the four HBsAg-positive haemodialysis patients, two (50%) were coinfected with TTV. Anti-HCV antibodies were detected in 19 patients, nine (47.4%) of whom were coinfected with TTV. One patient was HIV positive and was coinfected with TTV. Finally, one patient was positive for TTV, HBV and HCV and another was positive for TTV, HCV and HIV (not shown).

The TTV prevalence (73.3%) detected among blood donors was similar to that observed among blood donors from France (66%) (Biagini et al. 2006) and from the northern (60%) (Pinto et al. 2007) and southeastern (62%) (Niel et al. 1999) regions of Brazil, but was higher than that found in blood donors from India (43%) (Chattopadhyay et al. 2005) and Belgium (29.7%) (Ali et al. 2004). This study again highlights the widespread nature of TTV infection among blood donors around the world and indicates that the parenteral route of transmission

TABLE

Torque teno virus (TTV) infection in blood donors and haemodialysis patients

	Blood donors				Haemodialysis patients			
	TTV DNA negative	TTV DNA positive	Total		TTV DNA negative	TTV DNA positive	Total	
Variable	n (%)	n (%)	n (%)	p	n (%)	n (%)	n (%)	p
Gender								
Male	27 (26.7)	74 (73.3)	101 (67.3)	0.268	17 (39.1)	28 (60.9)	45 (58.4)	0.193
Female	13 (26.5)	36 (73.5)	49 (32.7)	-	8 (25)	24 (75)	32 (41.6)	-
Age (years)								
18-24	21 (36.8)	36 (63.2)	57 (38)	0.024[a]	0 (0)	1 (100)	1 (1.3)	0.316
25-38	10 (21.7)	36 (78.3)	46 (30.7)	-	4 (30.8)	9 (69.2)	13 (17.1)	-
39-53	9 (24.3)	28 (75.7)	37 (24.7)	-	4 (25)	12 (75)	16 (21.1)	-
≥ 54	0 (0)	10 (100)	10 (6.6)	-	18 (39.1)	28 (60.9)	46 (60.5)	-
Education								
Elementary school	12 (26.7)	33 (73.3)	45 (30)	0.795	19 (33.9)	37 (66.1)	56 72.7)	0.809
Junior college	16 (25)	48 (75)	64 (42.7)	-	4 (28.6)	10 (71.4)	14 (18.2)	-
University	12 (29.3)	29 (70.7)	41 (27.3)	-	3 (42.9)	4 (57.1)	7 (9.1)	-
ALT								
< 45 U/L	-	-	-	-	19 (31.7)	41 (68.3)	60 (82.2)	0.499
≥ 45 U/L	-	-	-	-	6 (46.2)	7 (53.8)	13 (17.8)	-
Blood transfusion								
No	40 (26.7)	110 (73.3)	150 (100)	-	11 (34.4)	21 (65.6)	32 (42.1)	1.000
Yes	0 (0)	0 (0)	0 (0)	-	15 (35.8)	29 (65.9)	44 (57.9)	-
Time on haemodialysis								
0-36 months	-	-	-	-	10 (25.6)	29 (74.4)	39 (50.6)	0.198
≥ 37 months	-	-	-	-	16 (42.1)	22 (57.9)	38 (49.4)	-
HBsAg								
Non-reactive	40 (26.7)	110 (73.3)	150 (100)	-	23 (34.3)	44 (65.7)	67 (94.4)	0.921
Reactive	0 (0)	0 (0)	0 (0)	-	2 (50)	2 (50)	4 (5.6)	-
Anti-HCV								
Non-reactive	40 (26.7)	110 (73.3)	150 (100)	-	15 (29.4)	36 (70.6)	51 (72.9)	0.128
Reactive	0 (0)	0 (0)	0 (0)	-	10 (52.6)	9 (47.4)	19 (27.1)	-

a: p statistically significant; ALT*:* alanine aminotransferase; HBsAg: hepatitis B surface antigen; HCV: hepatitis C virus.

may not necessarily play a major role in infection. This observation has been supported by the detection of TTV in saliva (Naganuma et al. 2008), breast milk (Iso et al. 2001), semen (Inami et al. 2000), faeces (Hamza et al. 2011) and water (Diniz-Mendes et al. 2008). The observation that the TTV prevalence increased with age in this group of blood donors was consistent with the results of other studies (Saback et al. 1999, Salakova et al. 2004).

To our knowledge, this study is the first to evaluate the prevalence of TTV infection in Brazilian haemodialysis patients. The prevalence (68.8%) detected among these patients was higher than the prevalence observed among patients requiring haemodialysis from other countries, which ranged from 16-58.5% (Martinez et al. 2000, Irshad et al. 2010). Such differences may be explained by the existence of different routes of virus transmission and lifestyle differences among different populations around the world.

This study did not demonstrate any association between TTV infection and the duration of haemodialysis treatment. This finding is in agreement with the results of previous studies (Chattopadhyay et al. 2005, Irshad et al. 2010). Consistent with the results of Irshad et al. (2010), no biochemical evidence of a link between liver disease and TTV infection was observed in the TTV DNA-positive haemodialysis patients. Furthermore, elevated serum ALT levels were more closely linked to HCV than to TTV.

In conclusion, the TTV prevalence among patients requiring haemodialysis is very high, although it is comparable to that observed in blood donors. The pathology of TTV and the host response to TTV infection warrant further investigation.

REFERENCES

Ali S, van Pelt JF, Verslype C, Nevens F, Fevery J, Yap SH 2004. TT virus infection in acute and chronic liver diseases and in patients regularly receiving blood products in Belgium. *Acta Gastroenterol Belg 67*: 161-165.

Alzahrani AJ, Cruz DMD, Obeid OE, Bukhari HA, Al-Qahtani AA, Al-Ahda MN 2009. Molecular detection of hepatitis B, hepatitis C and Torque teno viruses in drug users in Saudi Arábia. *J Med Virol 81*: 1343-1347.

Asim M, Singla R, Gupta RK, Kar P 2010. Clinical and molecular characterization of human TT virus in different liver diseases. *Indian J Med Res 131*: 545-554.

Biagini P, Gallian P, Cantaloube JF, Attoui H, Micco P, Lamballerie X 2006. Distribution and genetic analysis of TTV and TTMV major phylogenetic groups in French blood donors. *J Med Virol 78*: 298-304.

Chattopadhyay S, Rao S, Das BC, Singh NP, Kar P 2005. Prevalence of transfusion-transmitted virus infection in patients on maintenance haemodialysis from New Delhi, India. *Hemodial Int 9*: 362-366.

Devalle S, Niel C 2004. Distribution of TT virus genomic groups 1-5 in Brazilian blood donors, HBV carriers and HIV-1-infected patients. *J Med Virol 72*: 166-173.

Diniz-Mendes L, Paula VS, Luz SL, Niel C 2008. High prevalence of human Torque teno virus in streams crossing the city of Manaus, Brazilian Amazon. *J Appl Microbiol 105*: 51-58.

Hamza IA, Jurzik L, Uberla K, Wilhelm M 2011. Evaluation of pepper mild mottle virus, human picobirnavirus and Torque teno virus as indicators of faecal contamination in river water. *Water Res 45*: 1358-1368.

Inami T, Konomi N, Arakawa Y, Abe K 2000. High prevalence of TT virus DNA in human saliva and semen. *J Clin Microbiol 38*: 2407-2408.

Irshad M, Mandal K, Singh S, Agarwal SK 2010. Torque teno virus infection in haemodialysis patients in North India. *Int Urol Nephrol 42*: 1077-1083.

Iso K, Suzuki Y, Takayama M 2001. Mother-to-infant transmission of TT virus in Japan. *Int J Gynaecol Obstet 75:* 11-19.

Maggi F, Pifferi M, Tempestini E, Fornai C, Lanini L, Andreoli E, Vatterloni M, Presciuttini S, Pietrobelli A, Boner A, Pistello M, Bendinelli M 2003. TT virus load and lymphocyte subpopulations in children with acute respiratory diseases. *J Virol 77*: 9081-9083.

Martinez NM, Garcia F, Garcia-Valdecasas J, Bernal C, García F Jr, López I, Alvarez M, Piédrola G, Maroto MC 2000. Prevalence and viral persistence of TT virus in patients on haemodialysis. *Eur J Clin Microbiol Infect Dis 19*: 878-880.

Mushahwar IK, Erker JC, Muerhoff AS, Leary TP, Simons JN, Birkenmeyer LG, Chalmers ML, Pilot-Matias TJ, Dexai SM 1999. Molecular and biophysical characterization of TT virus: evidence for a new virus family infecting humans. *Proc Natl Acad Sci USA 96*: 3177-3182.

Naganuma M, Tominaga N, Miyamura T, Soda A, Moriuchi M, Moriuchi H 2008. TT virus prevalence, viral loads and genotypic variability in saliva from healthy Japanese children. *Acta Paediatr 97*: 1686-1690.

Niel C, de Oliveira JM, Ross RS, Gomes SA, Roggendorf M, Viazov S 1999. High prevalence of TT virus infection in Brazilian blood donors. *J Med Virol 57*: 259-263.

Niel C, Diniz-Mendes L, Devalle S 2005. Rolling-circle amplification of Torque teno virus (TTV) complete genomes from human and swine sera and identification of a novel swine TTV genogroup. *J Gen Virol 86*: 1343-1347.

Niel C, Moraes MT, Gaspar AMC, Yoshida CF, Gomes SA 1994. Genetic diversity of hepatitis B virus strains isolated in Rio de Janeiro, Brazil. *J Med Virol 44*: 180-186.

Nishizawa T, Okamoto H, Konishi K, Yoshizawa H, Miyakawa Y, Mayumi MA 1997. Novel DNA virus (TTV) associated with elevated transaminase levels in post transfusion hepatitis of unknown aetiology. *Biochem Biophys Res Commun 241*: 92-97.

Okamoto H, Takahashi M, Nishiza T, Ukita M, Fukuda M, Tsuda F, Miyakawa Y, Mayumi M 1999. Marked genomic heterogeneity and frequent mixed infection of TT virus demonstrated by PCR with primers from coding and non coding regions. *Virology 259*: 428-436.

Pinto WV, Assis MF, Lemos JA 2007. Prevalência do TTV em doadores de sangue na região metropolitana de Belém-Pará. *Cad Saude Colet 15*: 349-356.

Saback FL, Gomes SA, de Paula VS, da Silva RR, Lewis-Ximenez LL, Niel C 1999. Age-specific prevalence and transmission of TT virus. *J Med Virol 59*: 318-322.

Salakova M, Nemecek V, Konig J, Tachezy R 2004. Age-specific prevalence, transmission and phylogeny of TT virus in the Czech Republic. *BMC Infect Dis 4*: 56-64.

Vasilyev EV, Trofimov DY, Tonevitsky AG, Ilinsky VV, Korostin DO, Rebrikov DV 2009. Torque Teno Virus (TTV) distribution in healthy Russian population. *Virol J 6*: 134-137.