別紙様式第2

別紙(3)-3

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 2008年1月29日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①～③人血清ｱﾙﾌﾞﾐﾝ④人血液凝固第ＸⅢ因子 ⑤⑥ﾌｨﾌﾞﾘﾉｹﾞﾝ加第ＸⅢ因子		研究報告の公表状況	Clinical outcome of frequent exposure to Torque Teno virus (TTV) through blood transfusion in thalassemia patients with or without hepatitis C virus (HCV) infection Journal of Medical Virology 1, 2008, 80/2 (365-371)	公表国 米国	
販売名（企業名）	①アルブミンーベーリング②アルブミナー5% ③アルブミナー25%④フィブロガミン P⑤ベリプラスト⑥ベリプラスト P コンビセット（CSL ベーリング株式会社）					

研究報告の概要

問題点（サラセミア患者の輸血による TTV 感染）

サラセミア患者は、頻繁にウイルスに暴露された輸血に依存していて肝不全や肝障害を頻繁に合併している。HBV と HCV 検査によるスクリーニング導入以来、この患者グループでの輸血関連の肝炎は激減した。しかしながら既知の肝炎に感染していないサラセミア患者の 37%がいまだに ALT 異常値を示しているが、原因は特定できていない。

TTV は一般の人々の間で高率で広く分布しているが、特にサラセミア患者などの頻繁に輸血を受ける患者では 80%以上が複数の TTV の遺伝子型を保有している。

著者らはアラブ首長国連邦で定期的に輸血を受ける（年間 13-18 回）サラセミア患者 197 名の TTV の遺伝子型およびフェリチン、AST、ALT レベルを検査した。

フェリチン、AST、ALT レベルは TTV 陽性患者群が、陰性患者群より有意に高かったが、HCV と TTV 共に感染した患者群は、TTV 単独感染患者群に比べ ALT が有意に高かった。

TTV 陽性群において ALT 異常値率は、年齢による差はなく、年間の投与間隔や投与数に起因していないことが示唆された。

TTV ウイルスは 27 遺伝子型から成る少なくとも 5 グループに分類される。TTV DNA を RD プライマー、TT6/7/8/9 プライマーおよび NG プライマーの 3 種を用いて増幅して、遺伝子型を解析したが、TTV 感染患者のほとんどが複数の遺伝子型を保持しているので、フェリチン、AST、ALT を上昇に関与する遺伝子を特定できなかった。

筆者らは、TTV 感染が HCV 感染患者の肝疾患を重篤にさせるとは結論できないとしている。重篤な肝疾患の進展には TTV よりも HCV の方が重要な役割を果たしている。

109

使用上の注意記載状況・その他参考事項等

報告企業の意見	今後の対応
現在まで TTV の病原性は十分解明されていないので、当社製品の原料血漿の段階で TTV に対しての検査は実施されていない。 TTV と ALT に関する CPMP の見解(Plasma-derived medicinal products : position paper on ALT testing : CPMP/BWP/385/99）は、TTV DNA 値と ALT 値との間には明確な相関関係はないことを示唆しているとしている。 TTV 検査は実施していないが、本剤の製造工程（60℃10 時間の液状加熱）で不活化されると考えられる。 今後とも情報収集に努める。	今後とも情報収集に努める所存である。

16

Journal of Medical Virology 80:365–371 (2008)

Clinical Outcome of Frequent Exposure to Torque Teno Virus (TTV) Through Blood Transfusion in Thalassemia Patients With or Without Hepatitis C Virus (HCV) Infection

Yu-Wen Hu,[1,2]* Moslih I. Al-Moslih,[3] Mahmoud Talib Al Ali,[4] Samra Uzicanin,[1] Heather Perkins,[1] Qi-Long Yi,[1] Shabnam Rahimi Khameneh,[1] Jun Wu,[5] and Earl G. Brown[2]

[1]*Canadian Blood Services, Ottawa, Ontario, Canada*
[2]*Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada*
[3]*College of Health Sciences, University of Sharjah, Sharjah, UAE*
[4]*Thalassemia and Genetic Center, Al Wasal Hospital, Dubai, UAE*
[5]*Bloodborne Pathogens and Blood Safety Surveillance Division, Centre for Infectious, Disease Prevention and Control, Public Health Agency of Canada, Ottawa, Ontario, Canada*

As a consequence of the high prevalence of TorqueTeno virus (TTV) in blood donors, thalassemia patients frequently acquire various genotypes of this virus through therapeutic blood transfusions. At present, the clinical consequences of TTV infection remain indeterminate for these patients. Here, several hundred thalassemia patients were tested for the presence of TTV and its genotypes using a combination of PCR and clone-based DNA sequencing. Approximately 10% (12/118) of the patients aged 2–20 years remained negative for TTV including eight genotypes of SENV. Ferritin, aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT) levels were invariably lower in TTV-negative patients ($P=0.02$, <0.01, and 0.06, respectively) than in TTV-positive patients. Patients with TTV–HCV co-infection showed elevated ferritin and ALT levels compared with patients with TTV infection alone ($P<0.02$ and $P<0.01$). AST and ALT levels were within the normal range for all TTV-negative patients, whereas abnormal levels of AST and ALT were seen in a significant proportion of TTV-positive patients (30.7% and 33.6%, respectively) and patients with TTV–HCV co-infections (70.0% and 56.6%, respectively). Only TTV-positive patients (28.0%) and patients with TTV–HCV co-infections (36.3%) had hyper-ferritin levels (≥3,000 ng/ml). The genotype(s) of TTV responsible for the liver dysfunction could not be determined. However, high levels of AST and ALT were found to be correlated with detection of a higher number of TTV genotypes in the patients. The data suggests that frequent and persistent TTV infection through blood transfusion is associated with hepatic dysfunction and/or damage in transfusion dependent thalassemia patients. ***J. Med. Virol. 80:365–371, 2008.*** © 2007 Wiley-Liss, Inc.

KEY WORDS: TTV; HCV thalassemia patient; liver disease

INTRODUCTION

Hepatic dysfunction and/or damage are frequent complications in thalassemia patients who depend on blood transfusions that are associated with a high frequency of exposure to viruses. Accordingly, the incidence of transfusion related hepatitis for this group of patients has been markedly reduced since the implementation of blood screening for hepatitis B virus (HBV) and hepatitis C virus (HCV) nucleic acid and antibodies. However, over one third (37%) of thalassemia patients without infection by known hepatitis viruses still have an abnormal alanine-aminotransferase (ALT) pattern [Chen et al., 1999]. The exact cause of the ALT abnormality in those patients remains unknown [Okamoto and Mayumi, 2001].

A virus with a small single-stranded DNA genome was identified by Nishizawa et al. [1997] in Japan from patients with non-A-E transfusion acquired hepatitis in

Grant sponsor: Bayer-Canadian Blood Services-Hema-Quebec; Grant sponsor: University of Sharjah/Research Center, Sharjah, UAE (Partial Support); Grant number: 051019.

*Correspondence to: Yu-Wen Hu, Canadian Blood Services, 1800 Alta Vista Drive, Ottawa, Ontario, Canada K1G 4J5.
E-mail: yu-wen.hu@blood.ca

Accepted 13 October 2007
DOI 10.1002/jmv.21070
Published online in Wiley InterScience (www.interscience.wiley.com)

1997. With reference to the index patient, the virus was originally named TT virus however it is currently renamed as Torque Teno virus (TTV) the type species of the genus Anellovirus, in an unassigned family that is most closely related to the Circoviridae [Hino, 2002]. TTV is widely distributed geographically with a high rate of viremia within the general population [Simmonds, 1998; Cossart, 2000]. Mixed genotype infections are therefore common, particularly in frequently transfused patients such as thalassemia patients where 80% of them carry more than one genotype of TTV [Chen et al., 1999; Okamoto et al., 1999b]. This is presumably due to the high frequency of viral transmission through blood transfusion and the persistent nature of TTV viral infections [Chen et al., 1999; Gallian et al., 1999; Kanda et al., 1999; Kobayashi et al., 1999; Matsumoto et al., 1999; Oguchi et al., 1999; Prati et al., 1999]. TTV viruses have been classified into at least five groups consisting of more than 27 genotypes as a result of the extremely wide range of sequence divergence observed among TTV isolates [Okamoto et al., 1998; Tanaka et al., 1998; Takayama et al., 1999; Worobey, 2000; Okamoto and Mayumi, 2001]. Early studies indicate that TTV might cause some forms of cryptogenetic hepatitis, post transfusion hepatitis and/or other diseases, however these observations have not been confirmed in most subsequent studies although it has been suggested that certain TTV groups or genotypes (e.g., group 4, genotype 1 and genotype 21) might be especially pathogenic and associated with liver or other diseases [Simons et al., 1995; Okamura et al., 2000; Sugiyama et al., 2000; Bendinelli et al., 2001; Maggi et al., 2003; Pifferi et al., 2005; Szladek et al., 2005]. The clinical significance of TTV infection thus remains controversial. [Okamura et al., 2000; Sugiyama et al., 2000; Maggi et al., 2003; Pifferi et al., 2005; Szladek et al., 2005]. Considering that thalassemia patients frequently acquire multiple genotypes of TTV through repeated blood transfusion administered throughout their lifetime, the role of this virus in the development of clinical disease in this group of patients cannot be excluded [Chen et al., 1999]. In addition to TTV, a substantial proportion of thalassemia patients have acquired HCV infection through blood transfusion. It remains uncertain if HCV–TTV co-infections result in more severe biochemical and histological changes compared to TTV infection alone [Charlton et al., 1998; Watanabe et al., 1999; Yuki et al., 1999; Zein et al., 1999; Cleavinger et al., 2000; Meng et al., 2001; Tokita et al., 2002].

Since TTV cannot be cultivated in vitro, PCR is the only available tool for detection of TTV. It has been difficult to determine the clinical significance of TTV infections because diagnostic systems using one or two sets primers for PCR are unable to detect the entire spectrum of TTV genotypes and their variants that exist in individuals [Okamoto et al., 1999b, 2000; Maggi et al., 2003]. Obviously, this has impeded a proper assessment of TTV viral pathogenesis. We recently found that all TTV genotypes (except genotype 21), and all SENV genotypes (A–H) can be detected using three TTV primer sets [Hu et al., 2005]. This finding has provided a more accurate and efficient tool for TTV diagnosis. In this study, we used this efficient primer system combined with clone-based DNA sequencing to investigate the prevalence of various genotypes of TTV with respect to clinical outcome in various age groups of blood transfusion dependent thalassemia patients with or without HCV co-infection.

MATERIALS AND METHODS

Study Groups

A total of 197 thalassemia patients from the United Arab Emirates (UAE) who had received regular blood transfusions (13–18/year) were enrolled in this study, including 54 with hepatitis C virus (HCV) infection. Among these patients a younger group of 118 thalassemia patients (49 female and 69 male) ranging in age from 2 to 20 years (with a median age of 10.8 years) was tested for blood ferritin, AST and ALT levels to study the clinical outcome of TTV and HCV infections. The remaining thalassemia patients ($n = 79$: aged 21–53 years) were only tested for ALT in this study. All samples were negative in standard donor-screening tests including HIV, Human T Cell Leukemia Viruses, and hepatitis A, B and G.

Isolation of Viral DNA

Plasma (100 μl) was used for isolation of viral DNA with a silica gel based membrane while using microspin technology as described in the QIAamp blood kit (QIAGEN, Inc., Mississauga, Ontario).

Amplification and Detection of TTV DNA

Purified TTV DNA was amplified using three sets of nested primers derived from the conserved regions in 5′UTR and ORFs of the TTV genome following the procedure as previously described [Okamoto et al., 1999b; Hu et al., 2005]. They include RD037-038 plus RD051-052 [Okamoto et al., 1998], TT6-7 plus TT8-9 [Hohne et al., 1998], and NG 5′UTR based nested primers (NG054-147 plus NG133-132) [Okamoto et al., 1999a]. TTMV (TTV-like mini virus) was also tested in the thalassemia patients using two sets of TTMV specific primers as described previously [Hu et al., 2005]. The sensitivity of PCR used in this study had previously been evaluated by both DNA dilution and real-time PCR methods. The sensitivity was determined to be ≤ 10 copies/ml. To confirm that thalassemia patients testing negative for TTV were truly negative, the 12 TTV negative samples were subjected to additional PCR reaction conditions including different and lower stringency primer annealing temperatures and additional reaction cycles. PCR amplified TTV DNA was detected on a 1.5% agarose gel using ethidium bromide staining.

Direct DNA Sequencing

Direct DNA sequencing was performed using an automated DNA sequencer (Visible Genetics). A 5′

Cy5.5 labeled sense primer, RD051, was used to produce a sequence of approximately 150 bases from RD primer set PCR fragments. For the PCR products amplified using TT primers, a 5′ Cy5.5 labeled sense primer, TT8, was used to produce a sequence of approximately 150 bases in length. 5′UTR based primers NG133F and NG132R were used for sequencing the PCR products amplified by 5′UTR based primers.

Cloning Based DNA Sequencing

Clonal based DNA sequencing was performed using TOPO- TA™ vector (Amersham Pharmacia Biotech, USA) for cloning of purified PCR products. The TTV sequence was amplified from colonies of *Escherichia coli* using M13 primers (Amersham Pharmacia Biotech, USA). Fifteen clones from each individual were sequenced. Re-amplified PCR products were sequenced using the same procedure as for direct DNA sequencing.

Genotyping and Computer Analysis of Nucleotide Sequences

DNA sequences derived from DNA sequencing were compared using an on line database for the best possible match using the Basic Local Alignment Search Tool (BLAST) program at the National Center for Biotechnology Information home page (http://www.ncbi.nlm.nih.gov/). All further sequence analyses and comparisons were performed using the DNA-STAR Lasergene '99 software package (DNAStar, Madison, WI).

Statistical Analysis

The mean differences in AST, ALT and ferritin between three clinical groups were compared using Student's *t*-tests. Since the sample size for both TTV-negative and TTV + HCV positive groups is small compared to that of the TTV positive group, we performed a matching comparison to increase the comparison power. Each case in the TTV-negative group (or TTV + HCV positive group) was matched to a case of the same age and gender in the TTV-positive group. Paired *t*-test was performed for the matching comparison. In addition, we categorized the AST, ALT and ferritin into normal and abnormal groups using the clinical normal range as the cut-off value, and compared the abnormal rates in the three clinical groups. Chi-square tests were used in the comparison of rates. The SAS software package was used for the statistical analyses.

RESULTS

Clinical Significance of TTV Infection

Ferritin, AST and ALT levels. For a thorough analysis of the clinical data, we first investigated the impact of age and gender on the levels of clinical parameters (Table I). Ferritin, AST and ALT levels were found to be higher in patients aged 11–20 years than in younger patients aged 2–10 years. However, the difference did not reach statistical significance ($P = 0.13$, 0.10 and 0.10 for ferritin, AST and ALT, respectively). All three parameters were slightly increased in male relative to female patients, but again no significant difference was found between different genders ($P = 0.46$, 0.19 and 0.43 for ferritin, AST and ALT, respectively). The data suggest that age and gender are not major factors that significantly contributed to liver disease in thalassemia patients aged 2–20 years. However, since all three parameters were slightly higher in older patients and male patients in comparison with younger patients and female patients, a minor impact of age and gender on liver dysfunction could not be completely excluded. To improve the accuracy of data analysis, we therefore decided to use both age-sex matching and non-age-sex matching methods for evaluating the clinical significance of TTV infection in thalassemia patients.

The results in Table II indicate that by using an exact age-sex matching method, the ferritin, AST and ALT levels were found to be invariably lower in TTV-negative patients compared to the TTV-positive patients ($P = 0.02$, $P < 0.01$ and $P < 0.06$, respectively). The trends of the results generated by non-age-sex matching were, in general, consistent with those derived using the age-sex matching approach, where ferritin, AST and ALT levels were shown to be higher in TTV-positive patients than in TTV-negative patients ($P < 0.02$, $P > 0.05$ and $P > 0.05$, respectively), but in this case only ferritin was significantly elevated. The clinical data regarding TTMV (TTV-like mini virus) negative samples (8.5%, 10/118) was also analyzed and compared to the TTMV positive samples by age-sex matching method. The levels of ALT, AST and ferritin were not significantly different between TTMV-negative and TTMV-positive patients (data not shown). Of the TTV negative patients, 91.7% (11/12) were positive for TTMV. These data further support the observation that TTV, but not TTMV causes liver dysfunction and/or damage. Using age-sex matched groups, the levels of both ferritin and

TABLE I. Ferritin, AST and ALT Levels in Different Age and Gender Groups of TTV Infected Thalassemia Patients

Patients (no.)	Ferritin (ng/ml) (*P*)	AST (IU/L) (*P*)	ALT (IU/L) (*P*)
Age			
2–10 (45)	2290.9 ± 1331.2	35.1 ± 20.7	37.3 ± 47.2
11–20 (47)	2706.3 ± 1274.5 (0.13)	42.9 ± 24.2 (0.10)	53.2 ± 44.8 (0.10)
Gender			
Male (52)	2566.4 ± 131318.5	42.2 ± 24.4	48.8 ± 47.4
Female (40)	2364.1 ± 1296.5 (0.46)	35.9 ± 21.4 (0.19)	41.2. ± 43.8 (0.43)

TABLE II. Comparison of Ferritin, AST and ALT Levels Between TTV Negative Patients, Patients With TTV Infection Alone (TTV+) and With TTV–HCV Co-Infections (TTV + HCV+)

Patients (no.)	Age	Ferritin (ng/ml) (*P*)	AST (U/L) (*P*)	ALT (U/L) (*P*)
Age-sex matching				
TTV– (12)	9.5 ± 5.6[a]	1470.3 ± 514.7	27.1 ± 7.1	22.1 ± 12.2
TTV+ (12)	9.5 ± 5.6	2340.0 ± 733.5 (0.02)	38.8 ± 18.1 (<0.01)	49.3 ± 45.3 (0.06)
No age-sex matching				
TTV– (12)	9.5 ± 5.6	1470.3 ± 514.7	27.1 ± 7.1	22.1 ± 12.2
TTV+ (92)	10.8 ± 4.6	2477.7 ± 1305.6 (0.02)	39.5 ± 23.3 (0.08)	45.5 ± 45.8 (0.11)
Age-sex matching				
TTV+ alone (11)	13.2 ± 4.4	2340.0 ± 733.5	36.2 ± 22.0	27.6 ± 17.0
TTV + HCV+ (11)	13.2 ± 4.4	3112.9 ± 1477.8 (0.02)	53.1 ± 27.6 (0.13)	55.4 ± 27.6 (<0.01)
No age-sex matching				
TTV+ alone (92)	10.8 ± 4.6	2477.7 ± 1305.6	39.5 ± 23.3	45.5 ± 45.8
TTV + HCV+ (11)	13.2 ± 4.4	3112.9 ± 1477.8 (0.08)	53.1 ± 27.6 (>0.05)	55.4 ± 27.6 (>0.05)

[a]Mean ± SD; significant differences are in bold.

ALT were found to be higher in patients with HCV–TTV co-infections than in patients with TTV infection alone ($P = 0.02$ and $P < 0.01$). It was expected that patients with TTV–HCV co-infections would have more severe liver disease than TTV alone as HCV is a serious liver pathogen. Non-age-sex matched analysis showed that all three clinical parameters were higher in patients with TTV–HCV co-infections than in patients with TTV infection alone, but no significant difference was found between the two groups of patients. This suggests that the use of age-sex matching approach is necessary for the clinical data analysis.

Abnormality rate of ferritin, AST and ALT. We found that all 12 TTV-negative patients aged 2–20 years had normal AST (≤40 IU/L ranging from 14 to 40 IU/L) and ALT (≤50 IU/L, ranging from 10 to 48 IU/L) levels. However, AST and ALT levels were elevated in nearly one-third of TTV-positive patients aged 2–20 years (30.7%, 27/88 for AST; and 33.6%, 48/143 for ALT; Fig. 1). Abnormal levels of AST (70%, 7/10) and ALT (56.6%, 30/53) was even higher in patients with TTV–HCV co-infections than patients with TTV infection alone. Data resulting from analysis of abnormalities in three clinical parameters agreed completely with those obtained from the assessment of actual differences in ferritin, AST and ALT levels. Although the ferritin levels were significantly higher ($P = 0.02$) with respect to TTV and HCV positivity for matched patient groups, the ferritin baseline level of the patient group is high due to transfusion treatments. The ferritin level in TTV-negative thalassemia patients was five times higher (1470.3 ± 514 ng/ml; Table II) than normal (10–300 ng/ml). Therefore, we used the hyper-ferritin level value, which is ten times the normal limit (3,000 ng/ml) to calculate the abnormality rate in thalassemia patients. None of the TTV-negative patients had ferritin levels over 3,000 ng/ml with the highest level being 2,440 ng/ml. However, about one third of patients with TTV infection alone (28.0%, 26/93) and patients with HCV–TTV co-infections (36.4%, 4/11) had hyper-ferritin levels (3,000 ng/ml ranging up to 6,250 ng/ml).

One might expect that the ALT abnormality rate should be highest in older patients due to the greater duration of transfusion treatment. In fact, the ALT abnormality rate was the highest (33.6%, 48/143) in young patients (11–20 years) compared to the youngest patients aged 2–10 years (21.2%, 14/66) and the older patient group, between the ages of 21 and 53 (20.9%, 9/43; Fig. 2). This indicates that ALT elevation in thalassemia patients was not dependent or directly attributable to the time span or number of transfusions alone.

TTV Prevalence and Pathogenesis of Various Genotypes

As reported previously [Hu et al., 2005], RD primers are known to specifically amplify genotype 1 (1a and 1b) and TT6/7/8/9 primers detect genotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 14, 15, and 16; mainly in genogroups 1 and 3. TT6/7 primers specifically detect TTV genotypes 2 and 3. NG primers can detect almost all known TTV genotypes except genotype 21, including eight genotypes (A–H) of SENV. Use of the three nested sets of TTV primers

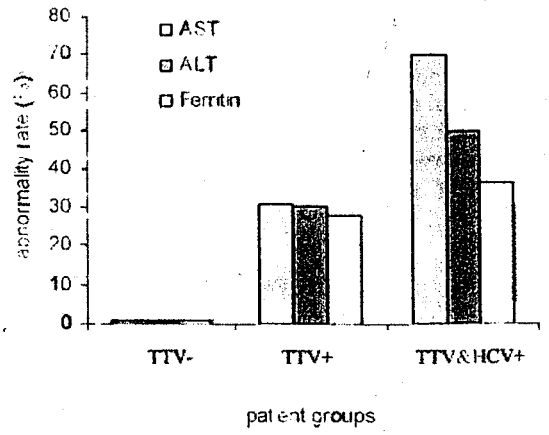

Fig. 1. Abnormality rate of ferritin, aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT) levels in TTV-negative patients (TTV–), TTV-positive patients (TTV+) and patients with TTV–HCV co-infections (TTV and HCV+).

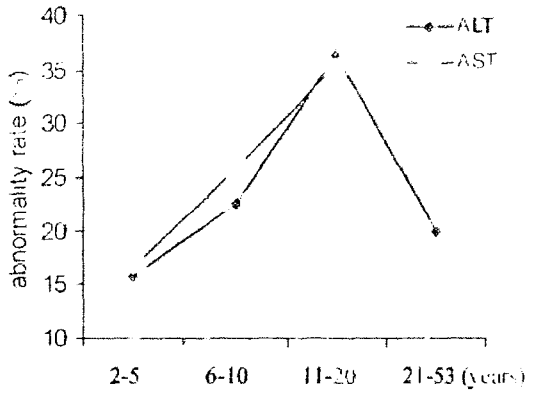

Fig. 2. Abnormality rate of AST (■) and ALT (◆) at different ages in TTV-positive thalassemia patients.

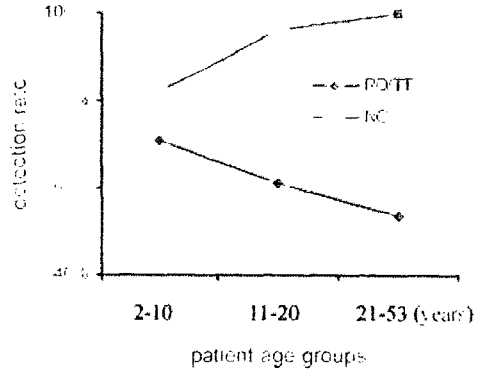

Fig. 3. TTV-positive rates of various genotypes in different age groups of thalassemia patients. The graphs indicate the viremia rates (%) of NG primer-specific genotypes (■), RD/TT6/7/8/9-specific genotypes (◆). The ages of patient groups (2–10, 11–20 and 21–53 years) are plotted against rate of infection.

allowed us to assess the prevalence of various genotypes in different age groups of thalassemia patients. Figure 3 shows that the positivity rate for the genotypes detected by NG primers increased significantly with age from younger (2–10 years) to older groups of patients (ages 11–20 years as well as 21–53 years) with detection rates of 81.8%, 45/55; 96.2%, 50/52; and 100.0%, 30/30, respectively (trend test $P < 0.0001$). In contrast, the detection frequency for the most prevalent genotypes that are detectable by RD/TT6/7/8/9 primers (i.e., genotypes 1–10 and 14–16) declined significantly from younger to older groups. The TTV-positive rate was 71.0%, 27/38; 61.5%, 32/52; and 53.6%, 30/56; for the younger and the two older groups of patients, respectively (trend test $P < 0.001$). The genotypes of amplified TTV viruses were confirmed by clone-based sequencing (see Materials and Methods Section, data not shown). The shifting of predominant TTV genotypes over a patient's lifetime suggests that younger patients have a greater susceptibility to the most prevalent genotypes relative to older patients. Presumably this is due to the fact that some of the older patients may become immune due to prior exposure and subsequent clearance of infection. As a result, these patients are protected from re-infection by some genotypes such as the genotypes detectable by RD/TT6/7/8/9 primers. However, in addition to being exposed to more TTV genotypes, the older patients have also been exposed to less prevalent TTV genotypes over time because of increased exposure due to repeated transfusions. Overall, the TTV viremia rate is higher in older patients. The genotype shifting during a patient's life-time provides strong evidence to indicate that both self-limited and chronic TTV infections exist and that persistence of the virus (at least certain genotypes) may not be life-long in thalassemia patients. The declining prevalence of some genotypes in older patients was paralleled by a decrease in the ALT abnormality rate (see Figs. 2 and 3). This suggests that the most prevalent genotypes such as 1, 2 and 3 may play a more important role in the development of liver disease in thalassemia patients. However, the exact genotype(s) responsible for the elevation of ferritin, AST and ALT levels could not be determined because the majority of TTV infected patients carried more than one genotype.

DISCUSSION

The role of TTV infection in liver disease has been the subject of much debate since the first TT virus was identified in 1997. In our present study, the data suggest that frequent and persistent TTV infection through blood transfusion is associated with abnormal AST, ALT and ferritin levels (seen in about one third of blood-dependent thalassemia patients). This supports the hypothesis that certain genotypes or variants of TTV cause disease when individuals are exposed to these genotypes or their variants for the first time and/or re-exposed to partially cross-reactive or non-cross reactive TTV strains [Bendinelli et al., 2001]. To further assess whether a specific genotype or group of TTV is associated with elevated ALT levels in thalassemiacs, we compared the genotypes infecting patients with abnormal ALT levels (>100 IU/L) and patients with normal ALT levels (<50 IU/L). A total of 480 clones from 32 samples (15 clones for each sample) were genotyped by DNA sequencing. We found that the majority (78.1%, 25/32) of the thalassemia patients were infected with more than one genotype. It was therefore difficult to judge which genotype was responsible for the elevation of ALT levels in patients with mixed genotype infections (data not shown). However, the number of mixed genotypes was found to be significantly increased in patients with abnormal ALT levels (three vs. two genotypes per patient, $P = 0.01$). It appears that ALT elevation is associated with a higher frequency of TTV mixed genotype infections, including transient and persistent infections through blood transfusion.

It is reasonable to propose that multiply transfused thalassemia patients are at a much greater risk of being infected by new and more pathogenic genotypes or strains than blood donors. In the UAE thalassemia patients average over 15 transfusion per year, where over one third of blood donors carry the virus. It would be expected that transfusion dependent thalassemia

patients could be infected by TTV at least six times per year or 60 times during the first 10 years of blood transfusion therapy [Al Moslih et al., 2004]. Thus, patients with a 10-year transfusion history could have been infected or re-infected by all genotypes existing in the UAE. In addition, the extent of virus replication in thalassemia patients may be higher due to the large viral inocula injected directly into the blood stream through transfusion. This is obviously different from the small amount of virus acquired through infection via the oral route in normal blood donors.

It was not possible to conclude that TTV infection enhances the severity of liver disease in HCV infected patients because very few patients infected with HCV alone were available for comparison with patients co-infected with TTV and HCV. It is obvious that HCV plays a more important role than TTV in the development of severe liver disease.

It is well known that TTV infections are persistent. Consequently, the presence of TTV-negative thalassemia patients was unexpected. We do not yet have an explanation for this observation. Perhaps TTV host dependent genetic factors play an important role in determining the resistance or outcome of TTV infection among patients.

Follow-up studies of TTV infection and clearance in TTV-negative and TTV-positive thalassemia patients will eventually provide clues to understanding the natural history and pathogenesis of TTV. Of equal importance, a thorough understanding of the immune response to TTV infection, including viral persistence, quasipecies evolution, and viral immune escape, is needed to characterize the disease causing potential of this new group of viruses.

ACKNOWLEDGMENTS

Y.-W. Hu, M.I. Al-Moslih and E.G. Brown designed the research and wrote the manuscript H.P., S.U. and S.K. performed the research O.-L.Y. and J.W. analyzed the data M.T.A. provided valuable samples.

REFERENCES

Al Moslih MI, Abuodeh RO, Hu YW. 2004. Detection and genotyping of TT virus in healthy and subjects with HBV or HCV in different populations in the United Arab Emirates. J Med Virol 72:502–508.

Bendinelli M, Pistello M, Maggi F, Fornai C, Freer G, Vatteroni ML. 2001. Molecular properties, biology, and clinical implications of TT virus, a recently identified widespread infectious agent of humans. Clin Microbiol Rev 14:98–113.

Charlton M, Adjei P, Poterucha J, Zein N, Moore B, Therneau T, Krom R, Wiesner R. 1998. TT-virus infection in North American blood donors, patients with fulminant hepatic failure, and cryptogenic cirrhosis. Hepatology 28:839–842.

Chen BP, Rumi MG, Colombo M, Lin YH, Ramaswamy L, Luna J, Liu JK, Prati D, Mannucci PM. 1999. TT virus is present in a high frequency of Italian hemophilic patients transfused with plasma-derived clotting factor concentrates. Blood 94:4333–4336.

Cleavinger PJ, Persing DH, Li H, Moore SB, Charlton MR, Sievers C, Therneau TM, Zein NN. 2000. Prevalence of TT virus infection in blood donors with elevated ALT in the absence of known hepatitis markers. Am J Gastroenterol 95:772–776.

Cossart Y. 2000. TTV—A virus searching for a disease. J Clin Virol 17:1–3.

Gallian P, Berland Y, Olmer M, Raccah D, de MP, Biagini P, Simon S, Bouchouareb D, Mourey C, Roubicek C, Touinssi M, Cantaloube JF, Dussol B, de LX. 1999. TT virus infection in French hemodialysis patients: Study of prevalence and risk factors. J Clin Microbiol 37:2538–2542.

Hino S. 2002. TTV, a new human virus with single stranded circular DNA genome. Rev Med Virol 12:151–158.

Hohne M, Berg T, Muller AR, Schreier E. 1998. Detection of sequences of TT virus, a novel DNA virus, in German patients. J Gen Virol 79:2761–2764.

Hu YW, Al Moslih MI, Al Ali MT, Khameneh SR, Perkins H, Diaz-Mitoma F, Roy JN, Uzicanin S, Brown EG. 2005. Molecular detection method for all known genotypes of TT virus (TTV) and TTV-like viruses in thalassemia patients and healthy individuals. J Clin Microbiol 43:3747–3754.

Kanda Y, Chiba S, Tanaka Y, Kami M, Saito T, Asai T, Izutsu K, Yuji K, Ogawa S, Honda H, Mitani K, Yazaki Y, Hirai H. 1999. TT virus in frequently transfused patients. Am J Med 106:116–117.

Kobayashi M, Chayama K, Arase Y, Tsubota A, Suzuki Y, Koida I, Saitoh S, Murashima N, Ikeda K, Koike H, Hashimoto M, Kumada H. 1999. Prevalence of TT virus before and after blood transfusion in patients with chronic liver disease treated surgically for hepatocellular carcinoma. J Gastroenterol Hepatol 14:358–363.

Maggi F, Pifferi M, Fornai C, Andreoli E, Tempestini E, Vatteroni M, Presciuttini S, Marchi S, Pietrobelli A, Boner A, Pistello M, Bendinelli M. 2003. TT virus in the nasal secretions of children with acute respiratory diseases: Relations to viremia and disease severity. J Virol 77:2418–2425.

Matsumoto A, Yeo AE, Shih JW, Tanaka E, Kiyosawa K, Alter HJ. 1999. Transfusion-associated TT virus infection and its relationship to liver disease. Hepatology 30:283–288.

Meng XW, Komatsu M, Goto T, Nakane K, Ohshima S, Yoneyama K, Lin JG, Watanabe S. 2001. Clinical significance of TT virus in chronic hepatitis C. J Gastroenterol Hepatol 16:202–208.

Nishizawa T, Okamoto H, Konishi K, Yoshizawa H, Miyakawa Y, Mayumi M. 1997. A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. Biochem Biophys Res Commun 241:92–97.

Oguchi T, Tanaka E, Orii K, Kobayashi M, Hora K, Kiyosawa K. 1999. Transmission of and liver injury by TT virus in patients on maintenance hemodialysis. J Gastroenterol 34:234–240.

Okamoto H, Mayumi M. 2001. TT virus: Virological and genomic characteristics and disease association. J Gastrenterol 36:519–529.

Okamoto H, Nishizawa T, Kato N, Ukita M, Ikeda H, Iizuka H, Miyakawa Y, Mayumi M. 1998. Molecular cloning and characterization of a novel DNA virus (TTV) associated with posttransfusion hepatitis of unknown etiology. Hepatol Res 10:1–16.

Okamoto H, Nishizawa T, Ukita M, Takahashi M, Fukuda M, Iizuka H, Miyakawa Y, Mayumi M. 1999a. The entire nucleotide sequence of a TT virus isolate from the United States (TUS01): Comparison with reported isolates and phylogenetic analysis. Virology 259:437–448.

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

Okamoto H, Takahashi M, Kato N, Fukuda S, Tanaka T, Miyakawa Y, Mayumi M. 2000. Sequestration of TT virus of restricted genotypes in peripheral blood mononuclear cells. J Virol 74:10236–10239.

Okamura A, Yoshioka M, Kikuta H, Kubota M, Ma X, Hayashi A, Ishiko H, Kobayashi K. 2000. Detection of TT virus sequences in children with liver disease of unknown etiology. J Med Virol 62:104–108.

Pifferi M, Maggi F, Andreoli E, Lanini L, Marco ED, Fornai C, Vatteroni ML, Pistello M, Ragazzo V, Macchia P, Boner A, Bendinelli M. 2005. Associations between nasal torquetenovirus load and spirometric indices in children with asthma. J Infect Dis 192:1141–1148.

Prati D, Lin YH, De MC, Liu JK, Farma E, Ramaswamy L, Zanella A, Lee H, Rebulla P, Allain JP, Sirchia G, Chen B. 1999. A prospective study on TT virus infection in transfusion-dependent patients with beta-thalassemia. Blood 93:1502–1505.

Simmonds P. 1998. Transfusion virology: Progress and challenges. Blood Rev 12:171–177.

Simons JN, Leary TP, Dawson GJ, Pilot-Matias TJ, Muerhoff AS, Schlauder GG, Desai SM, Mushahwar IK. 1995. Isolation of novel virus-like sequences associated with human hepatitis. Nat Med 1:564–569.

Sugiyama K, Goto K, Ando T, Mizutani F, Terabe K, Yokoyama T, Wada Y. 2000. TT virus infection in Japanese children: Isolates from genotype 1 are overrepresented in patients with hepatic dysfunction of unknown etiology. Tohoku J Exp Med 191:233–239.

Szladek G, Juhasz A, Kardos G, Szoke K, Major T, Sziklai I, Tar I, Marton I, Konya J, Gergely L, Szarka K. 2005. High co-prevalence of genogroup 1 TT virus and human papillomavirus is associated with poor clinical outcome of laryngeal carcinoma. J Clin Pathol 58:402–405.

Takayama S, Yamazaki S, Matsuo S, Sugii S. 1999. Multiple infection of TT virus (TTV) with different genotypes in Japanese hemophiliacs. Biochem Biophys Res Commun 256:208–211.

Tanaka H, Okamoto H, Luengrojanakul P, Chainuvati T, Tsuda F, Tanaka T, Miyakawa Y, Mayumi M. 1998. Infection with an unenveloped DNA virus (TTV) associated with posttransfusion non-A to G hepatitis in hepatitis patients and healthy blood donors in Thailand. J Med Virol 56:234–238.

Tokita H, Murai S, Kamitsukasa H, Yagura M, Harada H, Takahashi M, Okamoto H. 2002. High TT virus load as an independent factor associated with the occurrence of hepatocellular carcinoma among patients with hepatitis C virus-related chronic liver disease. J Med Virol 67:501–509.

Watanabe H, Shinzawa H, Shao L, Saito T, Takahashi T. 1999. Relationship of TT virus infection with prevalence of hepatitis C virus infection and elevated alanine aminotransferase levels. J Med Virol 58:235–238.

Worobey M. 2000. Extensive homologous recombination among widely divergent TT viruses. J Virol 74:7666–7670.

Yuki N, Kato M, Masuzawa M, Ishida H, Inoue T, Tabata T, Matsushita Y, Kishimoto H, Sasaki Y, Hayashi N, Hori M. 1999. Clinical implications of coinfection with a novel DNA virus (TTV) in hepatitis C virus carriers on maintenance hemodialysis. J Med Virol 59:431–436.

Zein NN, Arslan M, Li H, Charlton MR, Gross JBJ, Poterucha JJ, Therneau TM, Kolbert CP, Persing DH. 1999. Clinical significance of TT virus infection in patients with chronic hepatitis C. Am J Gastroenterol 94:3020–3027.