Surveillance and impact of occult hepatitis B virus, SEN virus, and torque teno virus in Egyptian hemodialysis patients with chronic hepatitis C virus infection

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A B S T R A C T
Objectives: Egypt ranks first regarding the prevalence of hepatitis C virus (HCV) infection. Many patients have concomitant diseases like kidney disorders requiring hemodialysis, a procedure carrying the hazard of transmitting other hepatitis viruses. The purpose of this study was to investigate for occult hepatitis B virus (HBV), SEN virus (SENV), and torque teno virus (TTV) among chronic HCV patients on maintenance hemodialysis to identify their impacts.

Methods: A total of 325 hemodialysis patients were enrolled and divided into two groups based on HCV RNA testing results. Blood samples were collected before hemodialysis. Sera were tested for hepatitis B core antibodies (anti-HBc) and hepatitis B surface antibodies (anti-HBs) using ELISA. HBV, SENV, and TTV DNA were detected by PCR. The serum alanine aminotransferase (ALT) level was measured.

Results: Anti-HBC and HBV DNA were detected in 73.1% and 50.8% of group 1 versus 36.4% and 22.6% of group 2. The serum ALT level was higher in group 1 than group 2. SENV was detected in 11.5% of group 1 versus 8.2% of group 2. TTV was detected in 29% of group 1 versus 27% of group 2.

Conclusions: There is an increased prevalence of occult HBV in our locality among chronic HCV patients undergoing hemodialysis. The existence of SENV and TTV viremia has no clinical impact.

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Introduction

Egypt ranks first among countries regarding the prevalence of hepatitis C Virus (HCV) infection (Amer et al., 2015). Many patients have associated diseases like kidney disorders that necessitate hemodialysis (HD). The HD procedures pose a risk of transmitting other viral hepatitis infections (Karkar et al., 2006). Among these are hepatitis B virus (HBV), SEN virus (SENV), and torque teno virus (TTV). The causal relationship of the latter two viruses in hepatic disease is controversial (Kazemi et al., 2015; Omar et al., 2008).

HBV is a double-stranded DNA virus belonging to the family Hepadnaviridae, genus Orthohepadnavirus (Datta et al., 2012). HBV infection is classified into three categories: acute, chronic, and occult hepatitis B infection (Inoue and Tanaka, 2016). Occult hepatitis B infection (OBI) is defined as the detection of HBV DNA in the serum or liver of individuals by precise laboratory tests in the absence of hepatitis B surface antigen (HBsAg), with or without serological markers of previous viral exposure (Raimondo et al., 2008). The clinical importance of OBI includes the possibility of HBV transmission in patients receiving blood transfusion and organ transplantation, especially the liver (Squadrito et al., 2014). If reactivated, it can be a reservoir for full-blown hepatitis (Nakamoto et al., 2014). In HCV co-infected patients and patients with liver disease due to alcohol abuse or other unidentifiable...
etiology, OBI may enhance the progression to cirrhosis and hepatocellular carcinoma (HCC) (Squadrito et al., 2014).

SENV, a member of the Circoviridae family, is a small single-stranded, non-enveloped circular DNA virus (Pirouzi et al., 2014). It is transmitted by parenteral and vertical routes (Sagir et al., 2004). Nine different strains (A through I) have been identified. Most clinical studies have focused on strains D and H because of their link with non-A-E hepatitis. Strains of SENV have been detected in hepatitis patients as well as in healthy individuals (Cakaloglu et al., 2008; Kojima et al., 2003; Omar et al., 2008).

TTV is a single-stranded circular DNA virus and is classified as a member of the Circoviridae family (Irshad et al., 2006). Five distinct genogroups have been identified (groups 1 to 5) (Peng et al., 2002). TTV is present in body fluids and internal organs, and is transmitted through enteric, vertical, and parenteral routes (Colacino and Heinz, 2012; Gerner et al., 2000).

The purpose of this study was to estimate the prevalence of OBI, SENV, and TTV in patients with chronic hepatitis C (CHC) on maintenance HD, in order to identify their impacts.

Materials and methods

Ethical approval

The study was approved by the Institutional Review Board, Faculty of Medicine, Zagazig University, Zagazig, Egypt. Written informed consent was obtained from each study participant. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki (Carpenter et al., 2003).

Subjects

Three hundred and twenty-five patients with end-stage renal disease, undergoing maintenance HD, participated in this cross-sectional study. They had undergone three sessions per week of HD for a duration ranging from 6 months to 9 years. These HD patients were divided into two main groups based on the results of HCV RNA testing by PCR. Group 1 included 130 HD patients who tested positive for HCV RNA, while group 2 included 195 HD patients who tested negative for HCV RNA. Their full clinical history was collected. Abdominal ultrasound, computed tomography, or magnetic resonance imaging were done to exclude any hepatic mass suspicious for HCC. The sociodemographic variables of the study participants were collected using a questionnaire. All of the HD patients were seen in the HD units of Zagazig University Hospital, Zagazig, Egypt, during the period between June 2017 and June 2018. The following exclusion criteria were applied: vaccination against HBV, HBsAg positivity, using potentially hepatotoxic drugs, history of alcohol abuse, or diagnosis of autoimmune hepatitis, hemochromatosis, alpha-1-antitrypsin deficiency, or Wilson disease.

Blood sampling

Five milliliters of peripheral blood was obtained by venous puncture from each participant before the dialysis session. Serum was separated without delay by centrifugation at 3000 rpm for 10 min. One fraction of serum was used for the determination of alanine aminotransferase (ALT) and for virological marker assays, and the other was divided into aliquots and stored at −70 °C for viral nucleic acid testing.

Biochemical parameters

The serum ALT level was assayed using an automated COBAS Integra 400 Plus instrument (Roche, Japan). Average serum ALT level values in our clinical laboratory are ≤40 IU/L for females and ≤60 IU/L for males.

Serum assays

Anti–HCV antibody was tested by third-generation ELISA (Ortho HCV 3.0; Ortho, Raritan, NJ, USA). Commercially available ELISAs were used to detect hepatitis B surface antibodies (anti-HBs) (DiaSorin, Saluggia, Italy) and hepatitis B core antibodies (anti-HBc) (Bio-Rad Fujirebio, Inc. Tokyo); the manufacturer protocols were followed. All patient data were recorded.

HCV RNA detection by PCR

Only patient samples testing positive for HCV antibodies were confirmed by HCV RNA testing. HCV RNA was detected using a qualitative reverse transcriptase PCR (TaqMan Real-Time PCR; Roche Applied Science, Basel, Switzerland), with a lower limit of detection of 15 IU/ml and with primers in the S’ non-coding region, as described previously (van Doorn, 1994).

Extraction of DNA from serum

DNA was extracted from all patient serum samples by QiAamp Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions.

HBV DNA detection by nested PCR

A nested PCR was used to detect HBV DNA in patient sera. For the first round, forward primer SA1 (5′-ATGCCTG-GATGTTGTCGTCCGG-3′) and reverse primer SA2 (5′-GCCAAGGGGGA-TAAAGGTTCA-3′) were used (Abbasi et al., 2016). The 25 μl PCR reaction mixture was prepared according to the manufacturer’s protocol (Promega Corporation, Madison, WI, USA) and contained 7 μl of the template DNA and 0.5 μl of each of the forward and reverse primers. Samples, in addition to the negative and positive controls, were subjected to a thermocycler (Biometra, Germany) at 94 °C for 5 min and 35 cycles of 94 °C for 1 min, 54 °C for 1 min, 72 °C for 1.5 min, and a final extension at 72 °C for 10 min. For the second round, 5 μl of the first round product was used for the template, and the inner forward primer SB1 (5′-TGAAGGTTTAAAGTGTATACCC-3′) and the inner reverse primer SB2 (5′-CATCTTCTGTTGTTTCTCTCTG-3′) were used (Abbasi et al., 2016). The PCR reaction mixture contents were similar to those used in the first round, and the following PCR conditions were programmed: 94 °C for 5 min, 30 cycles at 94 °C for 1 min, 45 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR products were analyzed by 1.5% agarose gel electrophoresis and staining with ethidium bromide. The presence of a PCR product with 416 base pairs (bp) indicated a positive reaction.

SENV (D and H) DNA detection by nested PCR

Five microliters of the extracted DNA was added to the Master Mix (Promega Corporation, Madison, WI, USA) with 20 pmol of each sense and antisense primer in a final volume of 25 μl. DNA was amplified by nested PCR, with forward primer Al-1F (5′-TWCYCMACGACG CAG CTA GAC CT-3′; W=A or T, Y=C or T, M=A or C) and a reverse primer Al-1R (5′-GGT TGT GGT CAG CAG AAC GGA-3′). During the first round ‘for all the SENV genotypes’, all samples, including the negative and positive controls, were subjected to the following conditions: 94 °C for 5 min and 35 cycles of 94 °C for 1 min, 54 °C for 1 min, 72 °C for 1.5 min, with a final extension at 72 °C for 10 min (Abbasi et al., 2016). Second-round PCR amplification was done with specific primers. Forward
and reverse primers for SENV-D included D-1148 F (5'-CTA AGC AGC CCT AAC ACTCATACG-3') and D-1341 R (5'-GCAGTTGACCCG-CAAGTGAACAGA G-3'), and for SENV-H they included H-1020 F (5'-TTG GCC TGC ACC TTC TGG TT-3') and H-1138 R (5'-GAA AAT GAT GGGTGACGTGTTAAGG-3') (Kojima et al., 2003). The amplification products were analyzed by 3% agarose gel electrophoresis and staining with ethidium bromide. A fragment of 119 bp was indicative of SENV-H, while a fragment of 195 bp was indicative of SENV-D (Abbasi et al., 2016).

**TTV DNA detection by semi-nested PCR**

Three primers for the ORF1 gene were used for the identification of TTV in a semi-nested PCR analysis, as described by Okamoto et al. (Okamoto et al., 1999). These were the forward primer TTV-F (5'-ACACACAGGAGACAGCTTACG-3'), the reverse primer TTV-R (5'-CTGCGATTATCCACATACGG-3'), and another forward primer TTV-FF (5'-GGCAACATGTTATGGATAGACTG-3'). One microgram of template DNA was added to the PCR Master Mix (Promega Corporation, Madison, USA) together with the sense and antisense primers (1 μM of each primer) in a final volume of 50 μL. All samples, including the negative and positive controls, were subjected to a thermocycler for gene amplification under the following conditions: for the first round, initial denaturation at 95 °C for 5 min, 30 cycles of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min, and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 6 min. The second round was carried out using 2 μL from the first round amplicon as a template. It was performed with TTV-FF and TTV-R primers for 25 cycles under the same conditions. The PCR products were analyzed by 1.5% agarose gel electrophoresis and staining with ethidium bromide. The presence of a PCR product of 271 bp indicated a positive reaction.

**Statistical analysis**

Quantitative data were represented as the mean value ± 1 standard deviation. Virus status frequencies were determined by direct counting. Associations between virus status and clinical/laboratory variables were analyzed by Pearson Chi-square test or Fisher’s exact test. All tests were two-tailed. Results were considered statistically significant when the p-value was ≤ 0.05. All analyses were performed using IBM SPSS Statistics version 24.0 (IBM Corp., Armonk, NY, USA).

**Results**

The sociodemographic and laboratory variables of group 1 (HCV RNA-positive) and group 2 (HCV RNA-negative) HD patients are summarized in Table 1. OBI, SENV, and TTV were detected in 66 (50.8%), 15 (11.5%), and 38 (29%) of 130 HCV RNA-positive patients and in 44 (22.6%), 16 (8.2%), and 53 (27%) of 195 HCV RNA-negative patients, respectively. There was a significant association between CHC and OBI.

**Table 2** demonstrates the analysis of the relationships during the duration of hemodialysis, transfusion history, and elevated ALT serum level and virus status. Less than half of the OBI-positive patients in both groups had received previous blood transfusions (45.5% and 22.7%, respectively). In both groups, a significant association between blood transfusion history and both SENV and TTV positivity was found (group 1: p = 0.024 and p = 0.006; respectively; group 2: p < 0.001 and p < 0.001; respectively). In group 2, less than half of the TTV-positive patients had a duration of HD ≥ 24 months and an elevated serum ALT level (22.6% and 7.5%, respectively).

**Table 2** also demonstrates that eight HCV RNA-positive patients were simultaneously co-infected with the three viruses. They were matched with 16 controls on maintenance HD who were negative for all the studied viruses. The statistical analysis revealed a significant association regarding the duration of HD, blood transfusion history, and elevated serum ALT level (p = 0.008, p = 0.021, and p = 0.003, respectively).

**Discussion**

HCV-infected patients undergoing maintenance HD are at high risk of infection. In addition to their weakened clinical condition, their prolonged and frequent exposure to many possible hazards, like blood transfusion, contributes to this vulnerability. Among these infections are HBV and others, including SENV and TTV, whose causal relationships with hepatic disease need further investigation. A large body of evidence supports the high prevalence of OBI in HCV-infected patients (Raimondo et al., 2005; Torbenson and Thomas, 2002). Moreover, the possibility of infection among HD patients is increased due to their low response to HBV vaccination (Aghakhani et al., 2010; McNulty et al., 2005). The early identification of OBI helps prompt patient management (Kwak and Kim, 2014). In the current work, OBI was diagnosed at a rate of 50.8%, which is comparable to that reported by researchers from the north coast of Egypt (47.1%) (Helaly et al., 2015), but higher than the rate identified in the Nile Delta (36.4%) (Ismail et al., 2010). This rate is far higher than that determined in the west of Egypt and in Upper Egypt (6% and 4.1%, respectively) (Bedewy and Ibrahim, 2006; El Makarem et al., 2012). Investigators from the Kingdom of Saudi Arabia, Iran, and Turkey concluded that OBI was absent among their HCV patients (Arababadi et al., 2009; Gadour et al., 2017; Goral et al., 2006). However, two working groups from the Turkey and one from Greece identified OBI at rates of 36.4% (Besik et al., 2003), 14.2% (Kanbay et al., 2006), and 20.4% (Sagris et al., 2006), respectively. Prevalence rates of OBI are influenced by several factors, including HBV endemcity and subtypes, patient characteristics, the sensitivity of diagnostic techniques, the type and number of samples, and the implementation of infection prevention and control (Goral et al., 2006; Raimondo and Policino, 2016; Raimondo et al., 2007).

The amplification of HBV DNA from serum or liver biopsy specimens is the gold standard for the diagnosis of OBI. Liver biopsy is an invasive procedure and a sample can be collected from

**Table 1** Sociodemographic and laboratory variables for the hemodialysis groups.

<table>
<thead>
<tr>
<th></th>
<th>Total (N = 325)</th>
<th>Group 1 (n = 130)</th>
<th>Group 2 (n = 195)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean ± SD</td>
<td>47.3 ± 10.2</td>
<td>48.1 ± 10.5</td>
<td>46.5 ± 9.8</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>130:175</td>
<td>40:70</td>
<td>90:105</td>
</tr>
<tr>
<td>HBV Anti-HBs</td>
<td>8 (2.5%)</td>
<td>3 (2.3%)</td>
<td>5 (2.6%)</td>
</tr>
<tr>
<td>HBV Anti-Hbc</td>
<td>166 (51.0%)</td>
<td>95 (73.1%)</td>
<td>71 (36.4%)</td>
</tr>
<tr>
<td>HBV DNA</td>
<td>110 (33.8%)</td>
<td>66 (50.8%)</td>
<td>44 (22.6%)</td>
</tr>
<tr>
<td>SENV DNA</td>
<td>31 (9.5%)</td>
<td>15 (11.5%)</td>
<td>16 (8.2%)</td>
</tr>
<tr>
<td>D genotype</td>
<td>22 (6.7%)</td>
<td>6 (4.6%)</td>
<td>16 (8.2%)</td>
</tr>
<tr>
<td>H genotype</td>
<td>9 (2.8%)</td>
<td>9 (6.9%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>TTV DNA</td>
<td>91 (28%)</td>
<td>38 (29%)</td>
<td>53 (27%)</td>
</tr>
<tr>
<td>Elevated serum ALT level</td>
<td>120 (36.9%)</td>
<td>52 (40%)</td>
<td>68 (33%)</td>
</tr>
</tbody>
</table>

HD, hemodialysis; HCV, hepatitis C virus; pos, positive; neg, negative; SD, standard deviation; HBV, hepatitis B virus; anti-HBs, hepatitis B surface antibody; anti-Hbc, hepatitis B core antibody; SENV, SENV virus; TTV, torque teno virus; ALT, alanine aminotransferase.

* p = 0.001,
* b p = 0.32,
* c p = 0.69,
* d p = 0.35.
Table 2

<table>
<thead>
<tr>
<th>Virus status</th>
<th>HD &gt;24 months</th>
<th>Transfusion history</th>
<th>Elevated serum ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV RNA-pos (n = 130)</td>
<td>35 (53.0%)</td>
<td>30 (45.5%)</td>
<td>30 (45.5%)</td>
</tr>
<tr>
<td>OBI-pos (n = 66)</td>
<td>29 (45.3%)</td>
<td>45 (70.1%)</td>
<td>22 (34.4%)</td>
</tr>
<tr>
<td>p-Value</td>
<td>Chi-square, p = 0.38</td>
<td>Chi-square, p = 0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Chi-square, p = 0.2</td>
</tr>
<tr>
<td>HCV RNA-neg (n = 195)</td>
<td>22 (50.0%)</td>
<td>10 (22.7%)</td>
<td>15 (34.1%)</td>
</tr>
<tr>
<td>OBI-pos (n = 44)</td>
<td>80 (53%)</td>
<td>75 (49.7%)</td>
<td>53 (35.1%)</td>
</tr>
<tr>
<td>p-Value</td>
<td>Chi-square, p = 0.73</td>
<td>Chi-square, p = 0.002&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Chi-square, p = 0.9</td>
</tr>
<tr>
<td>HCV RNA-pos (n = 130)</td>
<td>10 (66.7%)</td>
<td>13 (86.7%)</td>
<td>15 (34.1%)</td>
</tr>
<tr>
<td>SENV DNA-pos (n = 15)</td>
<td>52 (45.2%)</td>
<td>62 (53.9%)</td>
<td>44 (38.3%)</td>
</tr>
<tr>
<td>p-Value</td>
<td>Fisher exact, p = 0.17</td>
<td>Fisher exact, p = 0.024&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Chi-square, p = 0.26</td>
</tr>
<tr>
<td>HCV RNA-neg (n = 195)</td>
<td>8 (50%)</td>
<td>14 (87.5%)</td>
<td>8 (50%)</td>
</tr>
<tr>
<td>SENV DNA-pos (n = 16)</td>
<td>94 (52.5%)</td>
<td>71 (39.7%)</td>
<td>60 (31.5%)</td>
</tr>
<tr>
<td>p-Value</td>
<td>Chi-square, p = 0.85</td>
<td>Fisher exact, p = 0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Chi-square, p = 0.19</td>
</tr>
<tr>
<td>HCV RNA-pos (n = 130)</td>
<td>19 (50%)</td>
<td>29 (76.3%)</td>
<td>20 (52.6%)</td>
</tr>
<tr>
<td>TTV DNA-pos (n = 38)</td>
<td>45 (48.9%)</td>
<td>46 (50%)</td>
<td>32 (34.8%)</td>
</tr>
<tr>
<td>p-Value</td>
<td>Chi-square, p = 0.91</td>
<td>Chi-square, p = 0.006&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Chi-square, p = 0.059</td>
</tr>
<tr>
<td>HCV RNA-neg (n = 195)</td>
<td>12 (22.6%)</td>
<td>48 (90.6%)</td>
<td>4 (7.5%)</td>
</tr>
<tr>
<td>TTV DNA-pos (n = 53)</td>
<td>90 (63.4%)</td>
<td>37 (26.1%)</td>
<td>64 (45.1%)</td>
</tr>
<tr>
<td>p-Value</td>
<td>Chi-square, p &lt; 0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Chi-square, p &lt; 0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Fisher exact, p &lt; 0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HCV RNA-pos (n = 8)</td>
<td>7 (87.5%)</td>
<td>5 (62.5%)</td>
<td>7 (87.5%)</td>
</tr>
<tr>
<td>OBI/TTV DNA/SENV DNA-pos</td>
<td>4 (25.0%)</td>
<td>2 (12.5%)</td>
<td>5 (23.8%)</td>
</tr>
<tr>
<td>p-Value</td>
<td>Fisher exact, p = 0.008&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Fisher exact, p = 0.021&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Fisher exact, p = 0.003&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; HCV, hepatitis C virus; OBI, occult hepatitis B infection; SENV, SEN virus; TTV, torque tenu virus; HD, hemodialysis; pos, positive; neg, negative. 

<sup>a</sup> Significant difference.

a minority of cases; furthermore, its standardization is challenging (Raimondo et al., 2008). In this study, serum samples were used. However, HBV DNA was identified in a smaller number of patients testing anti-HBc-positive, who were classified as seropositive for OBI. It is not surprising that the anti-HBc test is known to be a less sensitive diagnostic marker of OBI (Comanor and Holland, 2006; Gerlich et al., 2007). Nevertheless, seropositive OBI constitutes the majority of OBI cases (Torbenson and Thomas, 2002) and anti-HBc positivity can be due to resolved HBV infections (Raimondo and Pollicino, 2016).

In the present work, SENV DNA was detected in 9.5% of the study participants (SENV-D 6.7% and SENV-H 2.8%), a rate falling within the range reported from Japan, Thailand, and Turkey (5–10%) (Serin et al., 2005; Shibata et al., 2001; Tangkijvanich et al., 2003) and comparable to that reported in a previous study from Egypt (7.1%) (Omar et al., 2008). However, it is lower than the rate reported from China (31%) (Mu et al., 2004) and higher than that identified in Italy and the United States (range 2–3%) (Bowden, 2001; Mushahwar, 2000). Contrary to Loutfy et al., who reported SENV-H to be the most prevalent genotype among HD patients (Loutfy et al., 2009), the present study results concluded that this genotype was the least predominant (Table 1). These variations might be attributed to the different exposure rates, the different routes of transmission, or the different rates of spontaneous clearance of genotypes (Dai et al., 2005).

Generally, the prevalence of TTV among HD patients ranges from 27.8% to 68.8% (Afkari et al., 2012; Irshad et al., 2010; Massaï et al., 2012; Rivero et al., 2009), and in the present work, the frequency of TTV among HD patients was 28% (Table 2). The wide variation in the prevalence rates of TTV may be related to the increasing number of TTV-infected cases over time, the medications used, or the suboptimal TTV titer of the test samples. Another reason legitimizing such a difference is the primer used. The group-specific primer is designed according to the nucleotide sequence of one TTV genotype, representing one TTV genogroup (groups 1 to 5). As a result of the high sequence heterogeneity between TTV genotypes, not all TTV genotypes could be identified (Vasconcelos et al., 2001). Moreover, the varying TTV infection rates could also be related to the multiple modes of TTV transmission (Brassard et al., 2015; Pujol et al., 2005).

In the present study, there was a significant association between CHC and OBI (Table 1). It is widely agreed that patients with CHC represent a category of individuals with a high prevalence of OBI (El Sheredy et al., 2015; Mahmoud et al., 2016). History of blood transfusion had no impact on OBI (Table 2), a finding that could be credited to the rigorous procedures routinely used in Egypt to make blood transfusion safe.

In contrast, a non-significant association was observed between SENV or TTV viremia and CHC (Table 1). However, there was a significant relationship between SENV/TTV and blood transfusion, which is in agreement with previous studies (Afkari et al., 2012; Elsayed et al., 2019; Hashish et al., 2005; Tzcan et al., 2009; Umemura et al., 2001) (Table 2). Since the pathological role of these two viruses is unidentified, their routine testing is not included among pre-donation screening tests. The clinical significance of SENV or TTV infection alone or in combination with HCV infection remains unclear (Ataei et al., 2012; Elsherbiny et al., 2015; Hosseini and Bouzari, 2016; Omar et al., 2008; Yuki et al., 1999). Consistent with previous studies (Omar et al., 2008; Yuki et al., 1999), the relationship between serum ALT elevation or HD duration and SENV or TTV infection was not evident (Table 2).

When the findings related to the eight HCV, HBV, SENV, and TTV co-infected patients were analyzed (Table 2), the duration of HD was found to be significantly associated with co-infection, which might be associated with a more prolonged exposure time and consequently greater possibility of disease transmission. The absolute correlation between co-infection and transfusion history may raise the possibility of infection prevention and control breaches during transfusion procedures, coupled with factors related to SENV and TTV, as mentioned before. The elevated serum
ALT level was found to be significantly higher in co-infected patients than in controls. This might perhaps be due to the interaction between the three viruses. Moreover, the natural history of infections, the available treatments, and the response to vaccines may differ from what is known for the general population. However, the small number of cases studied might be the cause of all these contradictions. Therefore, a study with a larger sample size could provide more useful information to better help in patient management. In conclusion, HCV patients undergoing HD can be at high risk of OBI and SENV and TTV transmission. The early identification of OBI helps in prompt patient management. Further prospective studies are recommended to assess the importance of simultaneous co-infection with the three agents in a background of CHC.

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Conflict of interest
The authors have no competing interests to declare.

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