Prevalence of TTV in blood donors and patients with acute viral hepatitis and genotyping by phylogenetic analysis

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SUMMARY

The aim of this study was to evaluate the prevalence of TTV in patients with acute hepatitis A and B, and to genotype TTV isolates by phylogenetic analysis. The authors evaluated sera of 82 patients who had acute hepatitis A (N = 40) and B (N = 42), and of 71 blood donors. TTV was detected by nested-PCR assay and phylogenetic analysis was performed using the neighbor-joining method. TTV was detected in 23% of patients with acute hepatitis and in 31% of donors. Mean aminotransferase levels were similar in patients who were TTV positive and TTV negative. A phylogenetic tree was drawn that showed TTV isolates of genotype 1, 2, 3, and 4. In conclusion, TTV infection is more frequent among blood donors than among patients with acute hepatitis A or B in Salvador-Bahia. TTV does not appear to increase the severity of necroinflammatory activity of acute hepatitis A or B. Using phylogenetic analysis TTV isolates of genotype 1, 2, 3, and 4 were encountered in the study population. **GED 22(4):129-132,2003**

Key words – Acute hepatitis
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Unitermos – *Hepatite aguda*
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INTRODUCTION

In 1997, a new virus designated transfusion transmitted virus (TTV) was isolated from the serum of a patient with post-transfusion non A-E hepatitis who was HGV-RNA negative[13]. TTV is a single stranded, circular, non-enveloped DNA virus with at least 3800 base pairs and at least two open reading frames[11]. It has been detected in amounts 10 to 100 times higher in the liver compared to serum and thus, could be hepatotropic[14]. Nevertheless, studies regarding the role of TTV in acute and chronic liver diseases have been controversial[6,12,17].

Is spite of being a DNA virus, TTV has a relatively high genetic diversity. In the initial study of Nishizawa, the authors suggested the existence of more than one genotype[13]. This was confirmed by several other studies that detected the existence of many genotypes and subtypes.

The aim of this study was to evaluate the prevalence of TTV in blood donors and in patients with acute hepatitis A and B, and to genotype TTV isolates by phylogenetic analysis.

MATERIAL AND METHODS

Patients and donors

We evaluated sera from 82 out of 310 patients who attended the acute hepatitis outpatient service at the Federal University of Bahia between 1995 and 1999. These 82 cases were selected because all had acute hepatitis A (N = 40) or B (N = 42) and they had stored serum samples available. All patients had signs and symptoms of acute hepatitis, as well as alanine and aspartate aminotransferases (ALT and AST) at least two-fold the upper limit of normal (ULN). Patients with acute hepatitis A were seropositive for anti-HAV IgM (immunoglobulin M antibody to hepatitis A virus) and seronegative for anti-HBc IgM (immunoglobulin M antibody to hepatitis B core antigen), HBsAg (hepatitis B surface antigen), and hepatitis C antibody (anti-HCV) by second-generation immunoassay. Patients with acute hepatitis B were seropositive for anti-HBc IgM and for HBsAg, and seronegative for anti-HAV IgM and for anti-HCV by second-generation immunoassay.

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We also analyzed sera of 71 consecutive volunteer blood donors who had donated blood in 1999 at the main Blood Center in the state of Bahia (HEMOBA). All met the required criteria for blood donation, were seronegative for serum HBV and HCV markers, and had normal ALT levels.

The institutional review boards approved the study and the protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Laboratory methods

TTV-DNA detection. TTV-DNA was detected in serum by a nested-PCR assay using sense primers NG059 and NG061, and anti-sense primer NG063 described previously[14]. The second anti-sense oligonucleotide (5' GTK GGK TAC CAY TTA GCT CTC ATT C 3') was denominated JR01. Nucleic acid was extracted as previously reported and amplified using standard PCR conditions[1]. First round of PCR was performed with primers NG059 and JR01 for 35 cycles (94°C, 30 s; 60°C, 45 s; 72°C, 60 s), plus an additional cycle of 72 °C for 7 min. Identical conditions and primers NG061 and NG063 were used for the second round of amplification. PCR products were subjected to electrophoresis on 1.5% agarose gel, and samples producing a single band of 271 bp were considered as positive. Stringent precautions to avoid contamination were taken as previously described[10].

Sequencing and phylogenetic analysis. Both strands of TTV PCR products were sequenced utilizing ABI Prism BigDye Terminator Ready Reaction Kit version 1.0 (Applied Biosystems, Foster City, CA). Reactions were analyzed by automated DNA sequencer (ABI model 377). Sequences were analyzed by visual inspection and using the Phred-Phrap-Consed software[2,4] and aligned using CLUSTAL X version 1.81[18]. TTV isolates were subjected to phylogenetic analysis using the Neighbor-joining method in program MEGA (version 2.1)[9]. The distance matrix was calculated with the Kimura 2-parameter model[8]. Previously published genomes were used as reference sequences. Bootstrap test pf phylogeny was performed with 1000 replications.

STATISTICAL ANALYSIS

Continuous variables are expressed as the mean ± SD. Comparison of means was performed with the Mann-Whitney U test. Proportions were compared utilizing the Chi-square test. All analyses were performed utilizing the SPSS package (SPSS for Windows release 11.5, SPSS Inc., Chicago, IL). A p value ≤ 0.05 was considered to be statistically significant.

RESULTS

The clinical features of patients with acute hepatitis are summarized in table 1. Fifty-five patients were male and 27, female. Mean age was older in patients with hepatitis B compared to patients with hepatitis A. Mean ALT and AST levels were higher in the group of patients with acute hepatitis B.

Serum TTV-DNA was detected in 19 of 82 patients (23%) with acute hepatitis, and in 22 of 71 blood donors (31%). TTV was detected in 8 of 40 (20%) patients with acute hepatitis A and 11 of 42 (26%) with acute hepatitis B. There was no statistically significant difference between mean age and mean aminotransferase levels of TTV positive and TTV negative patients (tables 2 and 3).

Phylogenetic analysis of TTV isolates was carried out in 26 of 41 subjects who were TTV positive. GenBank accession numbers for these sequences are AY137347 to AY137372. Six of these subjects had acute hepatitis B, two had acute hepatitis A, and 18 were blood donors. They were selected according to the availability of spare amounts of serum. We encountered TTV isolates of genotype 1a, 1b, 2, and 3 among blood donors and patients with acute hepatitis, and TTV genotype 4 among blood donors only (figure 1).

TABLE 1
Clinical features of the 82 study patients

Group	No. of patients	Gender (male:female)	Age (years) (mean ± SD)*	ALT (x ULN)[a] (mean ± SD)**	AST (x ULN)[a] (mean ± SD)***
Hepatitis A	40	30:10	14.28 ± 8.95	18.55 ± 14.48	12.05 ± 11.64
Hepatitis B	42	25:17	32.19 ± 12.99	30.93 ± 20.77	26.36 ± 21.62
Total	82	55:27	23.45 ± 14.32	24.89 ± 18.92	19.38 ± 18.80

a ALT and AST levels are expressed in multiple upper limit of normal. Normal range for ALT is 12-50 U/L; normal range for AST is 12-46 U/L.
* p value < 0.001
** p value = 0.002
*** p value < 0.001

TABLE 2
Prevalence of TTV-DNA in patients with acute hepatitis and blood donors

Group	N	TTV-DNA positive N (%)
Blood donors	71	22 (31)
Hepatitis A	40	8 (20)
Hepatitis B	42	11 (26)
Total	153	41 (27)

TABLE 3
Comparison between acute hepatitis patients with and without TTV-DNA

Clinical data	TTV-DNA positive	TTV-DNA negative	p value
No. patients	19	63	
Gender (male:female)	13:6	42:21	0.887
Age (yr, mean ± SD)	23.40 ± 12.50	24.80 ± 16.37	0.987
ALT (ULN)	23.58 ± 18.19	25.29 ± 19.26	0.750
AST (ULN)	16.58 ± 13.83	20.22 ± 20.08	0.700

ULN: upper limit of normal. Normal range for ALT is 12-50 U/L; normal range for AST is 12-46 U/L.

DISCUSSION

In this study we evaluated the prevalence of TTV in patients with acute hepatitis A, acute hepatitis B, and in volunteer blood donors. We found that TTV is highly prevalent among all groups studied, although, the virus was less frequently detected in patients with viral hepatitis A or B. Moreover, ALT and AST levels were lower in patients infected with TTV compared to patients who were TTV negative, although these differences were not statistically significant. These findings suggest that TTV does not increase the severity of necroinflammatory activity in patients with acute hepatitis caused by HAV or HBV.

The studies that have analyzed the role of TTV in acute and chronic liver diseases have been controversial. Initially, authors have found a greater prevalence of TTV-DNA in patients with cryptogenic chronic liver disease compared to patients with liver disease of known etiology and compared to controls[6,13,14]. However, subsequent investigations in several countries have found contradictory results[3,12]. In a Japanese study, TTV was detected in 29% of patients with hepatitis A, 24% of patients with acute hepatitis B, 43% of subjects with non A-E hepatitis, and in 37% of controls. There was no statistical difference between aminotransferase levels of TTV positive and TTV negative patients[7]. These results appear to be similar to ours.

Coinfection with TTV in chronic HCV-infected or HBV-infected children did not result in higher peak ALT levels during follow-up, suggesting that TTV has no synergistic pathogenic effect[5].

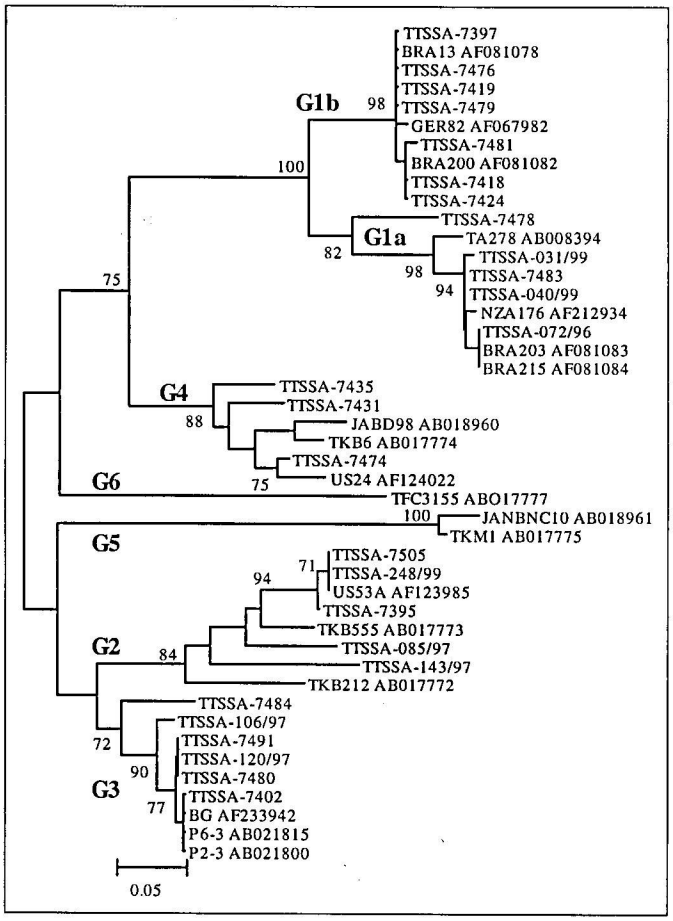

Fig. 1 - Neighbor-joining tree of 183 nt fragments of TTV (corresponding to nt 1960-2142 of strain TA278) from previous published reference strains and from the study population. For previously published references strains, the GenBank accession numbers are indicated. The genotype designations used correspond to designations of previous publications. They are indicated on the branches. Bootstrap test of phylogeny was performed with 1000 replications and values equal to or greater than 70 are indicated.

Other studies have also reported a high prevalence of TTV in Brazilian populations, including blood donors and patients with acute and chronic hepatitis[16]. Pinho et al found that 17 % of patients with cryptogenic chronic liver diseases and 40% of patients with chronic hepatitis B were TTV positive[15].

The distribution of TTV genotypes showed that genotype 1 was the most prevalent among the study population, nevertheless TTV genotypes 2, 3, and 4 were also found. Among 26 samples that were subjected to genotyping, 12 isolates were genotype 1. TTV genotypes 1 and 2 have been described in several countries including Japan, Thailand, United Kingdom, and Germany[6,12]. Other genotypes have also been reported in these regions.

In conclusion, TTV infection is more frequent among blood donors than among patients with acute hepatitis A or B in Salvador-Bahia. TTV does not appear to increase the severity of the necroinflammatory activity of acute hepatitis A or B. Using phylogenetic analysis, TTV isolates of genotypes 1, 2, 3, and 4 were found in the study population.

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Prevalência de TTV em doadores de sangue e pacientes portadores de hepatite viral aguda e genotipagem por análise filogenética

RESUMO

O objetivo desse estudo foi avaliar a prevalência da infecção pelo TTV em pacientes com hepatite aguda A e B, e genotipar os isolados do TTV através de análise filogenética. Foram avaliados soros de 82 pacientes que apresentaram hepatite aguda A (N = 40) e B (N = 42), e 71 doadores de sangue. O TTV foi determinado através "nested-PCR", e a análise filogenética foi realizada utilizando o método "neighbor-joining". O TTV foi detectado em 23% dos pacientes com hepatite aguda e em 31% dos doadores. Os níveis médios de aminotransferases foram semelhantes em pacientes TTV positivos e pacientes TTV negativos. Uma árvore filogenética foi construída e mostrou isolados do TTV dos genótipos 1, 2, 3 e 4. Em conclusão, a infecção pelo TTV foi mais freqüente entre doadores de sangue do que em pacientes com hepatite aguda A ou B, em Salvador-Bahia. O TTV não aumentou a gravidade da atividade necroinflamatória dos pacientes com hepatite aguda A ou B. Através de análise filogenética foram encontrados isolados do TTV dos genótipos 1, 2, 3 e 4 na população estudada. ***GED 22(4):129-132,2003***

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