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Association between Torque Teno Virus Frequency and Systemic Lupus Erythematosus: A Potential Trigger for Autoimmune Diseases

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ABSTRACT

Background: Infectious agents are considered as one of the possible etiological factors of systemic lupus erythematosus (SLE). It has been suggested that torque teno virus (TTV) may trigger autoimmune disorders, but few studies have been conducted on the relationship between this virus and autoimmune diseases, especially SLE. The present study aimed to evaluate the association between TTV frequency and SLE.
Materials & Methods: Serum samples were collected from a total of 116 participants, including 58 healthy people and 58 SLE patients who referred to the rheumatology clinic of Shahid-Beheshti hospital in Kashan, Iran from January 2020 to January 2021. After the extraction of viral DNA from the samples, a nested PCR test was performed using specific primers to detect TTV.
Findings: TTV was detected in 43 SLE patients (74.1%, 95% CI: 63.4-86.2) and 33 healthy individuals (56.9%, 95% CI: 44.1-69.0). A significant correlation was found between SLE and the presence of TTV (r= .32, p= .03). There was no correlation between the presence of TTV and musculoskeletal involvements, skin lesions, renal manifestations, and hematological manifestations (r< .05, p> .05). TTV was detected more frequently in patients with active lupus than in patients with quiescent disease, and this difference was significant (p= .048).
Conclusion: A significant association between TTV and SLE was observed in the present study; however, further studies are needed to investigate the role of TTV in the pathogenesis and clinical course of SLE.

Keywords: Autoimmune diseases, Systemic lupus erythematosus, Torque teno virus.

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Introduction

Systemic lupus erythematosus (SLE) is characterized by the production of autoantibodies and impaired nuclear autoantigen tolerance [1]. This complex illness could affect the skin, eyes, heart, kidneys, muscles, and joints [2]. The global SLE prevalence in adults is estimated to be around 61 cases per 100,000 people. The prevalence of SLE in Iran is estimated at 40 cases per 100,000 people [3]. According to previous research, some viruses may be responsible for the development of lupus erythematosus [4-6]. Epstein–Barr virus (EBV), cytomegalovirus (CMV), parvovirus B19, and retroviruses are possible causes of SLE [7-9]. Molecular mimicry is one of the primary ways that viral pathogens potentially trigger autoimmunity [10]. This occurs when the similarity between exogenous peptides and self-peptides leads to activation of autoreactive T or B cells [4]. Transfusion-transmitted virus (TTV) was first isolated from blood samples of patients without hepatitis A-G in 1997. After that, this new DNA virus was classified in the family *Anelloviridae*, and named torque teno virus [11]. Virions consist of a circular single-stranded DNA of 3,800 base pairs. Due to the genetic structure of the TTV genome, more than 30 genotypes of this virus have been divided into five groups. The TTV genome consists of a coding sequence of approximately 2.6 kb, two open reading regions (ORF1 and ORF2), and an untranslated region (UTR) of approximately 1.2 kb [12].

At first, researchers found that TTV was associated with hepatitis, but later studies reported its presence among more than 50% of the healthy population. Today, epidemiological studies report the presence of this virus in diseases such as Hodgkin's disease, liver diseases, aplastic anemia, acute respiratory diseases, bullous pemphigoid, and cancer [12-14]. Recent data on the biology of TTV suggest that it could trigger autoimmune diseases, but few studies have been done in this regard [7]. Gergely et al. (2005) [15] and Costa et al. (2012) [16] reported that the frequency of TTV in lupus patients was higher than in healthy controls., but these studies are not strong enough to confirm the link between TTV and systemic lupus erythematosus. Considering that the etiology of lupus disease is still unknown, finding a strong correlation between an infectious agent such as TTV and the disease is very valuable. If the lupus disease is found to be associated with a virus, the incidence of the disease could be reduced by inhibiting the viral infection. The research question of this study is whether there is a link between the presence of TTV and SLE.

Objectives: This study aims to investigate the possibility of an association between TTV and SLE by estimating the prevalence of TTV in SLE patients.

Materials and Methods

Study population: Blood samples from 58 healthy individuals and 58 SLE patients (without age or gender restrictions) who were referred to the rheumatology clinic at the Shahid-Beheshti hospital in Kashan, Iran from January 1, 2020 to January 1, 2021 were collected for this case-control research. Patients who meet at least 4 of the 11 American College of Rheumatology (ACR) criteria, including positive ANA, seven clinical signs (constitutional, hematologic, neuropsychiatric, mucocutaneous, serosal, musculoskeletal, renal), and three immunological signs (antiphospholipid antibodies, complement proteins, SLE-specific antibodies), were selected by two rheumatologists. Exclusion criteria were as follows: 1) suffering from other autoimmune diseases, 2) malignancy, 3) infectious diseases, 4) hepatitis, and 5) receiving a liver

transplant. The selected healthy people did not have at least four ACR criteria.

All participants completed informed consent forms. The Kashan University of Medical Sciences Ethics Committee (IR.KAUMS.MEDNT.REC.1397.100) authorized this study. The following information was collected from SLE patients' medical records: age, sex, disease duration, SLE clinical manifestations, and the disease activity. Lupus disease activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), and SLEDAI score ≤ 6 was considered as inactive lupus [17].

TTV detection: Viral DNA was extracted from sera using AmpliSens® RIBO-prep nucleic acid extraction kit (AmpliSens, Russa) by precipitation method. The polymerase chain reaction (nested PCR) was used to detect TTV. The first step of test was conducted with a reaction mixture consist of 12.5 µL of Sinaclon PCR master mix (Iran), 0.5 µL of TTV-NG054 primer (TTTGCTACGTCACTAACCAC), 0.5 µL of TTV-NG1321 primer (AGCCCGAATTGCCCCTTGAC), 10 µL of template DNA, and 1.5 µL of nuclease-free water. The second step of test was conducted with a reaction mixture consist of 12.5 µL of Sinaclon PCR master mix (Iran), 10 µL of the first product, 0.5 µL of TTV-NG 054 primer (TTTGCTACGTCACTAACCAC), 0.5 µL of TTV-NG1471 primer (GCCAGTCCCGAGCCCGAATTGCC), and 1.5 µL of nuclease-free water. The following PCR program was conducted: initial denaturation step at 94 °C for 7 min, followed by 40 (for the first step of PCR) or 35 (for the second step of PCR) cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 45 s, and extension at 72 °C for 1 min as well as a final extension step at 72 °C for 5 min. PCR products were electrophoresed on 2% agarose gel and visualized using a gel documentation system after staining with DNA safe stain (Sinaclon, Iran). The size of amplified fragment in the second step of PCR was 220 bp.

Statistical analysis: Chi-square test was used to evaluate the relationship between TTV and SLE clinical signs (musculoskeletal involvement, skin lesion, etc.) and between TTV and lupus activity. The same test was used to compare the frequency of TTV positivity in controls and SLE patients. Student's t-test was performed to compare the duration of the disease between TTV-positive and TTV-negative groups. SPSS software Version 27 was used to perform statistical analysis, and a p value below 0.05 was considered significant.

Findings

The mean age of SLE patients and controls was 41.3±13.6 and 39.5±14.2 years, respectively (p= .802). There were 49 women (84.5%) and nine men (15.5%) in each population group under study. TTV was detected in 76 samples by PCR method and observing a specific band on the electrophoresis gel (Fig. 1). The results showed that 43 (74.1%) of the SLE patients were TTV positive, and 15 (25.9%) were TTV negative (Table 1). In the control

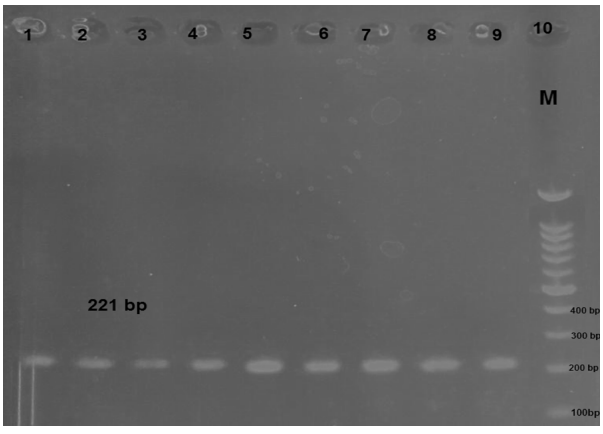

Figure 1) Electrophoresis of PCR products after performing nested-PCR with specific primers. Lane 1-8: positive samples, lane 9: positive control (a positive sample with the sequenced genome), lane 10: 100 bp ladder.

group, 33 individuals (56.9%) were TTV positive, while 25 (43.1%) were TTV negative. There is a positive relationship between SLE and TTV (p= .03). The frequency distribution of TTV among SLE patients according to their clinical signs showed that there was no correlation between the presence of TTV and musculoskeletal involvements, skin lesions, renal manifestations, or hematological manifestations (Table 2, p> .05). Musculoskeletal involvement was more prevalent in TTV-positive than in TTV-negative patients. TTV was detected more frequently in patients with active lupus than in patients with quiescent disease, and this difference was significant (p= .048). The average duration of the disease in TTV-positive and TTV-negative patients was 6.3±3.5 and 5.9±3.7 years, respectively.

Discussion

Systemic lupus erythematosus is an autoimmune disease with a chronic and relapsing history that mainly affects women of childbearing age [2]. Several viruses, including Epstein-Barr virus (EBV), cytomegalovirus (CMV), parvovirus B19, and retroviruses, are potential causes of the disease and linked to the pathogenesis of SLE [7, 8]. In the current study, genome of TTV was found more frequently in lupus patients than in healthy controls. The results showed a statistically significant link between TTV infection and SLE. None of the SLE symptoms evaluated in this study showed any correlation with TTV. Musculoskeletal involvement was more prevalent in TTV-positive than in TTV-negative patients.

In general, there are few and insufficient studies on the prevalence of TTV in lupus patients. For the first time (2005), Gergely et al. reported the frequency of TTV in SLE patients about 57%, which was statistically higher than the frequency of the virus in healthy people (33.16%; p< .001) [15]. In the present study conducted in Kashan, TTV was detected in 43 SLE patients (74.1%) and 33 healthy individuals (56.9%). The frequency of TTV infection in both study groups in the present research was higher than in Gergely's study, which may be due to the difference in the geographic distribution of

Table1) Frequency of TTV among SLE patients and the control group

Group	TTV Positive	TTV Negative	*P* Value
SLE	43 (74.1%)	15 (25.8%)	.03
Control	33 (56.9%)	25 (43.1%)	

Table 2) Frequency of TTV among SLE patients according to the clinical signs, lupus activity, and disease duration

Characteristic		TTV Positive N(%)	TTV Negative N(%)	*P* Value
Clinical sign	Musculoskeletal involvement	35 (73)	13 (27)	.09
	Skin lesion	21 (70)	9 (30)	.14
	Renal manifestation	14 (70)	6 (30)	.17
	Hematological manifestation	17 (74)	6 (26)	.19
Lupus activity		32 (74)	11 (26)	.048
Disease duration (mean year ± sd)		6.3±3.5	5.9±3.7	0.97

the virus.

Costa et al. (2012) detected TTV in 17 SLE patients (37%) and seven healthy controls (15.2%, p= .03) [16]. In their study, TTV prevalence was lower than that reported in the current study. Similar to our study, they did not find a relationship between TTV infection and SLE clinical manifestations. In the present study, TTV was detected more frequently in patients with active lupus than in patients with quiescent disease, and this difference was significant (p= .048). This evaluation was not done in Gergely's or Costa's study. Findings suggest the virus may play a role in the activation of lupus, which requires more extensive studies.

In addition, future research should also investigate the potential role of TTV in disease progression and whether it could be a target for therapeutic interventions. If future research confirms the association between TTV and SLE, it could have important clinical implications for the diagnosis, treatment, and management of SLE patients.

Seemayer et al. (2001) found no relationship between TTV prevalence and autoimmune disorders. They detected TTV in 10 of 84 (12%) patients with systemic sclerosis, 9 of 41 (22%) patients with rheumatoid arthritis, 3 of 43 (7%) patients with osteoarthritis, and 16 of 122 (13%) healthy controls [18]. They did not report TTV prevalence among SLE patients. TTV has been found to be more prevalent in individuals suffering from some autoimmune disorders; however, no evidence linking the virus with the pathology of these or any other diseases has been provided to date.

There are several potential mechanisms suggesting that TTV may contribute to the onset or development of SLE. According to the findings of Shirai and Hirose (2006), viruses with lymphocyte tropism, like TTV, can trigger auto-reactive lymphocytes [19]. Therefore, the virus could promote B cell proliferation and antibody production, resulting in the buildup of circulating immune complexes. In another potential mechanism, TTV may interfere with antigen processing and presentation after infecting immune system cells, including macrophages and T cells. This interference could lead to the generation of novel epitopes, which may result in autoimmunity. TTV has the potential to interfere with MHC class I molecule expression in a manner similar to cytomegalovirus. In another potential mechanism, TTV might promote the production of a number of cytokines that could change regulatory T cell behavior and increase the risk of SLE. Gergely et al. (2005), found cross reactive epitopes between the endogenous retroviral autoantigens, torque teno virus proteins, and the lupus autoantigen 70kU1snRNP [15]. In another possible way, molecular patterning of some TTV antigens and self-antigens may stimulate autoreactive lymphocytes, indicating the initiation of an autoimmune response. In addition, TTV-derived apoptosis-inducing protein (TAIP) can induce abnormal apoptosis and cause loss of regulatory lymphocytes necessary for autoimmunity control [20]. It could also stimulate autoreactive B cells through toll-like receptors (TLR).

It is important to note that treatment of SLE patients with corticosteroids and immunosuppressants may increase the risk of developing opportunistic infections such as TTV. Therefore, more detailed studies should be done to show whether this virus could trigger lupus or not. The present study had a relatively small sample size and was conducted in a specific geographic region. The generalizability of the findings may be limited, and larger studies with broader populations are needed to confirm the association between TTV and SLE.

Conclusion

A significant association between TTV and SLE was observed in the present study; however, further studies are needed to investigate the role of TTV in the pathogenesis and clinical course of SLE.

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Ethical permissions: The Kashan University of Medical Sciences Ethics Committee (IR.KAUMS.MEDNT.REC.1397.100) authorized this study.

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