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Assessment of Torque Teno Virus (TTV) Frequency in Healthy Blood Donors in the Central Region of Iran, Yazd

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ABSTRACT

Background: Torque teno virus (TTV) is a globally prevalent virus in humans, yet comprehensive knowledge about its prevalence, predominant transmission routes, and pathogenesis remains limited. This study aimed to assess the frequency of TTV infection among healthy blood donors in Yazd, Iran.

Materials and Methods: A total of 236 healthy blood donors, devoid of HIV/HBV/HCV infection markers, participated in the study from 2015 to 2016. Nested Polymerase Chain Reaction (PCR) utilizing a set of oligo primers for the 5ˈ- UTR region was employed to detect TTV DNA in serum samples.

Results: The TTV genome was identified in 161 out of 236 (61.2%) healthy blood donors. The mean age for men and women was 43 and 57 years, respectively. Of the participants, 156 were male, and 107 were female. Donor age exhibited a significant association with virus presence (P=0.007); however, gender did not show a statistically significant association with the frequency of TTV infection in healthy blood donors (P=0.3).

Conclusion: The study revealed a notably high frequency of the Torque teno virus in Yazd province, aligning with similar findings globally. Further investigations are warranted to elucidate the clinical implications of the virus in the healthy population.

Keywords: Torque teno virus (TTV); Blood donors; Polymerase chain reaction

INTRODUCTION

Human Torque teno virus (TTV) viremia is prevalent in the general population without directly inducing illness in humans [1]. TTV belongs to the Anelloviridae family, characterized by a negative-sense, single-stranded circular DNA structure[2]. While TTV infection typically does not manifest clinical

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symptoms, the detection of TTV genome sequences in sera and hepatic tissues has been associated with certain acute and chronic liver diseases of unknown etiology [3].
TTV exhibits widespread transmission among humans through various means, including blood transfusions, hemodialysis, hemophilia, intravenous drug use, and oral-fecal routes[4]. The virus can be identified in plasma, peripheral blood mononuclear cells, and diverse bodily fluids, such as saliva, feces, semen, and vaginal secretions [5, 6]. Given its potential role in conditions like cancer, hepatitis, respiratory diseases, and autoimmune disorders, ensuring the safety of blood transfusions becomes paramount in preventing irreversible complications associated with TTV [2, 7]. The global prevalence of TTV is notably high in individuals with liver diseases, including cirrhosis, non-A to G fulminant hepatitis, non-A to G liver disease, hepatocellular carcinoma, as well as in cases of acute respiratory and kidney diseases, HIV-positive individuals, and even healthy populations[8, 9].
Recognizing the significance of identifying TTV in contaminated blood, several health regulatory organizations, including those in Brazil and the United States, mandate comprehensive screening of donated blood for blood-borne viruses, including TTV. Additionally, the prevalence of TTV among blood donors has been investigated, revealing its presence in up to 20% of the Iranian population[10]. Since the initial discovery of TTV, debates have ensued regarding its association with human disease, and studies on TTV viremia prevalence have reported substantial variations, ranging from 2-23% to as high as 90% [11, 12].
Various methods have been proposed to identify and screen TTV in donated blood, including nucleic acid testing methods such as PCR, serological methods like ELISA, and PCR-based techniques like real-time PCR [13]. Due to its elevated sensitivity and specificity, PCR has earned the designation of the gold standard for identifying the TTV virus [14].
TTV remains a prevalent infection among healthy donors globally, and understanding its frequency in different regions serves as a valuable resource for elucidating transmission routes and potential disease causation. This study endeavors to contribute to this understanding by determining the frequency of TTV among healthy blood donors in the Yazd province of Iran.

MATERIALS AND METHODS

Study Population

A total of 236 human blood samples were prospectively collected from healthy blood donors attending the blood donation center in Yazd, Iran, between 2015 and 2016. The sample size was chosen based on the availability of healthy donors during that period and study constraints. Inclusion criteria for this study comprised individuals with negative PCR results for Hepatitis C Virus (HCV), Hepatitis B Virus (HBV), and Human Immunodeficiency Virus (HIV). Ethical approval for this study was obtained from the Ethics Committee of Shahid Sadoughi University of Medical Sciences-Yazd, indicated by the assigned ethical code. Prior to participation in the study, all subjects provided informed consent for inclusion. Data, including sex and age, were recorded for each donor.

Viral DNA Extraction

Viral DNA was extracted from serum samples using the High Pure Viral Nucleic Acid Kit (Roche Germany), following the manufacturer's instructions. Subsequently, DNA extracts were stored in DNase-free microtubes at -20°C.

Nested Polymerase Chain Reaction (Nested PCR)

The quality evaluation of the extracted genome involved amplifying the human β-globin gene using PCO3/PCO4 primers (Table 1). Subsequently, further investigations were conducted on samples that exhibited positive results. These DNA extracts were then used as templates for the detection of Torque Teno Virus (TTV) DNA through Nested Polymerase Chain Reaction (Nested PCR). The primers used for the first and second rounds were designed from the 5'UTR region of the TTV genome (Table 1).
The first round of amplification was conducted in a total reaction volume of 25 µl, comprising 5 µl of extracted DNA, 15 µl of master mix, 2 µl of primers (NG054/NG147), and 3 µl of water for 35 cycles. Each cycle consisted of denaturation at 94°C for 30 sec,

annealing at 60°C for 30 sec, and extension at 72°C for 30 sec. The second round involved 2 µl of primers (NG133/NG132) using 2 µl of the round 1 PCR product as a template for 25 cycles, with identical cycling parameters as the first round, except for the final extension step which was increased to 10 minutes to ensure complete duplex molecule formation. Negative controls, consisting of a PCR reaction mixture without a DNA template, were included in each set of PCR runs.

Gel Electrophoresis and Visualization

The PCR products were electrophoresed on a 1.5% agarose gel, stained with 1 µg/ml safe stain, and visualized under ultraviolet light (Figures 3-5).

Purification and Sequencing

The TTV-positive PCR products were purified using the QIAQuick PCR Purification Kit (Qiagen, United Kingdom) and subsequently subjected to automated sequencing (Figures 6). The obtained DNA sequences were compared using BLAST to confirm their identity as TTV sequences.

Table 1: Sequences of Primers Used in This Study

Locus	Oligonucleotide Sequence	Products Size, bp	Ref
NG054 sense 3-22	5' TTTGCTACGTCACTAACCAC 3'	151	15
NG147 antisense 211-233	5' GCGAGTCCCCGAGCCCGAATTGCC 3'		
NG133 sense 91-115	5' GTAAGTGCACTTCCGAATGGCTGA 3'	60	15
NG132 antisense 204-22	5' AGCCCGAATTGCCCCTTGAC 3'		
PCO3	5' ACACAACTGTGTTCACTAGC 3'	110	16
PCO4	5' CAACTTCATCCACGTTCACC 3'		

Statistical analysis

Statistical analysis was performed using IBM SPSS software (SPSS Inc., Chicago, IL), version 22.0, to determine any statistical relations. The Chi-square test or Fisher's exact test was utilized to analyze data, and a P-value <0.05 was considered statistically significant.

RESULTS

In total, 236 (male, 156; female, 107) healthy individuals were enrolled in this study. The mean age of participants was 43 and 57 years, respectively. TTV-DNA was detected in 161 out of 236 (61.2%) healthy blood donors (Figures 4,5). These results showed a significant relationship between the frequency of the infection with TTV and age groups (P-value= 0.007) (Figure 1). However, no significant differences were observed between the frequency of the TTV infection and gender groups (P-value= 0.3) (Figure 2).

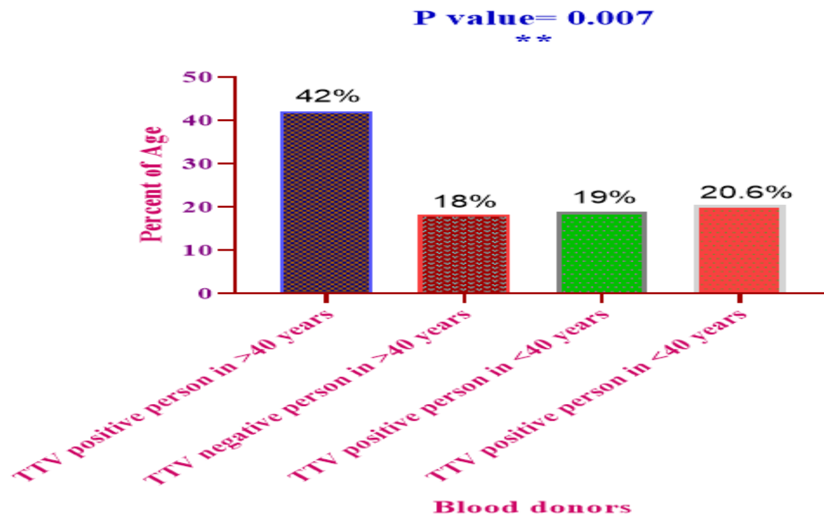

Figure 1. Torquetenovirus (TTV) frequency grouped by age intervals in total study population

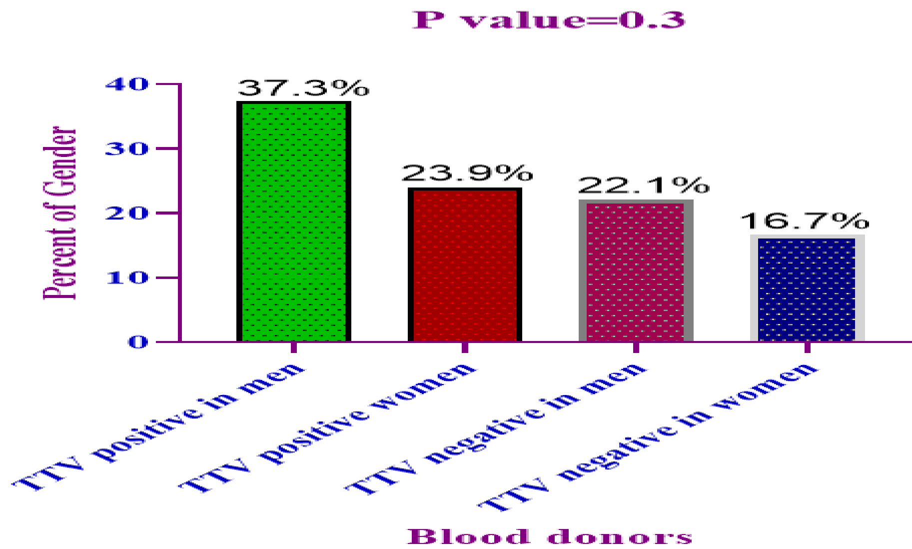

Figure 2. Torquetenovirus (TTV) frequency grouped by gender intervals in total study population

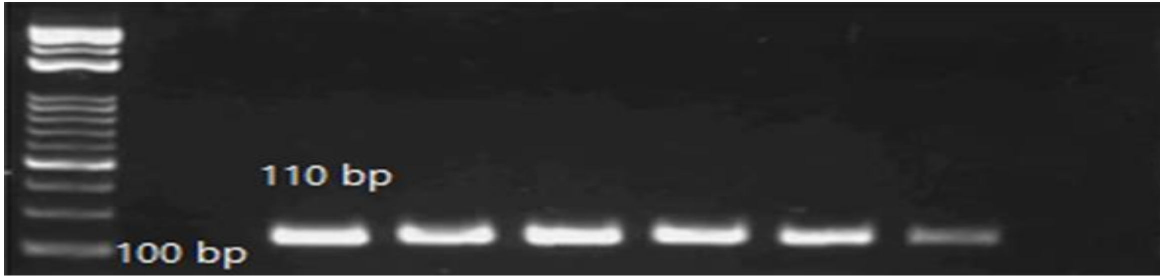

Figure 3. Positive bands detected in the polymerase chain reaction, indicative of the presence of the β-globin gene

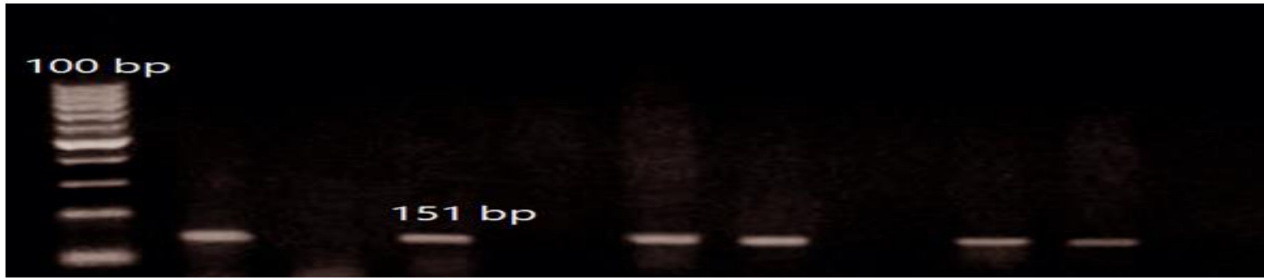

Figure 4. The positive bands detected in the first run of the nested-polymerase chain reaction indicate the presence of the TTV gene

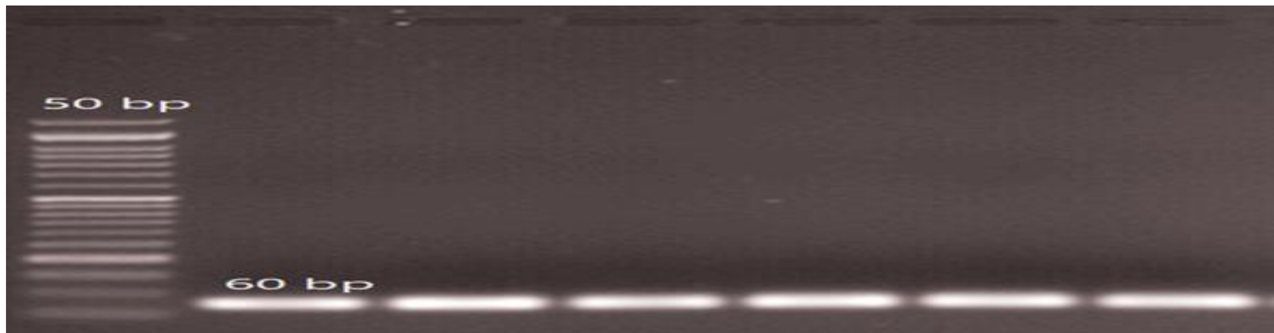

Figure 5. The positive bands detected in the second run of the nested-polymerase chain reaction indicate the presence of the TTV gene.

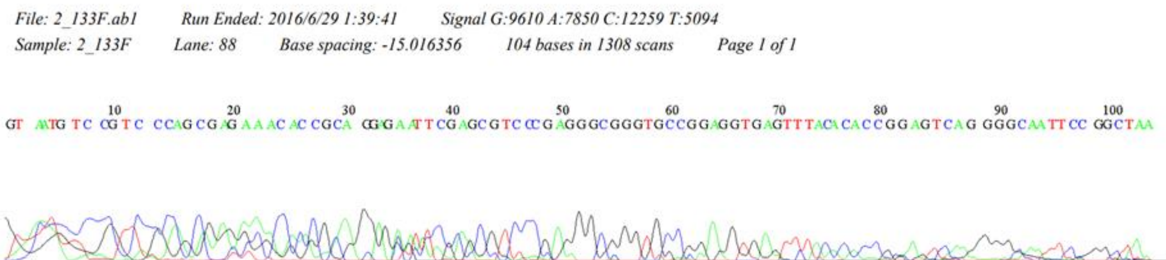

Figure 6. Representative electropherograms of PCR product of TTV ORF1 obtained from sanger sequencing

DISCUSSION

The rate of TTV infection in humans is relatively high and this infection is related to various diseases. However, there is no direct evidence to prove this association[8]. Recent studies have shown that TTV infection is a very common viral infection in the blood of high-risk groups such as people with HIV, HBV or HCV infection and healthy people worldwide[9]. On the other hand, considering the role of this virus in diseases such as cancer, hepatitis, respiratory diseases and autoimmune disorders, ensuring the safety of blood transfusion is very important in preventing irreversible complications related to TTV [17]. Therefore, more studies should be conducted to evaluate the frequency of TTV infection in regional blood donors to determine the pathology of TTV infection.

The recent study aimed to evaluate the frequency of TTV infection in healthy blood donors in Yazd, Iran. All Serum samples were obtained from healthy blood donors who had no markers of HIV, HBV, or HCV infection. In this study, the frequency of TTV DNA in healthy blood donors was 61.2% which was similar to that observed in Qatar (81.4%)[10], Russia (94%)[11], Brazil (69%)[5], Italy (65%)[18], and Iran (80%) [19] among healthy blood donors.

Moreover, the TTV infection in the current study was remarkably higher than those conducted in the USA and Europe; the viremia rates in healthy blood donors in the USA varied from 1% to 10%. Moreover, in European countries ranged from 1-13% [20, 21]. Other studies were performed in Yazd in 2020, and the prevalence of TTV was reported at 4% in healthy subjects[22]. Also, the TTV genome was detected in 3.2% of healthy individuals in Pakistan[23]. Thus, considerable variation in the frequency of TTV has been observed in healthy populations in various countries.

The considerable variation in viremia rates in this study and the cited studies could be associated with different factors, such as sample size, the diagnostic tests used, the geographical distribution of the population, and the selection of the different target amplified and PCR primers including primers that amplified regions in the ORFs or the UTRs[24]. According to studies, targeting sequence amplified UTRs vs. ORF demonstrated a higher rate of TTV viremia detection [25]. Furthermore, the overall high prevalence of TTV viremia in the current study, and other studies may be because the virus spread easily not only through blood transfusion, but also through other routes including the oral-fecal mode, mother-to-child, breast milk, saliva, and other body fluids[10]. According to studies, TTV viremia is relatively frequent around the world and in different ethnic groups[9], thus it is important to investigate the impact of TTV infection in healthy individuals.

The findings of the present study showed that TTV infection is associated with age among blood donors. According to a study performed in Brazil, the presence of TTV was significantly associated with age[26]. No significant relationship was found between TTV infection and gender. A study in Qatar found no significant differences between men and women[10]; however, a study in Italy showed that the TTV viremia in men was higher than in women. Hence, subsequent studies are needed to confirm this hypothesis [18].

CONCLUSION

This study found a high frequency of TTV in healthy blood donors, in confirmation of other studies in the healthy population. TTV significantly was associated with age among healthy blood donors. However, no interaction was detected between gender and TTV infection. More studies are needed to investigate transmission routes and pathogenesis of TTV to decrease the risk of transmission among healthy blood donors.

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CONFLICT OF INTERESTS

None to declare.

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Ethical approval

The study was approved by the Ethics Committee of Yazd University of Medical Sciences, Yazd, Iran, under number IR.SSU.MEDICINE.REC.1395.135.

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