

The first evidence of TT virus infection in Slovakia

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Recently, the presence of a novel DNA virus (TTV) has been associated with either acute or chronic hepatitis of unknown aetiology, suggesting a possible aetiological role. TT virus is transmitted mainly parenterally and the clinical importance of its infection is not clear yet. This paper gives the first report about evidence of TT virus infection in Slovakia. Corresponding with literature data the first cases were confirmed in patients with multiple risk factors as repeated reception of blood transfusions and blood products, invasive or surgical interventions, intravenous drug abuse, chronic hepatitis B and C, non-sterile injections, hazardous sexual contacts. A short characteristic of these first TTV-positive cases is given.

Key words: TTV, hepatitis non-A-non-E, PCR detection.

Introduction

Currently there are six known types of viruses causing viral hepatitis which account for most cases of acute and chronic viral hepatitis. But there are cases of viral hepatitis in which none of known viruses is proved to be the causative agent (SCHRÉTER, 2002).

These facts thanks to progress in modern laboratory molecular techniques led to increased efforts to isolate another potential hepatotropic virus. The isolation of TT virus in 1997 as one of the new candidates for list of hepatotropic viruses was the result of this effort. TT virus (transfusion-transmitted virus, TTV) was described in as-

sociation with increased aminotransferase activity in a patient with post-transfusion hepatitis of unknown etiology (T.T. are patient's initials) (NISHIZAWA et al., 1997). TTV is a non-enveloped single-stranded DNA virus, yet unclassified (OKAMOTO et al., 1998).

There is an assumption that not only blood transfusions themselves, but also the other blood products, mostly clotting factor concentrates (factors VIII and IX) are accountable for TT virus transmission (SIMMONDS et al., 1998). Intravenous drug use and sexual activity are also supposed to be route of TT virus transmission (CAO et al., 1999; KREKULOVA et al., 2001; MASIA et al., 2001). Fecal excretion of TT virus suggests the

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possibility for not only parenteral but also fecal-oral route of viral transmission (OKAMOTO et al., 1998).

Prevalence of TT virus ranges from 1.9% to 37%, respectively, in general population or in healthy voluntary blood donors in different countries. TT virus prevalence among blood donors in the Czech Republic varies from 0% (URBÁNEK et al., 2000) to 13.5% (KREKULOVA et al., 2001). Data from Slovakia have not been available yet.

TTV infection is quite frequently observed in HBV and HCV infected patients. Coinfection of HBV infected patients with TT virus differs from 18% to 35% (COLOMBATTO et al., 1999; KANDA et al., 1999; MASIA et al., 2001). Data about HCV and TTV coinfection are similar to above within the range from 8% to 42% (IKEDA et al., 1999; KANDA et al., 1999; TRIMOULET et al., 2000; MASIA et al., 2001), in a study from the Czech Republic it was 34.5% (URBÁNEK et al., 2000).

Material and methods

Sera from 170 patients with diverse risk for TTV infection were collected during the period from December 2001 till March 2003.

DNA extracted from 200 μ L of serum by QIAamp DNA Blood Mini Kit (QIAGEN) was used as a template for PCR reaction. TTV DNA was identified by semi-nested PCR as described previously (OKAMOTO et al., 1998). In brief, first PCR utilized NG 059 sense primer (5'-ACAGACAGAGGAGAAGGCAACATG-3') and NG 063 antisense primer (5'-CTGGCATTTTACCATTTCCAAAGTT-3'). PCR protocol consisted of initial denaturation (96°C/6 min) followed by 35 cycles (94°C/30 s; 60°C/45 s; and 72°C/45 s), and final extension (72°C/2 min). Second PCR was carried out with NG 061 sense primer (5'-GGCAACATGTTGTG GATAGACTGG-3') and the same NG 063 antisense primer for 25 cycles under the same PCR conditions. PCR products (271 bp) were detected by agarose gel electrophoresis, with using of 2 % agarose, stained with ethidium bromide, and visualised under UV light. DNA 50-bp ladder (Invitrogen) was used as DNA molecular weight size marker.

Results and discussion

First 20 serum samples were tested, of these 4 samples were positive for TTV (Fig. 1). All of these four cases were patients with relatively high risk of TTV infection (Tab. 1).

The first patient was 21 year-old male. He was treated from childhood for a severe form of hemophilia type A. He has been repeatedly receiving blood transfusions since 1985, later he

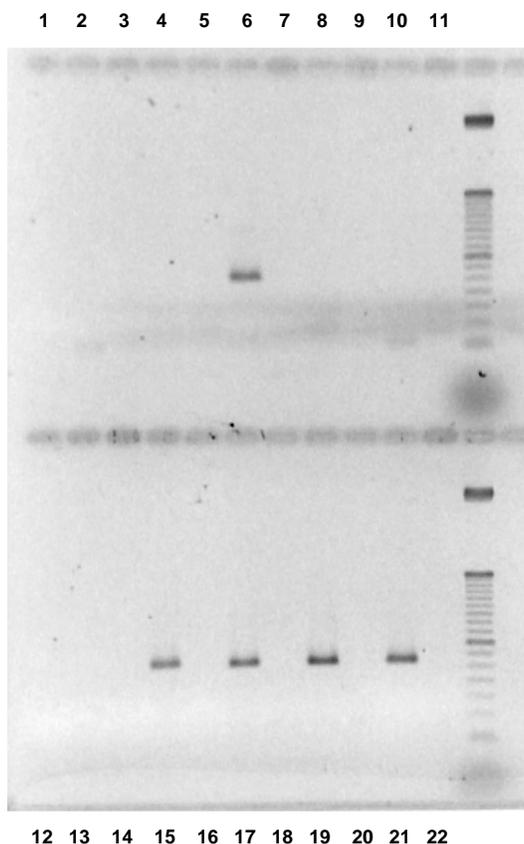


Fig. 1. First positive TTV tested samples (molecular size 271 bp) confirmed by semi-nested PCR method (lanes 6, 15, 17, 19). Lanes 1–20: PCR products of tested samples. Lane 21: positive control. Lane 22: negative control.

was treated with factor VIII concentrates. During the years 1991-1994 he had three times knee surgery because of secondary changes caused by hemophilia. Finally, chronic hepatitis C was diagnosed in 2002.

The second patient, a 25 year-old male, is an intravenous drug addict since 1998. He was diagnosed with acute hepatitis A in 2000. Diagnosis of chronic hepatitis C was confirmed at the same time. From other risk factors he had one surgery in childhood, during the years 1997-2001 he was repeatedly unprofessionally tattooed and pierced and had hazardous sexual female partners.

The next patient, a 48 year-old female, had undergone six surgeries all together. Two surgeries were performed during childhood. She re-

Table 1. Present coincident risk factors of TTV infection and ALT values of TTV positive patients.^a

Patient	B	C	Transfusions	IDA	Surgeries	Other	ALT μ kat/L
1	no	yes	multiple	no	3 \times	piercing	0.42
2	no	yes	no	yes	1 \times	tattoo, piercing, risk sexual partners	1.92
3	no	yes	2 \times	no	6 \times	no	0.90
4	yes	no	5 \times	no	2 \times	contact with hepatitis B	5.32

^aB, hepatitis B; C, hepatitis C; IDA, intravenous drug abuse.

ceived multiple transfusions after Caesarian sections in 1980-1982. She had increased aminotransferase levels for couple of years and from 2002 she had confirmed chronic hepatitis C.

Last patient, a 44 year-old male, had a complicated course of pancreatitis in 2000 with repeated surgical revisions and multiple blood transfusions. Since 2001 he had confirmed chronic hepatitis B. His wife had hepatitis B as well.

Diagnosis of chronic hepatitis B and C in all patients was confirmed by serological as well as molecular methods.

This paper gives the first report about the evidence of TT virus infection in Slovakia. The first cases were confirmed in patients with multiple risk factors as repeated reception of blood transfusions and blood products, invasive or surgical interventions, intravenous drug abuse, chronic hepatitis B and C, non-sterile injections, and hazardous sexual contacts. Our results correspond with the literature data (SIMMONDS et al., 1998; CAO et al., 1999; COLOMBATTO et al., 1999; IKEDA et al., 1999; KANDA et al., 1999; TRIMOLET et al., 2000; KREKULOVA et al., 2001; MASIA et al., 2001). There are plans to examine a larger cohort of patients in a near future, to determine the prevalence of TTV infection in Slovakia in different risk groups, and to contribute to the knowledge about the clinical importance of this infection.

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