Transfusion Transmitted Virus (TTV) Infection in Polytransfused Egyptian Thalassemic Children

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Transfusion Transmitted Virus (TTV) is a naked, single stranded DNA virus, which has been discovered in the serum of a patient with post-transfusion hepatitis of unknown etiology. TTV infection is widespread in the general population, however, the mode of its transmission is still unclear. This study was conducted to search for TTV-DNA positivity rates and to evaluate its clinical significance in both Egyptian polytransfused thalassemic children and healthy children. Serum samples from 33 polytransfused thalassemic children and 30 healthy children were studied for the presence of TTV-DNA by nested PCR using primer sets generated from the untranslated region (UTR) of the viral genome. TTV-DNA was found positive in 39.4% of polytransfused children versus 40% of healthy children, the high rate of viremia observed in healthy children indicates that transfusion route is not the only mode of TTV spread. 60.6% of the polytransfused group were HCV positive. Fifty five percent of HCV positive cases showed TTV-DNA positivity, suggesting common route of infection. No significant difference between ALT and AST values in TTV-DNA positive polytransfused children compared to negative cases. ALT and AST values were statistically significantly higher in HCV positive patients compared to negative cases. On the other hand no significant difference was observed of ALT and AST values in isolated HCV infection versus TTV and HCV co-infection. Thus although TTV infection is quite common among Egyptian, no convincing evidence was found to support its involvement in the pathogenesis of hepatitis in children, more data are still needed for a better understanding of the natural history of TTV infection.

Key words: TTV virus-polytransfused-thalasemia-children

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INTRODUCTION

A novel human DNA virus, designated as transfusion transmitted virus (TTV), was found by molecular cloning in a Japanese adult with post-transfusion hepatitis of unknown etiology (Nishizawa et al., 1997). TTV, a small non enveloped virus with a single-stranded negative polarity circular DNA genome of 3.8 kb, was initially thought to be a circovirus similar to chicken anemia virus, porcine circovirus and other viruses of animals. But it is now under consideration as the possible type species of an independent virus family (Tanaka et al., 2001).

In fact, the epidemiology of TTV infection is not yet fully resolved. A high prevalence of TTV infection has been observed in individuals with parenteral risk exposure such as recipients of blood products (Okamoto et al., 1998a) and intravenous drug users (Biagini et al., 1998). It was also shown that TTV infection is present worldwide among blood donors, ranging from 1% in Taiwan to 62% in Brazil (Boris, 1999). In Egypt, the reported incidence is 29% (Gad et al., 2000) and 35.5% of Egyptian healthy blood donors (Zebrak et al., 2002). However, the prevalence of TTV infection in population at low risk of parenteral exposure, such as blood donors, indicates that TTV is not solely transmitted parenterally. A vertical transmission has been documented (Schreiber et al., 2000) and a community acquired transmission seems probable (Okamoto et al., 1998b).

Some studies, however, revealed that the prevalence rates of TTV infection were not significantly different between diseased patients and healthy individuals (HSU et al., 2003).

Furthermore, little is known about the clinical significance and the natural history of TTV infection. Most previous studies regarding the epidemiology and clinical significance of TTV have been confined to adult patients. A few studies in children also revealed that children can acquire TTV infection early in life by non-parenteral routes and these children seldom develop raised transaminase level or clinical signs of liver diseases (Davidson et al., 1999; Salakova et al., 2004). However, whether or not in children a parenterally-acquired TTV infection or co-infection of TTV with other hepatotropic viruses results in similar clinical features requires further study. Without such information, the understanding of the natural history of TTV infection in children would be incomplete.

In the current case control study, TTV infection was investigated in healthy as well as polytransfused thalassemic children aiming to assess the frequency of TTV infection in them and to investigate the possible relation of viremia with hepatic dysfunction.

MATERIALS AND METHODS

This case control study included thirty three patients with thalassemia major, 17 boys and 16 girls, with a mean age of 7.5 years, in the age range, 3 to 12 years. They were regularly followed in the hematology unit of Banha Children Hospital (Banha, Egypt) and the National Research Centre (Cairo, Egypt) during the year 2005. All patients received regular transfusions, mostly every 2 or 3 weeks. The mean number of blood transfusion of the patients were 5.57 ± 3.20 (range 7-100). Thirty healthy children matched in age and sex with the patients and without any history suggestive of liver disease or history of previous blood transfusion were selected as controls.

Both patients and controls were vaccinated against hepatitis B virus (HBV) infection and were having the serological pattern of vaccination. Thus, we did not retain the markers of HBV infection in the present study. All the patients were receiving deferoxamine therapy according to the current protocols and the mean of their serum ferritin was 2224.82 ± 1961.3 ng mL⁻¹ (range 337-9480 ng mL⁻¹).

Informed consent was obtained from the parents of each healthy and thalassemic studied before sampling.

Patients and controls were subjected to the following

- Complete history taking.
- Full clinical examination.
- Laboratory investigation included:
  - Serum ferritin levels.
  - Serum ALT (alanine amino transferase)
  - Serum AST (aspartate transferase)
  - Anti HCV-antibodies (anti-hepatitis C-virus)

Blood samples of the patients were collected at the time of transfusion. Serum samples were separated from 2 mL⁻¹ of blood within 2 h of venipuncture, aliquoted carefully to avoid contamination and degradation of viral nucleic acid and were stored at -70°C until tested.

- Serum ALT and AST were measured on the Olympus AU400 apparatus.
- Anti HCV was assayed by Eliza (DiaSorin enzyme immunoassay kit).
- Serum ferritin was measured by Eliza (Biocheck, Ferritin Enzyme Immunoassay).

Detection of TTV virus by nested PCR: The nucleotide sequence of TTV is conserved to a higher extent in the untranslated Region (UTR) than in coding regions, such as the N22 region in open reading frame.
As a result, TTV-DNA is detected more frequently by PCR with UTR primers (Okamoto et al., 1999a).

UTR PCR, which detects TTV of essentially all 16 genotypes, was carried out with nested primers according to the method described by Okamoto et al. (2000). DNA was extracted from serum samples using DNA extraction Kit (Fermentas) according to the manufacturer's instructions.

The first round PCR was performed for 35 cycles with primers NG 133 (sense, 5' - GAG GCA TCC CCG AAT GGC TGA G-3', representing nucleotides nt 91 to 115) and NG 352 (antisense, 5' - GAG CCT CGC TRG CCC GGC CAG-3' [nt 229 to 252], R = A or G).

The second round was performed for 25 cycles with NG 249 (sense, 5' - GAG TTT CCT CGC TCG GCC CCG GC-3' [nt 111 to 133]) mixed with an equal amount of the primer with the underlined four nucleotides replaced by ATGC and NG 351 (antisense, 5' - CCC ATR GCC CGC CCA TGC CCG AGC-3' [nt 221 to 244]).

The amplification product of the first round PCR was 162 bp and that of the second round PCR was 134 bp. The PCR products were run on electrophoresis in 3% agarose gel, stained with ethidium bromide and photographed under ultraviolet light (Fig. 1).

**Statistical analysis:** SPSS for Windows, version 7.0 computer program was used for statistical analysis. A p-value of less than 0.05 was considered statistically significant. The t-test was used to compare between 2 independent means. Non-parametric Mann-Whitney U test was used when parametric tests were not applicable. Data are represented as the mean±SD.

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**RESULTS**

The TTV positive cases among polytransfused thalassemic children comprised 39.4% while those of healthy children comprised 40% (Table 1).

In the 33 polytransfused thalasemic children only two patients showed isolated TTV infection, nine patients showed isolated hepatitis C virus (HCV) infection, eleven patients showed co-infection by both agents and eleven patients were free from both agents as shown in Table 2.

The main laboratory data of thalassemic children are summarized in Table 3. The comparison between different thalassemic groups as regards laboratory data are shown in Table 4. ALT and AST were statistically significantly higher in children with HCV infection compared to negative cases. On the other hand, there was no significant difference between TTV positive and negative cases as regards these two parameters. A Comparison of ALT and AST in isolated HCV infection versus HCV and TTV co-infection is shown in Table 5, there was also no significant difference between these 2 groups regarding these 2 parameters.

### Table 1. Distribution of TTV-DNA positivity in polytransfused children and controls

<table>
<thead>
<tr>
<th>TTV-DNA</th>
<th>Polytransfused children</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive n (%)</td>
<td>13 (39.4)</td>
<td>20 (66.6)</td>
</tr>
<tr>
<td>Negative n (%)</td>
<td>20 (60.6)</td>
<td>10 (33.3)</td>
</tr>
</tbody>
</table>

**Table 2. Distribution of isolated HCV-AB positive, TTV-DNA positive, co-infection, and free cases in polytransfused children**

<table>
<thead>
<tr>
<th>Isolated HCV-AB</th>
<th>Isolated TTV-DNA</th>
<th>Co-infection</th>
<th>Free children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive cases</td>
<td>2 cases</td>
<td>1 case</td>
<td>11 cases</td>
</tr>
<tr>
<td>Negative cases</td>
<td>1 case</td>
<td>11 cases</td>
<td>11 cases</td>
</tr>
</tbody>
</table>

AB: Antibodies, HCV: Hepatitis C Virus, TTV: TT Virus

### Table 3. Laboratory data of polytransfused thalassemic children

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean±SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>7.5±4.5 (3-12)</td>
</tr>
<tr>
<td>ALT (U L⁻¹)</td>
<td>64.6±44.2 (21-215)</td>
</tr>
<tr>
<td>AST (U L⁻¹)</td>
<td>63.6±48.8 (17-239)</td>
</tr>
<tr>
<td>Protein (g mL⁻¹)</td>
<td>23.2±31.3 (14-90)</td>
</tr>
</tbody>
</table>

ALT: Alanine Amino Transferase, AST: Aspartate Transferase

### Table 4. Comparison of laboratory data among different thalassemic groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TTV infected</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U L⁻¹)</td>
<td>75.2±5.2 (9)</td>
<td>0.27</td>
</tr>
<tr>
<td>AST (U L⁻¹)</td>
<td>70.3±4.2 (19)</td>
<td>0.54</td>
</tr>
<tr>
<td>Protein (g mL⁻¹)</td>
<td>23.2±3.1 (14-90)</td>
<td>0.61</td>
</tr>
</tbody>
</table>

HCV infected

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Positive n = 20</th>
<th>Negative n = 13</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U L⁻¹)</td>
<td>69.5±30.8 (7)</td>
<td>77.6±50.0 (7)</td>
<td>0.11</td>
</tr>
<tr>
<td>AST (U L⁻¹)</td>
<td>61.5±30.4 (6)</td>
<td>77.5±45.0 (6)</td>
<td>0.01</td>
</tr>
<tr>
<td>Protein (g mL⁻¹)</td>
<td>23.2±3.1 (14-90)</td>
<td>27.6±3.1 (14-90)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

ALT: Alanine Amino Transferase, AST: Aspartate Transferase, HCV: Hepatitis C Virus, TTV: TT Virus, * p<0.05

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Fig. 1: The analysis of PCR product in a 3% agarose gel by electrophoresis and stained with ethidium bromide. Lane 1: Shows 50 bp DNA ladder. Lane 6, 14: Amplification product of the first round PCR: 162 bp. Lane 8, 9, 15: Amplification product of the second round PCR: 134 bp.
Table 5: Comparison of ALT and AST in isolated HCV infection versus HCV and TTV co-infection

<table>
<thead>
<tr>
<th></th>
<th>Isolated HCV infection n = 9</th>
<th>HCV and TTV Co-infection n = 11</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>76.66±54.9</td>
<td>78±57.11</td>
<td>0.96</td>
</tr>
<tr>
<td>AST</td>
<td>82.66±71.83</td>
<td>73.63±46.66</td>
<td>0.73</td>
</tr>
</tbody>
</table>


DISCUSSION

Transfusion Transmitted Virus (TTV) is prevalent worldwide with the highest rates detected in the African and South American countries (Salakova et al., 2004).

In the present study, using UTR primers, the prevalence of TTV-DNA was nearly equal in polytransfused Egyptian children (39.4%) and healthy children (40%). These findings agree with Yarar et al. (2005) who used primers of ORF/N/1 but disagree with Hsu et al. (2003), in which the prevalence of TTV-DNA was significantly higher in polytransfused thalassemic children than healthy children by using either N-22 primers or UTR primers. This finding is also against the initial report that the most common route of TTV infection is via transfusion of contaminated blood and blood products (Nishizawa et al., 1997).

The discordance between the different PCR assay may be due to the high variability of the TTV genome or mixed infections with various TTV genotypes in which other strains have much higher titers than type 1 (Okamoto et al., 1999a, b).

Although UTR primers have high sensitivity, the conserved region amplified by this primer set has lower heterogeneity and is not appropriate for genotyping (Hsu et al., 2003).

In the present study, all the TTV-DNA positive healthy children were without transfusion history, which supports the hypothesis that TTV can be transmitted by routes other than transfusion (Salakova et al., 2004).

Furthermore, variation in the TTV prevalence in children from 5.1% in Japan (Goto et al., 1999) to 54% in the Democratic Republic on Congo (Davidson et al., 1999) is also suggestive of the possible involvement of other specific environmental factors in the acquisition of TTV infection. This agrees with Salakova et al. (2004) and Komatsu et al. (2004), in which the main source of TTV infection in children is presumed to be their mothers, transmitted via other routes in the course of daily contact. Indeed, if a serological test evidencing a past and resolved TTV infection was available, such an assay, combined to PCR, would reflect the total TTV exposure. All the epidemiological studies of TTV to date, including the present study, underestimate the true prevalence of TTV exposure since they were based only on the detection of viral DNA by PCR.

The pathogenic role of TTV in liver disease remains controversial (Komatsu et al., 2004). In this study TTV viremia was not associated significantly with abnormal ALT and AST levels. This agrees with Odemis et al. (2004) and Yarar et al. (2005).

So far, many studies have failed to demonstrate an association between TTV infection and liver disease. It is thus important to observe isolated infections with this agent.

On the other hand, there was no statistically significant difference between the mean ALT and AST in isolated HCV positive patients versus TTV and HCV co-infected patients, this suggests that TTV does not increase the severity of chronic liver disease, which agrees with Hsu et al. (2003) and Par et al. (2004).

Present study showed also a significantly high prevalence of TTV infection in polytransfused thalassemic children with hepatitis C positive versus negative patients (55% of HCV positive cases are co-infected with TTV) implying that HCV and TTV may share common modes of transmission which agrees with Salakova et al. (2004).

CONCLUSIONS

Present results indicate that TTV infection is quite common among Egyptian children. No convincing evidence was found to support the involvement of TTV in the pathogenesis of hepatitis in children. Thus, it is evident that more data are still needed for a better understanding of the natural history of TTV infection and further study is needed to clarify the role of TTV. Nevertheless, the spectrum of diseases studied in association with TTV infection is very narrow and justifies keeping TTV in the category of "orphan" viruses.

REFERENCES


