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Immunological Investigation of Torque Teno Virus among Blood Donors in Mosul City, Iraq

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ABSTRACT

Objective: *This study aimed to detect Torque Teno virus antigens using the ELISA technique and to evaluate the immune status by measuring the levels of inflammatory cytokines, namely Tumor Necrosis Factor (TNF) and Interleukin-10 (IL-10).* ***Method****: This study was conducted on 150 blood samples collected from blood donors at the main blood bank in Mosul, Iraq. The study used an enzyme-linked immunosorbent assay (ELISA) to detect viral antigens. The immune response was assessed by measuring the levels of tumor necrosis factor-alpha (TNF-a) and interleukin-10 (IL-10).* ***Results****: the ELISA test results revealed a low prevalence of TTV antigen (9.33%), with the highest rates recorded among the oldest age group (34–41 years) at 4.67%.* ***Novelty****: The results revealed the presence of immune regulatory dysfunction in this group of patients, as they exhibited signs of both immune activation and suppression simultaneously. This underscores the importance of continuous immunological monitoring for this category of patients.*

INTRODUCTION

Blood donation is a life-saving procedure that involves the temporary loss of a certain volume of blood. Before donation, donors must meet several eligibility criteria. However, despite these criteria, several adverse effects or complications can occur [1]. Although considered safe, the adverse effects of blood donation have recently increased with the emergence of new diseases, heightening the need for reliable and effective blood donation management [2]. The risk of transfusion-transmitted diseases, such as HIV, syphilis, and hepatitis B and C viruses, is low, but in developing countries, particularly in Africa, blood safety is still not assured [3]. Torque Teno Virus (TTV) is a small, non-enveloped, single-stranded circular DNA virus belonging to the Anelloviridae family, first discovered in 1997 in a patient with post-transfusion hepatitis of unknown etiology. TTV has since been recognized as a ubiquitous component of the human virome, with a global prevalence exceeding 90% in some populations [4]. Early studies of TTV indicate that it is hepatotropic and most likely replicates in liver cells [5]. TTV has an extremely high prevalence and is regarded as a part of the human virome, the replication of which is controlled by a functioning immune system [6]. Despite its widespread presence, the clinical significance of TTV remains largely unclear, as it has not been definitively associated with any specific disease [7]. TTV exhibits various features that facilitate its application as an immune biomarker: high prevalence rates, nearly ubiquitous distribution, stable viral loads with little intra-individual variability, and insensitivity to antiviral drugs [8]. The discovery of TTV also unlocked a variety of interesting functions for viruses [9]. As soon as the virus was discovered, it became evident that TTV was

neither linked to hepatitis nor any other recognized diseases because it was shown to be common in both healthy and sick individuals [10]. Given the growing interest in the human virome and its impact on health and disease, further research into TTV is warranted to elucidate its biological significance and potential applications in clinical practice [11].

Ethical Considerations

This research was approved by the Ethics Committee of the College of Science at the University of Mosul, Iraq. Written informed consent was obtained from all participants and from the legal guardians of underage individuals after explaining the objectives of the study, the sample collection process, and the laboratory procedures that would be performed on their samples, document number 58909 (9/12/2024).

RESEARCH METHOD

This study was conducted on 150 blood samples collected from blood donors at the Main Blood Bank in Mosul, Iraq, during the period from December 2024 to February 2025. In addition, 15 blood samples from healthy individuals were included as a control group for the immunological cytokine measurements. Five milliliters of venous blood were collected from each patient in plain tubes without anticoagulant. Serum was separated from the blood components by centrifugation at 3000 rpm for 10 minutes. The obtained serum samples were then stored at −20°C until further analysis. The ELISA technique was used to detect Torque Teno Virus (TTV) antigens present in serum samples, following the manufacturer's instructions provided with the commercial kit (Human transfusion transmitted virus (TTV) ELISA Kit Catalogue Number: (SL1737Hu) belongs to Sun Long Biotech Co., LTD company made in China. The assay is based on the sandwich ELISA method, in which specific antibodies are pre-coated onto the wells of the microplate. Serum samples were added, followed by the enzyme, a Horseradish Peroxidase (HRP)-conjugated antigen specific for TTV, which was added to each Microplate well and incubated, so the antibody-antigen-enzyme labeled antibody complex is formed. Following a wash to remove any unbound reagent, the TMB substrate solution is added to each well. The final step involved measuring the absorbance at 450 nm using an ELISA reader.

The level of Tumor Necrosis Factor-alpha (TNF-α) in serum was also measured by the ELISA technique. The standard curve was run using the standard solutions of the kit (Human Tumor Necrosis Factor α (TNF-α) ELISA Kit), Catalogue Number: SL1761Hu is a product of Sun Long Biotech Co., LTD company made in China, and the results were calculated by reading the absorbance at 450 nm using an ELISA reader.

In addition to measuring Tumor Necrosis Factor-alpha, the level of Interleukin-10 (IL-10) was also measured using the ELISA technique. A commercial kit (Human Interleukin 10 (IL-10) ELISA Kit Catalogue Number: SL0967Hu, from Sun Long Biotech Co., LTD company in China was used with the standard solutions provided by the company. The result was interpreted by measuring the absorbance at 450 nm using an ELISA reader.

Statistical analysis was made using SPSS software version 26. The data means and standard deviations were calculated. To compare the patient group and the control group, the unequal variance T-test (Welch's t-test) was applied. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Results

The study showed that the maximum TTV infection rate (TTV Ag by ELISA) was found in healthy blood donors, is 10.6 %. Despite being classified as a ubiquitous virus, the low percentage of TTV-positive samples in this study could be attributed to several factors.

Table 1. TTV detection in groups studied with ELISA.

Age/years	Number	Positive	Percentage
(18-25)	28	2	1.33%
(26-33)	73	5	3.33%
(34-41)	49	7	4.67%
TOTAL	150	14	9.33%

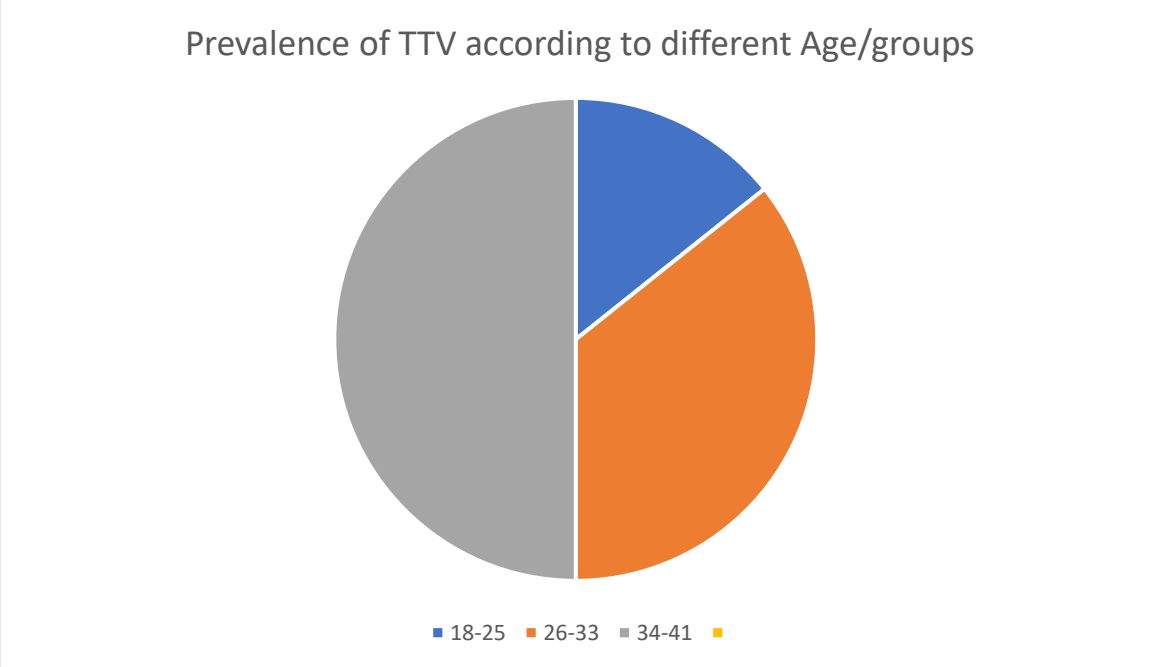

Figure 1. Prevalence of TTV according to different Age groups.

From an immunological perspective, the levels of the cytokines Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-10 (IL-10) were measured in 30 blood samples using the ELISA method. These results were compared with a control group consisting of 15 healthy individuals. The mean concentration of TNF-α in blood donors was normal and showed no statistically significant differences ($p > 0.05$).

Similarly, the mean IL-10 concentration in samples was 19.52 ± 6.6 pg/mL, compared to 16.87 ± 2.01 pg/mL in the control group, which showed a difference reaching statistical significance (p = 0.007).

Table 2. Comparison between IL-10 and TNF Concentration according to age groups (years) in Patient Samples.

Age/years	IL-10			TNF		
	Mean	P- value	SD	Mean	P- value	SD
(18-25)	16.56	0.007	2.20	23.10	0.388	3.88
(26-33)	23.29	0.221	10.07	23.98	0.139	1.64
(34-41)	18.71	0.597	7.81	22.63	0.444	8.54

Discussion

The study showed that the maximum TTV infection rate (TTV Ag by ELISA) was found in healthy blood donors, is 10.6 %. Despite being classified as a ubiquitous virus, the low percentage of TTV-positive samples in this study could be attributed to several factors. Torque Teno virus is a DNA virus and therefore its genome has a wide scope to either integrate directly into the human genome or to promote certain mutations in the cellular genome that lead to oncogenesis. Extensive studies are needed before these microorganisms can be assumed to be a safer organism that does not harm the human body [12]. Several articles have been published on the prevalence and risk factors of TTV infection in ESRD patients. Most are studies on the epidemiology of TTV in maintenance hemodialysis (HD) patients [13]. The present results are consistent with several previous studies [14]. It has been reported that the frequency of TTV infection may vary according to the genomic region tested and the geographical region studied, and dialysis patients showed a TTV positivity rate of 17% [15]. These results indicate that TTV-DNA is transmitted to recipients by blood and blood products. Therefore, blood transfusion is one of the most effective means of TTV transmission [16].

The results indicated a statistically significant difference in IL-10 levels exclusively among individuals aged 18-25 years with a probability value of 0.007 (P< 0.05), potentially reflecting heightened immune sensitivity or age-related regulatory shifts within this group. Older groups (26-33 and 34-41) had higher IL-10 levels, but P-values > 0.05, identifying individual variation (higher SD in 26-33). This could be explained by the fact that IL-10 is an anti-inflammatory cytokine, and studies show that IL-10 levels increase with age as the body attempts to regulate chronic inflammation [17]. Suggesting that these findings may be modulated by factors such as the chronicity of the disease or sustained immunological responses over time. The absence of detectable TNF levels in TTV-positive samples may be due to several factors. First, TTV is often considered a non-pathogenic or commensal virus in healthy individuals, and therefore may not elicit a strong immune or inflammatory response [18]. Second, TTV may exist in a latent phase

or at a low viral load, conditions that may not sufficiently activate the immune system to produce inflammatory cytokines such as IL-6 and TNF [19]. Moreover, recent studies suggest that TTV may have the ability to suppress or regulate immune responses, which allows it to persist in the body without triggering a noticeable immune reaction. For example, [20] reported that TNF responses may be reduced in TTV-infected individuals, especially in immunocompetent persons.

CONCLUSION

Fundamental Finding : As a result of this study, the study results indicate a moderate prevalence of the virus, as the ELISA method showed an overall rate of 10.3% positivity for the TTV antigen in Mosul. The study demonstrated an age-related variation in IL-10 levels, with statistically significant values in the younger age group (18-25), while the study showed relative stability in TNF values, with no statistically significant values. **Implication :** The presence of the virus in healthy blood donors indicates that TTV can be transmitted through blood transfusions without symptoms, raising questions about its potential risk and its impact on the immune system. **Limitation :** The study was limited to a specific number of blood donors, which may not fully represent the general population, as this was a cross-sectional study, it only provides a snapshot of TTV immune markers at a single time point, without follow-up over time, and the study focused on selected immune markers, and did not cover other important cellular or molecular immune responses that may play a role in TTV infection. **Future Research :** Further studies on additional immunological markers are necessary to gain a clearer understanding of the impact of TTV on the immune system, focus on the impact of TTV infection in specific patient populations with altered immune status, such as cancer patients, organ transplant recipients, and individuals with chronic liver diseases, employ advanced molecular techniques, including gene sequencing, to identify different genotypes of TTV and explore their association with immune responses, and investigate the possible co-infection and interaction between TTV and other transfusion-transmissible viruses such as HBV, HCV, and HIV, and assess their combined effects on the immune system.

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