

# Prevalence and genetic diversity of torque teno virus in patients with systemic lupus erythematosus in a reference service in Mato Grosso do Sul

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## ABSTRACT

Recent studies on the torque teno virus (TTV), genus *Anellovirus*, have allowed formulating the hypothesis that TTV may trigger autoimmune rheumatic diseases or have some pathogenic role in them. **Objectives:** To determine the frequency of TTV infection in patients with systemic lupus erythematosus (SLE), the genetic diversity of TTV, the correlation between TTV infection and SLE clinical manifestations, and SLE clinical course and serological profile. **Patients and methods:** Serum samples were obtained from 46 SLE patients treated at the University-Affiliated Hospital of Campo Grande (NHU/FAMED/UFMS), Brazil. For controls, serum samples were obtained from 46 healthy volunteer blood donors. Viral DNA was extracted from samples using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) and amplified using nested PCR. **Results:** Positivity for TTV was found in 17 (37%) of SLE patients and in only seven (15.2%) of the controls (z test,  $P = 0.03$ ). There was no correlation between TTV infection, SLE clinical manifestations, SLE clinical course, and the serological profile of the patients evaluated. **Conclusion:** Further studies on the presence of TTV in SLE patients are required.

**Keywords:** *Anellovirus*, autoimmune diseases, systemic lupus erythematosus, torque teno virus.

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## INTRODUCTION

In 1997, Nishizawa et al.<sup>1</sup> isolated a new virus from serum samples of a patient with post-transfusion hepatitis of unknown etiology. The virus was initially denominated transfusion-transmitted virus (TT virus or TTV). Later the virus name was changed to torque teno virus, maintaining the initials TTV.<sup>2,3</sup>

The viral particle of TTV has no outer lipid envelope, and its genome is formed by a single strand of circular DNA with approximately 3,800 base pairs.<sup>4</sup> But, unlike other DNA viruses,

TTV has high genetic heterogeneity.<sup>5</sup> Because of such genetic diversity, more than 30 genotypes of TTV are known and classified into five major genogroups.<sup>6</sup>

Initially TTV has been associated with hepatic disease and high mortality among patients with acute infection by the hepatitis B virus (HBV), but such association has not been confirmed in more recent studies.<sup>5</sup>

Epidemiological studies have evidenced the presence of TTV in several pathological conditions, such as Hodgkin's disease,<sup>7</sup> aplastic anemia,<sup>8</sup> idiopathic pulmonary fibrosis,<sup>9</sup>

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acute pulmonary disease,<sup>10</sup> bullous pemphigoid,<sup>11</sup> worsening of prognosis of laryngeal carcinoma,<sup>12</sup> and reduced survival of patients with the human immunodeficiency virus (HIV).<sup>13</sup> However, these studies could not characterize the real meaning of the presence of TTV in those illnesses.<sup>14</sup>

Currently, the role of viral infections as triggering factors of autoimmune diseases has been discussed.<sup>15</sup> Recent knowledge about TTV biology has supported the formulation of a hypothesis that TTV can trigger autoimmune rheumatic diseases.<sup>14,16</sup> However, studies relating TTV infection to such rheumatological diseases are scarce.

In 2005, Gergely et al.<sup>17</sup> reported a 56.87% frequency of TTV infection in systemic lupus erythematosus (SLE) patients, significantly higher than that in healthy controls (33.16%;  $P < 0.001$ ). In addition, first-degree relatives of SLE patients had a significantly lower frequency of infection (51.28%) than that of their SLE family members (66.11%;  $P = 0.0184$ ), but significantly higher than that of healthy controls with no SLE family member (33.16%;  $P = 0.0026$ ). It has been speculated that genetic factors for susceptibility to SLE could play a role in TTV infection.<sup>17</sup>

This study aimed at assessing the following: frequency of TTV infection and its gene diversity in SLE patients; and the existence of a correlation between TTV presence, clinical manifestations, and the antinuclear antibody serum profile of SLE patients.

## PATIENTS AND METHODS

This study assessed 46 serum samples randomly collected from SLE patients, aged from 14 to 51 years, cared for at the Rheumatology Outpatient Clinic of the University-Affiliated Hospital of Campo Grande of the Universidade Federal de Mato Grosso do Sul (NHU/FAMED/UFMS). As controls, 46 serum samples were obtained from healthy blood donors of the Hemonúcleo of the NHU/FAMED/UFMS.

All patients selected should meet at least four of the 11 American College of Rheumatology revised criteria for the classification of SLE,<sup>18</sup> agree to participate in the research after being instructed about it and understanding it, and sign the written informed consent. No patient could have other infectious diseases, malignant neoplasias, or other autoimmune diseases.

From the medical records stored at the NHU/FAMED/UFMS, the following data of the selected SLE patients were assessed: gender; age; SLE clinical manifestations; duration of disease; and previous and current treatments. In addition, the presence of the following antibodies was assessed: ANA (indirect immunofluorescence, substrate: HEp2 cells); anti-DNA

(indirect immunofluorescence, substrate: *Crithidia luciliae*); anti-Ro; anti-La; anti-Sm; and anti-RNP (ELISA technique).

For the purpose of analyzing the results, patients' treatment was considered as follows: low immunosuppression, when chloroquine and/or prednisone were used at the dosage of up to 20 mg/day; high immunosuppression, when prednisone (dose greater than 20 mg/day) and/or azathioprine and/or methotrexate and/or cyclophosphamide and/or cyclosporine were used.

For extracting the viral DNA from the serum, QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) was used according to the manufacturer's instructions. The viral DNA extracted was then amplified by use of the nested polymerase chain reaction assay (nested PCR) described by Devalle and Niel.<sup>19</sup> All oligonucleotides used in the PCR assay (T1S and T2S, primer sense; T1A, T2G1A, T2G2A, T2G3A, T2G4A e T2G5A, primer antisense) were described by Devalle and Niel in 2004.<sup>6</sup>

The relation between TTV and SLE was assessed by use of the Fisher exact test. The same test was used to assess the relation between SLE and TTV genogroups, between TTV and clinical signs, between TTV and serology results, and between TTV and the treatment used. The percentages of TTV-positive individuals in the control group and the group of SLE individuals were compared by use of the z test. Regarding the duration of SLE, TTV-positive and TTV-negative individuals were compared by use of the Student's *t* test.<sup>20</sup> The statistical analysis was performed by use of the programs SigmaStat, version 2.0, and SPSS, version 13.0, adopting the significance level of  $P < 0.05$ .

This study was submitted and approved by the Ethics Committee on Research of the UFMS.

## RESULTS

Regarding the SLE individuals ( $n = 46$ ), their mean age was  $31.8 \pm 9.4$  years (mean  $\pm$  standard deviation), and 44 (95.7%) were females. The mean duration of SLE was  $6.0 \pm 4.1$  years. In the control group, mean age was  $38.8 \pm 11.5$  years, and 45 (97.8%) were females (Table 1).

**Table 1**

Relative and absolute frequency of individuals regarding TTV and SLE. Campo Grande, 2011

SLE	TTV	
	Positive	Negative
Yes	37.0%* (n = 17)	63.0% (n = 29)
No	15.2% (n = 7)	84.8% (n = 39)

Data are shown as relative frequency (absolute frequency).

\* Significant difference as compared with controls.

In the z test, P value = 0.03.

Of the SLE individuals, 17 (37.0%) were TTV-positive and 29 (63.0%) were TTV-negative. Of the control individuals, only 7 (15.2%) were TTV-positive, while 39 (84.8%) were TTV-negative. A significant relation was observed between SLE and the presence of TTV (Fisher's exact test,  $P = 0.02$ ), with a significantly higher percentage of TTV-positive SLE individuals than TTV-positive controls ( $z$  test,  $P = 0.03$ ) (Table 2).

Among the TTV-positive SLE individuals ( $n = 17$ ), seven (41.2%) had genogroup-1 TTV, none (0.0%) had genogroup-2 TTV, seven (41.2%) had genogroup-3 TTV, seven (41.2%) had genogroup-4 TTV, and seven (41.2%) had genogroup-5 TTV (Table 2).

Regarding coinfection in the several genogroups, nine (52.9%) had TTV of a single genogroup, while the other eight (47.1%) had TTV of more than one genogroup. Specifically, six patients (35.3%) had TTV of two genogroups, one (5.9%) had TTV of three genogroups, one (5.9%) had TTV of four genogroups, and no individual had TTV of the five genogroups (Table 2). No relation between SLE and the amount of TTV genogroups was observed (Fisher's exact test,  $P = 0.19$ ).

**Table 2**

Relative and absolute frequency of individuals in the control and SLE groups, according to TTV positivity and TTV genogroup. Campo Grande, 2011

Characteristic	Frequency	
	SLE	Control
TTV		
Positive	37.0% (n = 17)*	15.2% (n = 7)
Negative	63.0% (n = 29)	84.8% (n = 39)
Genogroup		
G1	41.2% (n = 7)	0.0% (n = 0)
G2	0.0% (n = 0)	0.0% (n = 0)
G3	41.2% (n = 7)	42.9% (n = 3)
G4	41.2% (n = 7)	57.1% (n = 4)
G5	41.2% (n = 7)	14.3% (n = 1)
Coinfection		
One genogroup	52.9% (n = 9)	85.7% (n = 6)
Two genogroups	35.3% (n = 6)	14.3% (n = 1)
Three genogroups	5.9% (n = 1)	0.0% (n = 0)
Four genogroups	5.9% (n = 1)	0.0% (n = 0)
Five genogroups	0.0% (n = 0)	0.0% (n = 0)

Data are shown as relative frequency (absolute frequency).

\* Significant difference as compared with controls ( $z$  test,  $P = 0.03$ ).

**Table 3**

Relative and absolute frequency of SLE individuals, according to the clinical sign observed and its relationship with the TTV. Campo Grande, 2011

Clinical sign	TTV	
	Positive	Negative
Erythema	41.2% (n = 14)	58.8% (n = 20)
Discoid skin lesion	50.0% (n = 2)	50.0% (n = 2)
Photosensitivity	37.8% (n = 14)	62.2% (n = 23)
Ulcer	44.4% (n = 12)	55.6% (n = 15)
Arthritis	37.5% (n = 15)	62.5% (n = 25)
Serositis	33.3% (n = 5)	66.7% (n = 10)
Renal manifestation	24.0% (n = 6)	76.0% (n = 19)
Neurological manifestation	66.7% (n = 2)	33.3% (n = 1)
Hematological manifestation	39.3% (n = 11)	60.7% (n = 17)

Data are shown as relative frequency (absolute frequency).

No relationship between TTV and each of the clinical signs assessed in this study was observed (Fisher's exact test,  $P$  range: 0.07–1.00).

Table 3 shows the clinical signs analyzed in the SLE individuals. No relation was observed between TTV and each of the clinical signs assessed in this study (Fisher's exact test,  $P$  range: 0.07–1.00).

Table 4 shows the serology data. No relation between TTV and ANA was observed (Fisher's exact test,  $P = 0.23$ ). Similarly, no relation was observed between TTV and the autoantibodies assessed in this study (Fisher's exact test,  $P$  range: 0.12–1.00).

**Table 4**

Relative and absolute frequency of SLE individuals, according to the serology observed and its relationship with the TTV. Campo Grande, 2011

Serology	TTV	
	Positive	Negative
Antinuclear antibody (ANA)	34.3% (n = 12)	65.7% (n = 23)
Anti-DNA antibody	30.8% (n = 8)	69.2% (n = 18)
Anti-Sm antibody	14.3% (n = 1)	85.7% (n = 6)
Anti-Ro antibody	31.8% (n = 7)	68.2% (n = 15)
Anti-La antibody	14.3% (n = 1)	85.7% (n = 6)
Anti-RNP antibody	33.3% (n = 2)	66.7% (n = 4)

Data are shown as relative frequency (absolute frequency).

No relationship was observed between TTV, ANA and the autoantibodies assessed in this study (Fisher's exact test,  $P$  range: 0.12–1.00).

SLE treatment of TTV-positive SLE individuals ( $n = 17$ ) was as follows: low immunosuppression, three individuals (17.6%); and high immunosuppression, 14 (82.4%). However, among TTV-negative SLE individuals ( $n = 29$ ), SLE treatment

was as follows: low immunosuppression, ten individuals (34.5%); and high immunosuppression, 19 (65.5%). No relation was observed between the treatment used and TTV (Fisher's exact test,  $P = 0.32$ ) (Table 5).

**Table 5**

Relative and absolute frequency of SLE individuals, according to their immunosuppression level and its relationship with the TTV. Campo Grande, 2011

Immunosuppression	TTV	
	Positive	Negative
High	82.4% (n = 14)	65.5% (n = 19)
Low	17.6% (n = 3)	34.5% (n = 10)

Data are shown as relative frequency (absolute frequency).  
In the Fisher's exact test,  $P$  value = 0.32 (non-significant).

## DISCUSSION

This study showed a lower prevalence of TTV infection than that reported in the literature,<sup>21,22</sup> both for SLE patients ( $n = 17$ , 37.0%) and controls ( $n = 7$ , 15.2%).

For viral DNA detection, the following are critical: DNA extraction methods; and the region of the viral genome recognized by initiator oligonucleotides used in the PCR assay.<sup>23</sup>

De Paula et al.,<sup>24</sup> comparing four different methods of RNA extraction from the hepatitis A virus (HAV) in serum and fecal samples, have concluded that the methods using phenol-chloroform are more effective for viral RNA extraction in serum samples, while those using guanidine are more effective in fecal samples. Although TTV is a DNA virus, that might be valid and explain the low prevalence found, because the QIAamp DNA Blood Mini Kit used in this study does not contain phenol-chloroform, but guanidine.

Niel et al.,<sup>25</sup> using a phenol-chloroform-based DNA extraction method, have reported a prevalence of TTV infection in healthy blood donors of 62% ( $n = 45$ ), and of 71% ( $n = 37$ ) in patients with non-A, non-C hepatitis. Devalle et al.,<sup>6</sup> using the same oligonucleotides directed to the UTR region used in this study and a phenol-chloroform-based DNA extraction method, have found a prevalence of 46% ( $n = 11$ ) in blood donors.

A statistically significant association between SLE and TTV infection was found in this study. Thus, when TTV infects susceptible individuals, it can be a triggering factor for the development of SLE, leading to the disease through several ways.

Shirai et al.<sup>26</sup> have reported that viruses with lymphocyte tropism, such as TTV, have potential for the polyclonal activation of B and T cells, including auto-reactive lymphocytes.

Thus, TTV would lead to the proliferation of B cells, causing an increase in the production of antibodies, resulting in accumulation of circulating immune complexes, which could harm the host.<sup>27</sup> Or it could also stimulate the expansion of auto-reactive T cells already present inside the host, which, due to their small number, were unable to cause an autoimmune disease.<sup>28,29</sup>

When infecting the host cells, several viruses alter the cell metabolism through their proteases.<sup>30</sup> After infecting cells of the immune system, such as macrophages and T cells, TTV could interfere with antigen processing and presentation, generating new epitopes, which could trigger autoimmunity. Similarly to cytomegalovirus, TTV could also interfere with the expression of MHC class I molecules.<sup>31</sup> Thus, TTV could stimulate the induction of several cytokines that can divert regulatory T cells to susceptibility to SLE.

In some TTV isolates, a putative protein with apoptotic potential, called TTV-derived apoptosis-inducing protein (TAIP), was identified. Such protein was capable of inducing apoptosis in human cancer cell lines, such as the cells of hepatocellular carcinoma, osteosarcoma, and small-cell lung carcinoma.<sup>32,33</sup> Abnormalities in apoptosis could lead to the loss of regulatory lymphocytes essential to control autoimmunity. In addition, the excessive production of apoptotic remainders, or even the production of apoptotic remainders qualitatively different due to abnormal apoptosis, in association with genetically virus-mediated or induced defective clearance, could stimulate auto-reactive B cells through toll-like receptors (TLR).<sup>34</sup> In a study published in 2009, Rocchi et al.<sup>35</sup> reported that TTV DNA could induce splenic cells of mice to produce and secrete IF $\gamma$ , IL-6, and IL-10, and that this effect could be mediated by TLR 9.

In addition, the molecular mimicry between TTV antigens and autoantigens could stimulate auto-reactive lymphocytes, signaling to the development of an autoimmune response.<sup>36</sup>

Gergely et al.<sup>17</sup> have reported a cross-reactivity of the HRES-1/p28 nuclear autoantigen, TTV specific peptides, and 70kU1snRNP lupus autoantigen. The clinical significance of that reactivity, however, is yet to be established.

The association found between TTV infection and SLE could, however, be due to the immunosuppression caused by the treatment based on corticosteroids and immunosuppressants, such as azathioprine and cyclophosphamide. In this case, TTV infection should be considered opportunistic. The relation between TTV infection and the immunosuppression level of patients was assessed, and no significant statistical difference was observed between individuals on low or high level of immunosuppression. Although these data suggests no relationship between immunosuppression and TTV infection,

this finding should be carefully analyzed, because one of the groups (TTV-positive on low immunosuppression) had only three individuals, which hinders statistical assessment.

Niel et al.<sup>37</sup> have suggested that, in Brazil, coinfection with several TTV genotypes is common. However, in our study, most TTV-infected individuals, both in control and SLE group, were infected by only one genogroup (85.1% and 52.9%, respectively). No statistical difference was observed between these data.

Among lupus patients, all TTV genogroups were found, except for genogroups 2 and 4, which are the least common in Brazil.<sup>6</sup>

In this study, the most prevalent SLE clinical manifestations (arthritis, photosensitivity, malar erythema, hematological changes, oral or nasal ulcers, and kidney alterations) were similar to those reported by Bezerra et al.<sup>38</sup> No statistically significant relationship between TTV and each of the clinical signs assessed in this study was observed. However, the neurological manifestations were more frequent in TTV-positive

individuals, while the other manifestations, mainly the renal ones, predominated among TTV-negative individuals. In 2005, Gergely et al.<sup>17</sup> could associate the detection of TTV DNA neither with SLE systemic alterations nor with the SLEDAI score.

Elevated seroprevalence of TTV has been observed in several regions worldwide, even in apparently healthy individuals.<sup>22</sup> Considering that currently all the pathogenic potential of TTV is based on epidemiological surveys, some researchers have challenged that potential.<sup>14</sup> Thus, there is still no evidence to support the indication for treatment of TTV infection. In addition, there is still no evidence that TTV eradication from the body can change the course of any ongoing disease.

However, based on this study and on the literature published about this topic, TTV could use more than one mechanism to trigger autoimmune disease. However, further studies are required to determine the real pathogenic potential of TTV inside the immune system, such as its capacity to induce an altered autoimmune response, to modify the clinical course of SLE, or to trigger SLE in genetically predisposed individuals.

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