TT Virus DNA Among Hemodialysis Patients in Alexandria

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Since TTV was discovered a few years ago, it was a target for many studies; however, there is still a poor understanding of its mechanism of transmission and pathogenic potential. In the present study, we aimed to evaluate the presence of TTV among hemodialysis patients. One hundred and twenty eight serum samples obtained from patients undergoing hemodialysis were tested for the presence of TTV DNA using PCR technique, and the levels of liver enzymes were also determined. TTV DNA was detected in 83 (64.8%) out of the 128 hemodialysis patients. There was significant positive statistical relation between the presence of TTV DNA and history of blood transfusion. However, there was no significant effect of the duration of dialysis or the levels of liver enzymes on the presence of TTV DNA. This significant high rate of isolation highlights the need to further investigations and the development of sensitive and simple detection methods to understand the epidemiology and natural history of TTV infection.

Key words: TTV, hemodialysis, PCR.

INTRODUCTION

In 1997, Nishizawa First reported a virus which was associated with post transfusion hepatitis and named it TTV, after the initials of the index patient, and also stand for “Transfusion transmitted virus” but recently TTV established for “Torque teno virus”. (1,2) TTV is an unenveloped single stranded and circular DNA virus, which consists of approximately 3852 nucleotides and its size ranges from 30 to 50 nm. (3) TTV was primarily belonged to circoviridae family. (4) But recent taxonomy established that the virus is belonging to a novel family called circinovirdae. (5)

Epidemiological studies suggest that TTV is transmitted by blood transfusion, hence its name. (6) Being unenveloped, TTV is shed via the bile into feces of infected individuals, for possible fecal-oral rout of transmission, the dual transmission modes of TTV may enhance its deep, wide penetration into general population. (7) Being existed in many body fluids enhance its ability to be transmitted by maternal, sexual and respiratory routes. (8-10) Species-specific TTVs are reported in high frequencies in farm animals, Hence, TTV is a ubiquitous virus, being highly prevalent in many species. (11)

Till now, several attempts have been made to link TTV to the etiology of a specific disease. Several studies showed the presence of TTV DNA in patients with broad spectrum liver disorders such as acute, chronic hepatitis B and C, Fulminant hepatic failure, cryptogenic cirrhosis, hepatocellular carcinoma. (12-14) However, the association between TTV and hepatitis remain doubtful. The laboratory studies recently reported the presence of TTV DNA in specific human cancers, most notably in cancers of gastrointestinal tract, lung, and breast cancers and number of multiple myelomas. (15)

Chronic Renal failure (CRF) is a condition resulting from a multitude of pathologic processes that lead to derangement and insufficiency of renal excretory and regulatory function. (16) Renal failure is the result either of primary renal disease or of renal damage in a multi-system disorder. (17) Chronic hemodialysis (HD) is directly responsible for the maintenance of life for CRF patients. Although the benefits of this therapy are unquestioned, many complications have been associated with HD. The dialysis setting has been recognized as a high-risk environment for the transmission of blood-borne infections to both patients and health care workers. There is a high risk of indirect and direct transmission of infectious agents in chronic hemodialysis, as vascular access is needed on a regular basis. This results in an increased potential for acquiring nosocomial infections via equipment, environmental surfaces or the hands or gloves of any careers, which become contaminated by potentially infectious blood or other body fluids. (18)

The natural history of TTV in dialysis is an area of avid research since TTV has a propensity for establishing long-term infection in HD patients. A high prevalence of
TTV infections has been reported among patients undergoing hemodialysis. However, the transmission route of the virus is still unknown and the question of any association between duration of HD or previous transfusion and TTV infection is still a matter of controversy. (19, 20)

The aim of this study is to estimate the prevalence of TTV DNA among hemodialysis patients in Alexandria Using PCR technique. Also determination of the levels of serum transaminases: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) among those patients.

MATERIAL AND METHODS

One hundred and twenty eight hemodialysis patients were included in this study, 66 males and 62 females, their ages ranged from 14 to 70 years with the mean age of 42 years.

Blood samples were obtained from patients attending different hemodialysis units in Alexandria: Al-Mowasa Gamal Abd El Naser (60 blood sample), El-Meiry (54 blood sample) and Al-Mowasa El-Kabareey hemodialysis unit (14 blood sample). Samples were collected throughout the period of September, October and November, 2005.

Detection of TTV DNA by PCR technique

Total DNA from 200 ul serum was extracted using QIAamp DNA Mini Kit (QIAGEN Companies). Pupe Taq ready-to-go PCR Beads (Amersham Biosciences) were used for amplifications. These beads contain all the reagents that are necessary for PCR reaction except primers and template DNA. The primers employed were: T801 sense primer (5’ – GCTACGTCACAAACCACGTG-3) and T935 antisense primer (5’- CTBCGGTTGTGAAAACCTCACCC-3’).

Ten µL of the extracted DNA was added to each PCR bead together with the sense and antisense primers with a final volume of 25 ul. The tubes were transferred to the thermocycler (Perkin Elmer) where they were subjected to initial denaturation (95 C for 10 min), then 55 cycles of denaturation (94°C for 20 seconds), annealing (60°C for 20 seconds) and extension (72°C for 30 seconds), followed by final cycle of extension (72 C for 5 min). The PCR products were separated by electrophoresis on 2% agarose gel, stained with ethidium bromide, and photographed under UV light.

Determination of serum ALT and AST levels

Sera were tested for ALT and AST by RANDOX, Alanine Aminotransferase and Aspartate Aminotransferase EC 2.6.1.2 IFCC kit. This is an optimized standard method according to the concentrations recommended by the IFFCC (UV Method).

RESULTS

Out of the 128 hemodialysis patients who attended different hemodialysis units in Alexandria; 20 patients were serologically positive for anti HCV, 8 patients were serologically positive for HBsAg and 100 hemodialysis patients were serologically negative for both HCV and HBV.

Table 1 shows that TTV DNA was detected by PCR technique in 83 (64.8%) out of 128 hemodialysis patients. Out of the 100 HD patients free from hepatitis B or C, 63 (63%) were positive for TTV DNA, while 37 (37%) were negative.

From the 8 patients who had HBV, 7 were positive for TTV DNA and only one was negative. While the remaining 20 patients who had HCV, 13 were positive for TTV DNA and 7 were negative. The results were statistically insignificant X² = 1.95.

Table 2 demonstrates that there was a highly significant positive relation between the presence of TTV DNA and history of blood transfusion among hemodialysis patients X² = 3.803. As Out of 93 patients with positive history of blood transfusion, 65 (69.9%) were TTV DNA positive, while the remaining 28 (30.1%) were TTV DNA negative. While out of 35 patients with negative history of blood transfusion, 18 (51.4%) were TTV DNA positive, while the remaining 17 (48.6%) were TTV DNA negative.

Table 3 shows that there was no significant statistical relation between the presence of TTV DNA and the duration of dialysis MCP = 4.179.
Table 4 shows that out of 82 patients with normal ALT levels, 52 (63.4%) were positive for TTV DNA, while out of 46 patients with elevated ALT levels 31 (67.4%) were positive for TTV DNA. The result was not statistically significant $X^2 = 0.204$.

Table 5 demonstrates that similar finding was observed regarding the AST levels, as there was no significant statistical relation between TTV infection and ALT levels among hemodialysis patients $X^2 = 0.679$.

Table (1): TTV DNA among 128 hemodialysis patients.

<table>
<thead>
<tr>
<th>History of liver disease</th>
<th>TTV DNA</th>
<th>Total</th>
<th>Test of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ ve</td>
<td>- ve</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NO %</td>
<td>NO %</td>
<td></td>
</tr>
<tr>
<td>Negative hepatitis B and C</td>
<td>63 63</td>
<td>37 37</td>
<td>100 100</td>
</tr>
<tr>
<td>HBV</td>
<td>7 87.5</td>
<td>1 12.5</td>
<td>8 100</td>
</tr>
<tr>
<td>HCV</td>
<td>13 65</td>
<td>7 35</td>
<td>20 100</td>
</tr>
<tr>
<td>Total</td>
<td>83 64.8</td>
<td>45 35.2</td>
<td>128 100</td>
</tr>
</tbody>
</table>

$x^2 = 1.95$

Table (2): The relation between TTV DNA and history of blood transfusion.

<table>
<thead>
<tr>
<th>Blood transfusion</th>
<th>TTV DNA</th>
<th>Total</th>
<th>test of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ ve</td>
<td>- ve</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NO %</td>
<td>NO %</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>65 69.9</td>
<td>28 30.1</td>
<td>93 100</td>
</tr>
<tr>
<td>Negative</td>
<td>18 51.4</td>
<td>17 48.6</td>
<td>35 100</td>
</tr>
<tr>
<td>Total</td>
<td>83 64.8</td>
<td>45 35.2</td>
<td>128 100</td>
</tr>
</tbody>
</table>

$x^2 = 3.803^*$

* Significant
Table (3): The relation between TTV DNA and duration of dialysis.

<table>
<thead>
<tr>
<th>Duration of dialysis</th>
<th>TTV DNA</th>
<th>Total</th>
<th>Test of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ ve</td>
<td>- ve</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NO</td>
<td>%</td>
<td>NO</td>
</tr>
<tr>
<td>&lt; 1</td>
<td>17</td>
<td>68</td>
<td>8</td>
</tr>
<tr>
<td>&lt; 5</td>
<td>40</td>
<td>71.4</td>
<td>16</td>
</tr>
<tr>
<td>&lt; 10</td>
<td>15</td>
<td>62.5</td>
<td>9</td>
</tr>
<tr>
<td>&lt; 15</td>
<td>7</td>
<td>46.7</td>
<td>8</td>
</tr>
<tr>
<td>&gt; 15</td>
<td>4</td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>83</td>
<td>64.8</td>
<td>45</td>
</tr>
</tbody>
</table>

MCP = 4.179

Table (4): The relation between TTV DNA and ALT levels.

<table>
<thead>
<tr>
<th>ALT level</th>
<th>TTV DNA</th>
<th>Total</th>
<th>Test of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ ve</td>
<td>- ve</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NO</td>
<td>%</td>
<td>NO</td>
</tr>
<tr>
<td>Normal</td>
<td>52</td>
<td>63.4</td>
<td>30</td>
</tr>
<tr>
<td>Elevated</td>
<td>31</td>
<td>67.4</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>83</td>
<td>64.8</td>
<td>45</td>
</tr>
</tbody>
</table>

x^2 = 0.204
Table (5): The relation between TTV DNA and AST levels.

<table>
<thead>
<tr>
<th>AST level</th>
<th>TTV DNA + ve</th>
<th>TTV DNA - ve</th>
<th>Total</th>
<th>Test of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO</td>
<td>%</td>
<td>NO</td>
<td>%</td>
</tr>
<tr>
<td>Normal</td>
<td>55</td>
<td>64.7</td>
<td>30</td>
<td>35.3</td>
</tr>
<tr>
<td>Elevated</td>
<td>28</td>
<td>65.1</td>
<td>15</td>
<td>34.9</td>
</tr>
<tr>
<td>Total</td>
<td>83</td>
<td>64.8</td>
<td>45</td>
<td>35.2</td>
</tr>
</tbody>
</table>

DISCUSSION

Dialysis patients are at high risk of acquiring parental infection. The frequency of TTV infection among patients on maintenance hemodialysis varies widely. The geographical distribution, the methods used for TTV DNA testing, the size of the study group, and the presence of various demographic, virological or clinical features of dialysis patients all help account for the differences. (6)

In the present study, TTV DNA was detected by PCR technique in 83 (64.8%) out of 128 hemodialysis patients. The prevalence of TTV DNA in various hemodialysis patients was studied by many investigators.

Okomoto et al. (1998) (21) found a high prevalence of TTV (46%) in patients on maintenance hemodialysis. Gallian et al. (22) showed that the prevalence of TTV DNA among patients with ESRD was 48%. In another study, in a series of 115 patients undergoing hemodialysis from Japan, TTV DNA was detected in 51%. (23)

Many studies gave different rates of TTV detection among hemodialysis patients ranging from 13.8% to 68%. (24-27) Gallian and Coworkers (22) highlighted that the prevalence of TTV was higher in HD patients originating from Africa 42.8% as against 24.3% in European patients.

In this study, a statistical significant association between history of blood transfusion and prevalence of TTV DNA was detected among hemodialysis patients.

In agreement with other studies, as that conducted by Simmonds et al. (28) (1998), who showed that in hemophiliacs the prevalence of TTV increased with the amount of clotting factor treatment received and was also dependent on whether the blood concentrates tested had been virally inactivated. (29)

In a French study including multi transfused patients, TTV was related to receiving a high number of blood transfusions. In a study by Baysen et al. (26) (2003) they found a significant association between TTV viral load and number of transfusion.

In another study Prescoit et al. (2004) (30) they detected the highest prevalence of TTV in hemophiliacs and IVDU, which supports the importance of parenteral route of transmission of TTV.

In contrast, Ikeuchi et al. (24) (1999) found no association between the amount of blood received in the past and TTV viremia and concluded that blood transfusion was not an important route of TTV transmission. In a study by Abe et al. (31) they found that TTV viremia is widespread in the general population in many countries. Such as
extremely high prevalence of TTV infection in general population suggests that TTV may be transmissible not only by blood but also by non parenteral route.

In this study, the presence of TTV was unrelated to the duration of dialysis. The same finding was reported in many studies. Yuki et al. (32) (1999) found that TTV infection was found irrespective of time on HD treatment. Gallian et al. (22) (1999) found no significant relationship between TTV infection and duration of HD treatment. Also, two other studies (6, 27) in 1999, found that patients on maintenance hemodialysis become infected soon following the initiation of dialysis suggesting that whether or not a patient is receiving hemodialysis is a more important factor than the duration of dialysis.

In agreement with previous studies, no relation between TTV infection and the duration of hemodialysis was found by Boysen et al. (2003). (26) In contrast in a study by Sawada et al. (1998), showed that the prevalence of TTV infection may depend on the number of years that patients have been receiving hemodialysis. (20)

In this work, there was no statistical significant correlation between the presence of TTV DNA and elevation of ALT and AST levels.

Several studies have shown the same results. A study by Gallian et al. (22) 1999 found no significant relationship between TTV infection and either biological markers of hepatic cytolysis (transaminases). Kato et al. (33) (2000) reported that the mean level of ALT did not differ between TTV DNA positive and negative individuals in the presence or absence of other hepatotropic viruses. The same results also reported by Gad et al. (34) (2000). Lefrere et al. (35) (2000), found that, the majority of TTV carriers had no biochemical evidence of liver diseases. Furthermore, Choi et al. (36) (2003), in a study on patients on maintenance hemodialysis in Korea, reported the absence of relation between TTV and liver disease.

In contrast, several studies have shown a correlation between TTV titer and elevation of serum ALT levels. Itoh et al. (37) (1999), reported a higher prevalence of TTV in blood donors with elevated ALT levels (32%) than in those with normal levels (16%). According to the authors these findings strengthen the association of TTV with non A to G hepatitis. Utsunomiya and colleagues (38) (1999) reported that ALT activity was more frequent in TTV-positive patients than TTV-negative individuals on maintenance hemodialysis. Rodriguez-Inigo et al. (39) (2000), in a study to detect TTV DNA in hepatocytes by hybridization, stated that a statistically significant positive correlation between the ALT levels and the number of TTV infected hepatocytes as well as with intrahepatic TTV DNA titers was found, assuming that TTV might be responsible for some cases of cryptogenic hepatitis.

In conclusion, TTV was isolated at high frequency among hemodialysis patients, so strict adherence to the CDC guidelines during dialysis is recommended. This significant high rates of isolation highlights the need to the development of sensitive and simple detection methods and serodiagnostic antibody assays to understand the epidemiology and natural history of TTV infection. The continued search for TTV in non liver related clinical setting will help to determine whether the virus contribute to other human diseases.

REFERENCES


5- Springfield C, Bugert J, Schnizler P. TT virus as a human pathogen; significance


