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## Detection of TT Virus-DNA among Children with Collagen Vascular Diseases Receiving Blood Transfusions

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The novel viral agent, TT Virus (TTV), is a non-enveloped, single-stranded and circular virus, DNA virus that was originally identified in the serum of patients with non-A to E post-transfusion hepatitis. TTV transmission can occur by the parenteral route, but faecal-oral transmission may also occur. The aim of this study was to detect the occurrence of TT virus DNA in children with collagen vascular diseases who received multiple transfusions of blood or one of its products and to determine the risk factors for infection with TT virus. The study included 80 children with collagen vascular diseases who received repeated transfusions of blood or its products: 20 patients with dermatomyositis (DM), 20 patients with Juvenile Rheumatoid Arthritis (JRA) and 40 patients with Systemic Lupus Erythematosus (SLE), aged 8-18 years. Serum samples were analyzed for TTV-DNA, HCV and HBsAg. Samples were also analyzed for biochemical liver profiles. TTV-DNA is positive in 35% of Egyptian children suffering from autoimmune diseases who received multiple transfusions of blood or blood products. The positivity of TTV-DNA in DM, JRA and SLE were 30, 50 and 30%, respectively. The highest coinfection with TTV and HBsAg was 66.7% in SLE followed by 20% of JRA. Multiple infections of TTV, HBsAg and HCV were 16.7% in SLE. HEV was negative in all studied groups.

**Key words:** TT virus, autoimmune disease, children, HCV, HbsAg, blood transfusion

## INTRODUCTION

Recent molecular studies have demonstrated that TT virus (TTV), the first known human circovirus, is a single-stranded, nonenveloped DNA virus with an approximately 3.8 kb circular viral genome<sup>[1]</sup>. TTV has been suggested as an etiologic agent of non-A to -E hepatitis, but its role in liver disease is still unclear<sup>[2]</sup>. TTV is widespread in the general population, with a reported prevalence of 1.9% in Scotland<sup>[3]</sup>, 10% in the United States<sup>[4]</sup>, 12% in Japan<sup>[5]</sup> and 81% in Egypt<sup>[6]</sup>.

The significance of TTV infection in children has been relatively overlooked. Prevalence of TTV infection in childhood was 5.1% in Japan<sup>[7]</sup> 17% in Brazil<sup>[8]</sup>, 25% in Taiwan<sup>[9]</sup> and 54% in the Democratic Republic of Congo<sup>[10]</sup>. Although previous studies suggest TTV transmission through transfusion of contaminated blood and blood products, these data do not support the high prevalence of TTV infection observed in apparently healthy children with no history of such exposure<sup>[3]</sup>.

TTV transmission can occur by the parenteral route<sup>[3]</sup> and the relatively high prevalence of TTV among drug addicts, haemodialysis patients and haemophilia patients suggests that this is an important transmission route<sup>[11]</sup>. Shedding of TTV in bile and faeces has been demonstrated, suggesting that faecal-oral transmission may also occur<sup>[12]</sup>.

Previous studies on serum or plasma have demonstrated an age-associated acquisition of TTV in children<sup>[2,9]</sup>. In 90 children under the age of 15 years from Taiwan, Hsieh and associates demonstrated a TTV prevalence of 17% (4 of 23) among children younger than 1 year of age but no evidence of infection (0 of 30) among newborn infants<sup>[9]</sup>. Using T primers, Yokozaki *et al.*<sup>[13]</sup> found TTV DNA in the blood of 94.1% (32 of 34) children less than 6 years of age but failed to detect TTV in cord blood (n = 48) and in serum from 2- to 3-day-old infants (n = 48). TTV viremia was found at 12 months of age among 37 children (54%) who had been TTV negative at 3 months, in a longitudinal study of 68 children from the Democratic Republic of Congo<sup>[10]</sup>. While in Japan, TTV was demonstrated between the ages of 6 and 14 months after birth<sup>[2]</sup>. Another study in Italy done on 101 pregnant women, TTV infection was found in 8.9% (9 cases), but none of the neonates born to TTV-infected women had evidence of TTV infection. In contrast, Schroter and associates demonstrated 47.8, 95.4 and 73.9% prevalences of TTV DNA in the sera of pregnant women and their newborn infants and in their breast milk, respectively<sup>[14]</sup>.

The failure to detect TTV in children under 6 months of age and the high prevalence (40%) in children 7 to 12

months of age ( $p=0.1$ ) is consistent with the published data on age-specific TTV infection in children, which coincides with their increased interaction with the environment and the increased susceptibility to infectious agents<sup>[15]</sup>.

TTV viremia was detected in (61/105, 58%) women attending an antenatal clinic and in (6 /68,4%) infants. Most infants acquired the infection at  $\geq 3$  months postpartum. Surprisingly, TTV infection was detected in a large proportion of children with TTV-negative mothers (13/30, 43%)<sup>[10]</sup>.

Serum TTV DNA was not detected in any infant at 1 month of age, but was detected for the first time between 1.5 and 8 months after birth<sup>[16]</sup>.

The aim of this study was to detect the occurrence of TT virus DNA in collagen vascular diseases children who received multiple transfusions of blood or one of its products and determine the risk factors for infection with TT virus.

## MATERIALS AND METHODS

**Patients group:** Eighty serum samples were collected from new paediatric Hospital, Cairo University. All of them received multiple times transfusion of blood or its products and The mean duration of disease was  $4.2 \pm 1.5$  years. Twenty serum samples (4 males and 16 females) from dermatomyositis (DM), twenty samples, (16 males and 4 females) from Juvenile Rheumatoid Arthritis (JRA) and forty samples (22 males and 18 females) suffered from Systemic Lupus Erythromatosis (SLE), regularly attending the Pediatric Allergy and Immunology Clinic. Their ages ranged from 8-18 years with mean age of  $12.8 \pm 3.39$  years. 16 patients received plasma transfusion, 4 intravenous immunoglobulin, 2 human albumin transfusion and all of them received blood transfusion.

### Serologic assays:

**Virological examination:** Patients sera were separated, aliquoted and stored at  $-20^{\circ}\text{C}$ . HbsAg, HEV (IgG and IgM) and HCV antibodies were detected by enzyme immunoassay Kits (Bioket, S.A, Barcelona).

TTV DNA was detected by PCR using primers derived from 5' non-coding region and open reading frame 2 (ORF2) as described by Takahashi *et al.*<sup>[17]</sup>. Briefly an aliquot of 200  $\mu\text{L}$  of each patient's serum was obtained to isolate viral DNA with the QIAamp Blood Kit (Qiagen, Chatsworth, CA). The DNA was eluted with 50  $\mu\text{L}$  distilled water according to the manufacturer's recommendations. The TTV DNA isolated from 200  $\mu\text{L}$  serum was amplified by PCR in 50  $\mu\text{L}$  of buffer containing 50  $\text{mmol L}^{-1}$  Tris-HCl (pH 8.3), 50  $\text{mmol L}^{-1}$  KCl,

1.5 mmol L<sup>-1</sup> MgCl<sub>2</sub>, 200 mmol L<sup>-1</sup> deoxynucleoside triphosphate, 2 U of Ampli-Taq Gold (Perkin Elmer Applied Systems) using 30 pmol of primers: T801 (5'-GCT ACG TCA CTA ACC ACG TG-3', sense, nucleotide 6-25) and T935 (5'-CTB CGG TGT GTA AAC TCA CC-3', antisense, nucleotide 204-185; B=mixture of G, C and T). Amplification conditions were 95°C for 8 min followed by 55 cycles at 95°C for 25 sec, 60°C for 25 sec and 72°C for 30 sec, with a final step of 72°C for 5 min in a thermal cycler (Perkin Elmer Cetus, Norwalk, CT). The PCR products (measuring 199 base pairs) were visualized after electrophoresis in a 2% agarose gel and ethidium bromide staining.

**Biochemical analyses:** Analysis of biochemical liver profiles takes place at the time of the samples collection for all study subjects. ALT and AST were assayed according to the method of Reitman and Frankel<sup>[18]</sup>, using Randox kit. Total and direct bilirubin was determined according to the method of Automated Jendrassik-Grof method<sup>[19]</sup>.

**Statistical analysis:** Data were analyzed with standard computer program SPSS version 11 (SPSS Inc, USA). All numeric data were expressed as mean±SD. Data were analyzed using student t test to compare mean values of different variables. Chi-square test was used to compare proportions of qualitative data. For all tests a probability (p) of less than 0.05 was considered significant.

## RESULTS

Analysis data indicated that liver functions of the total serum bilirubin, ALT and AST in all studied patients were within the normal level. Furthermore there were no statistical differences between the mean values of bilirubin, ALT and AST in the three studied groups (DM, JRA and SLE) when compared to each other. Comparison of serum bilirubin and ALT in patients positive for TTV-DNA to those negative for TTV showed no significant difference (p>0.05). The same findings were found on comparing HBsAg and anti-HCV positive patients to negative ones.

Virological detection of tested samples indicated that all samples were negative for HEV infection.

Distribution of TTV in the three groups of collagen vascular diseases of children is presented in Fig. 1. TTV-DNA was positive in 28/80 patients (35%) included in the study. TTV-DNA was positive in 6/20 patients (30%) with DM, 10/20 patients (50%) with JRA and 12/40 patients (30%) with SLE. No significant difference in the prevalence of TTV positivity was found between the three groups ( $\chi^2 = 1.32, p>0.05$ ) or on comparing each group to the other two (p>0.05) (Fig. 1).

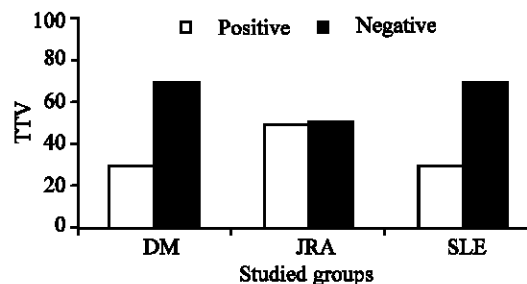


Fig. 1: Prevalence of TTV-DNA among children with autoimmune disease

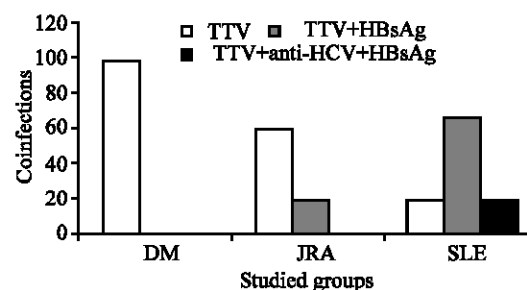


Fig. 2: Prevalence of multiple infections TTV-DNA, HCV and HbsAg in children with autoimmune disease

It is evident from the Fig. 2 that the presence of TTV-DNA alone or in combination with HBsAg or HCV or both was equally distributed.

The highest coinfection with TTV and HBsAg was 66.7% in SLE, followed by JRA (20%), while the multiple infections with TTV, HBsAg and HCV in SLE was 16.7%. There was no coinfection with TTV and HbsAg in DM group; also, multiple infections with TTV, HCV and HBsAg were not encountered in DM and RA.

No significant relationship could be found between TTV-DNA on the one hand and HBsAg ( $\chi^2 = 1.43, p>0.05$ ) and anti-HCV ( $\chi^2 = 1.28, p>0.05$ ) on the other hand.

In JRA group, TTV-DNA positivity was not influenced by gender or age, HBsAg or anti-HCV positivity (p>0.05). In groups DM and SLE, same findings were obtained.

## DISCUSSION

Among eighty pediatric patients suffering from collagen vascular diseases and received blood or blood products, TTV-DNA was detected in the serum of 28/80 patients (35%). Similar result reported that TTV with collagen diseases and nephrotic syndrome patients who received multiple blood or blood product transfusions was 35%<sup>[19]</sup>.

The prevalence of TTV-DNA among Egyptian blood donors; was 85%<sup>[20]</sup>, while in other study was

29%<sup>[21]</sup>. TTV has been found worldwide with a relatively high prevalence in the general population<sup>[3]</sup>.

This gives evidence that transfusion is not the sole route for transmission, TTV has been detected in bile and faces, so it may be transmitted by enteric route<sup>[5]</sup> serum<sup>[22]</sup>, saliva, nose and throat swabs, breast milk<sup>[23]</sup>, semen<sup>[24]</sup>, cervical swabs<sup>[20]</sup>. TTV could be isolated from liver<sup>[25]</sup>.

Mixed infection with multiple strains of TTV was common particularly in patients receiving a significantly greater number of blood transfusion<sup>[11]</sup>. The overall prevalence of TT viremia was 28% compared to 5.3% in blood donors from the same region.

No significant relationship was found between TT viremia and hepatitis C virus, transaminases, age and sex<sup>[26]</sup>. While, Tanaka *et al.*<sup>[23]</sup> reported that there was a significant difference between anti HbsAg and TTV positive and negative groups.

TTV prevalence in HD patients was 19% and among those patients HCV was also positive in 39.9%, while HBsAg coinfection was not detected. ALT levels were normal in TTV positive patients<sup>[27]</sup>.

TTV-DNA among Egyptian hemodialysis patients was reported to be higher than blood donors and there is no relationship between TTV positivity and age, sex, history of blood transfusion, positive markers for HBsAg or HCV<sup>[21]</sup>.

This study showed that the highest coinfection of TTV and HBsAg was 66.7% in SLE, followed by JRA (20%), while the multiple infections of TTV, HBsAg and HCV in SLE was 16.7%. There is no infection with TTV and HBsAg in DM group; also, TTV, HCV and HBsAg in DM and JRA.

In conclusion, the study showed that the prevalence of TTV-DNA among collagen vascular diseases patients receiving multiple blood or blood product transfusion is 35%. No clinical or laboratory evidence of acute liver insult among TTV-DNA positive patients could be found as evidenced by absence of hepatomegaly and jaundice and normal total serum bilirubin and ALT. Therefore, the present study supports previous studies conducted polytransfused patients<sup>[28]</sup>.

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