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Prevalence of Torque Teno Virus (TTV) in Blood Donor From Baghdad-Iraq

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Abstract

Objective: To determine the rate of TTV infection in Iraqi blood donors. And evaluate any association with demographic and risk factors.

Methods: This cross-sectional study conducted on serum samples from 359 participants who attended the blood donation unit of Al-Imammain Al-Kadhimain Medical City Hospital and National Blood Transfusion Center in Baghdad. The study sample consists of 351 males and only 8 females; their mean age is (36.04 ± 9.35 SD). For a period from November 2022 to January 2023. A nested polymerase chain reaction (PCR) was used to detect TTV untranslated region.

Results: The TTV virus was detected in 93.9% (337 out of 359) of blood donors. However, no significant association was found between demographic data (sex, age) and risk factors such as history of (medical surgery, tattooing, cupping practice, marital status, multiple sexual partners, animal husbandry , travel history), under line medical condition (diabetes mellitus and hypertension), viral hepatitis co-infection and occupation with TTV infection rate.

Conclusion: This study showed a high prevalence of TTV among healthy blood donors with no significant association with demographic data and risk factors. These results support that TTV as a part of the human virobiome and normally found in the majority of populations.

Keywords: Torque Teno virus, blood donors, polymerase chain reaction

Introduction

Two decades ago, Nishizawa and his group first isolated the prototype of the Human Torque Teno virus (HTTV) in a Japanese patient who was suffering from hepatitis of unknown etiology with rising alanine aminotransferase (ALT) in serum after receiving 30 units of blood during heart surgery.[1] TTV is a small, obligate intracellular virus with negative polarity and single-strand circular DNA, belonging to the large family Anelloviridae (AV), mainly classified into three genera: Alphatorquevirus (Torque teno virus), Betatorquevirus (Torque teno Mini virus), and Gammatorquevirus (Torque teno Midi virus), according to International Committee on Taxonomy of Viruses (ICTV), 2020.[2] However, the acronym (TTV) is derived from the Latin words "torque" (necklace) and "tenuis" (narrow), according to the nature of circular DNA.[3]

The main character of TTV is a great genetic diversity compared to other DNA viruses. Despite highly conservative untranslated regions (UTR), approximately 90% of all TTVs have similar UTR sequences and are used with specific primers for TTV detection.[4] Moreover, TTV exists in almost all populations worldwide,[5] with variable range according to the geographic regions; the prevalence ranges between (4% in Iran and 96% in Jordan) and may reach 100% in immune-compromised patients.[6,7] The high rate of TTV worldwide may be due to the presence of TTV in most clinical samples, such as peripheral blood, saliva, nasal secretions, tears, liver, gingival tissue, breast milk, semen, urine, bile juices, cerebrospinal fluids, and synovial fluid.[8,9] According to that, there are several modes of transmission (airborne, fecal-oral route, blood transfusions, transplacental during pregnancy, breastfeeding after birth, and sexual contact could be another mode of transmission).[10–12]

The researchers also observed TTV in animals and environments, leading to easy circulation within the community.[13,14] Several studies considered TTV a "commensal" virus that could be a part of the human virobiome without causing clinical outcomes or any particular pathogenesis.[15] Recently, TTV has been used as an endogenous immunological biomarker, a prognostic biomarker for viral infection and inflammatory diseases, and a virological marker for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) patients to distinguish between viral loads in severe, moderate, and mild cases; it is also used to estimate the efficiency of vaccines,[16] on the other hand, it could be used as an environmental or anthropic pollution biomarker.[17]

This study aimed to determine the rate of TTV infection in blood donors and evaluate any related demographic (age and sex) and risk factors (history of medical surgery, tattooing, cupping practice, marital status, multiple sexual partners, animal husbandry, travel history), underline medical conditions (diabetes mellitus and hypertension), viral hepatitis infection, and occupation with TTV infection rate.

Methods

Participants

The cross-sectional study was conducted on 359 of blood donors who attended the blood donation unit of Al-Imammain AL-Kadhimain Medical City Hospital and the National Blood Transfusion Centre in Baghdad from November 2022 to January 2023. A questionnaire was used to collect data by private interviews with participants, including sex, age, history of (medical surgery, marital status, multiple sexual partners, tattooing, cupping practice, travel), animal husbandry, underline medical condition (diabetes mellitus and hypertension), viral hepatitis co-infection, and occupation. The Institutional Review Board (IRB) at the College of Medicine/Al-Nahrain University granted the research project ethical approval on November 13, 2022 (No. 202207179).

Sample Collection

Five ml of whole blood was drawn from each participant in sterile gel tubes and allowed to clot at 25°C for 20 min. Then,

after being centrifuged at 3000 rpm for 10 minutes, serum samples were divided into aliquots in sterile eppendorf tubes, which were then stored at –20°C until used.

DNA Extraction

DNA was extracted from 200µl aliquots of serum by using the Easy Pure ReliaPrep Blood gDNA Miniprep System (Promega/ USA) kit to detect the presence of TTV nucleic acid in serum sample following the manufacturer's instructions.

PCR Amplification of TTV DNA

For DNA amplification of TTV, nested polymerase chain reactions (PCR) were used to amplify UTR sequences with specific primers (NG133 and NG147) for the first PCR round and (NG132 and NG134) for the second PCR round, as reported by Sarairah et al. and Cancela et al.[18,19] The PCR reaction (25 µl) for the first rounds of PCR was prepared by adding 5 µl of the template, 1.5 µl of each primer, and 12.5 µl of one Taq PCR master mix, Taq Green Master Mix (Promega USA). Complete the final volume by adding 4.5 µl of nuclease-free water. The PCR reaction (25 µl) for the second rounds of PCR was prepared by adding 2µl of the DNA template from the initial round of nested PCR then, 1.5 µl of each primers were added and 12.5 µl of one Taq PCR master mix (Promega, USA) was added, to completed the final volume adding 7.5 µl of nuclease-free water.

Positive and negative controls were included within each round of PCR to validate the results. Table 1 and Table 2 display the customized thermal cycler settings according to Cancela et al.[19] with modifications to optimize the outcome. The amplicon obtained from the first and second runs equals 143bp and 110bp nucleotides, respectively. They were observed in a 3% agarose gel, as shown in the Figures 1 and 2.

Quality Control

Both positive and negative controls were performed in each PCR round. The positive control was TTV DNA provided from a previous study,[20] while the negative control consist of all PCR components without adding the DNA template.

Table 1. **PCR program for first round reaction**

Step	Temperature	Time	Cycles description	Cycles
1	95°C	5 min	Initial Denaturation	1
2	94°C	30 s	Denaturation	35
	60°C	40 s	Annealing	
	72°C	45 s	Extension	
3	72°C	7 min	Final extension	1

Table 2. **PCR program for Second round (Nested)reaction**

Step	Temperature	Time	Cycles description	Cycles
1	95°C	5 min	Initial Denaturation	1
2	94°C	30 s	Denaturation	30
	60°C	40 s	Annealing	
	72°C	45 s	Extension	
3	72°C	7 min	Final extension	1

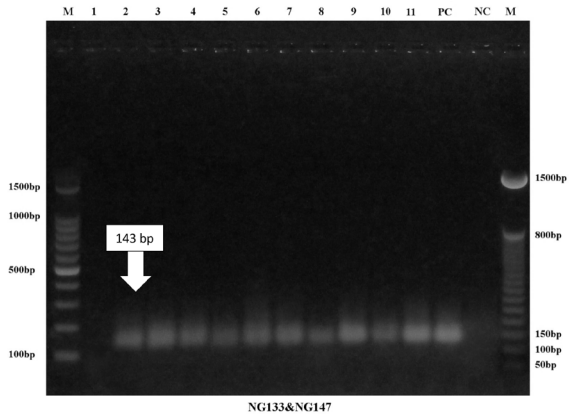

Fig. 1 **Gel-electrophoresis of first-round PCR products (5'UTR region) using 3% agarose in tris-acetate-EDTA (TAE) buffer Lane M: (100 bp DNA marker), Lane 1: negative sample, Lanes 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 positive samples, Pc: positive control, Nc: Negative control; and Lane M: (50 bp DNA marker).**

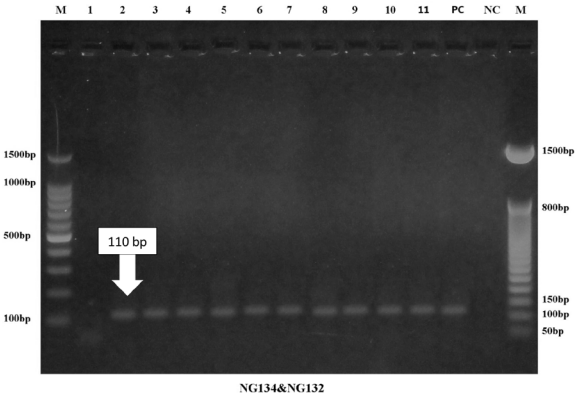

Fig. 2 **Gel-electrophoresis of second-round PCR products (5'UTR region) using 3% agarose in tris-acetate-EDTA (TAE) buffer Lane M: (100 bp DNA marker); Lane 1: negative sample; Lanes 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 positive samples; Pc: positive control; Nc: Negative control; and Lane M: (50 bp DNA marker).**

Statistical Analysis

Data analysis was performed using a statistical package for social sciences (SPSS). Comparison is obtained using the Chi-square (χ2-test), whereas numerical data is presented as the mean and standard deviation. The P-value below 0.05 has been considered statistically significant, below 0.01 has been recommended as highly significant, and above 0.05 has been considered non-significant.

Results

This study detected the prevalence of TTV DNA infection on three hundred fifty-nine (359) serum samples based on 5-UTR A amplification by using nested PCR. The results indicated that 93.9% (377 out of 359) were positive for TTV among the studied population, as illustrated in Figures 1 and 2.

Demographic and Risk Factors of Studied Population

According to demographic data (age and sex), this study showed a high rate of TTV infection with no significant

association with age and sex and TTV infection. According age, highest rate of TTV infection was detected within the age group (41–50), 75 out of 79 (94.90%). Regarding to sex, high rate of TTV in females; 8 out of 8 (100%) were TTV DNA positive, while in males 329 out of 351(93.70%) were positive, as shown in Table 3.

The results also showed no significant association between the high rate of TTV infection with all risk factors, as shown in Table 4.

Discussion

Torque Teno Virus detected in high quantity in the peripheral blood of both (healthy and non–healthy) individuals. Normally found in the human as blood virobiome in all age groups of populations and could be transmitted by blood transfusion[21] without causing specific pathogenesis.[22] In the present study, the prevalence of TTV-DNA in blood donors was high (93.9%) 337 out of 359. This result agrees with many studies

Table 3. **Demographic data and TTV infection**

Parameters		Total No. %	TTV-DNA UTR region		*P*-value
			Positive No. %	Negative No. %	
Age groups (years)	<31	119(33.14%)	112(94.10%)	7(5.90%)	0.954
	31–40	129(35.93%)	120(93.00%)	9(7.00%)	
	41–50	79(22.0%)	75(94.90%)	4(5.10%)	
	>50	32(8.91%)	30(93.80%)	2(6.20%)	
	Total	359(100.00%)	337(93.9%)	22(6.10%)	
Sex	Female	8(2.22%)	8(100.00%)	0(0.00%)	0.600
	Male	351(97.7%)	329(93.70%)	22(6.30%)	
	Total	359(100.00%)	337(93.90%)	22(6.10%)	

Table 4. **The risk factors and TTV infection**

Parameters		Total No. (%)	TTV-DNA UTR region		*P*-value
			Positive No. (%)	Negative No. (%)	
History of surgical procedures	Yes	112(31.19%)	105(93.80%)	7(6.20%)	0.558
	No	247(68.80%)	232(93.90%)	15(6.10%)	
	Total	359(100.00%)	337(93.90%)	22(6.10%)	
Marital status	Yes	289(80.50%)	271(93.80%)	18(6.20%)	0.566
	No	70(19.49%)	66(94.30%)	4(5.70%)	
	Total	359(100.00%)	337(93.90%)	22(6.10%)	
Multiple sexual partnerships	Yes	13(3.62%)	13(100.00%)	0(0.00%)	0.433
	No	346(96.3%)	324(93.60%)	22(6.40%)	
	Total	359(100.00%)	337(93.90%)	22(6.10%)	
History of tattoo	Yes	63(17.58%)	61(96.80%)	2(3.20%)	0.223
	No	296(82.45%)	276(93.20%)	20(6.80%)	
	Total	359(100.00%)	337(93.90%)	22(6.10%)	
History of cupping practices	Yes	157(43.73%)	148(94.30%)	9(5.70)	0.482
	No	202(56.26%)	189(93.60)	13(6.40)	
	Total	359(100.00%)	337(93.90%)	22 (6.10%)	
Travel history	Yes	160(44.56%)	150 (93.80%)	10 (6.20%)	0.551
	No	199(55.43%)	187(94.00%)	12(6.00%)	
	Total	359(100%)	337(93.90%)	22(6.10%)	

(Continued)

Table 4. **The risk factors and TTV infection—Continued**

Parameters			Total No. (%)	TTV-DNA UTR region		*P*-value
				Positive No. (%)	Negative No. (%)	
Co-infection	HBs Ag	Negative	356(99.1%)	334(93.82)	22(6.17)	0.827
		Positive	3(0.83%)	3(100%)	0(0%)	
		Total	359(100%)	337(93.87%)	22(6.10%)	
	HCV Ab	Negative	355(98.8%)	333(93.90%)	22(6.10%)	0.776
		Negative	4(1.114)	4(100%)	0(0%)	
		Total	359(100%)	337(93.90%)	22(6.10%)	
Diabetes		Yes	31(8.63)	27(87.10%)	4(12.90%)	0.11
		No	328(91.36%)	310(94.50%)	18(5.50%)	
		Total	359(100%)	337(93.90%)	22(6.10%)	
Hypertension		Yes	47(13.09%)	39(84.80%)	7(15.20%)	0.014
		No	313(87.18%)	298(95.20%)	15(4.80%)	
		Total	359(100%)	337(93.90%)	22(6.10%)	
Animal husbandry		Yes	158(44.01%)	145(91.80%)	13(8.20%)	0.106
		No	201(55.9%)	192(95.50%)	9(4.50%)	
		Total	359(100%)	337(93.90%)	22(6.10%)	
Occupation		Free work	140(38.99%)	130(92.90%)	10(7.10%)	0.430
		Military	128(35.65%)	123(96.10%)	5(3.90%)	
		Employee	56(15.59%)	50(89.30%)	6(10.70%)	
		Student	13(3.62%)	12(92.30%)	1(7.70%)	
		Health worker	19(5.29%)	19(100%)	0(0.00%)	
		Housewife	3(0.835%)	3(100%)	0(0.00%)	
		Total	359(100%)	337(93.90%)	22(6.10%)	

that reported a high rate of TTV infection among blood donors such as (98%) in China,[23] (95.5%) in Jordan,[18] (95%) in Taiwan[24] and (94%) in Russia.[25]

The techniques used to diagnose TTV may significantly impact the variable wide range of TTV infection rates in different studies.[26,27] The two major regions could be targeted for TTV detection, such as the UTR, and the other region, the open reading frame ORF1. The current study used the 5-UTR A region rather than the ORF1 for many reasons. The UTR region was more conserved than the ORF1 region.[28] Another reason is that more genotypes of TTV might be detected by 5-UTR PCR.[29]

According to demographic data (age & sex), this study showed no significant association between age groups and TTV infection rate. These results disagree with studies that showed the TTV infection rate might increase with age due to an inadequate immune response due to immunosenescence.[30,31] Regarding the sex showed a higher rate of positive TTV (100.00%) in 8 out of 8 females than males (93.90%), 329 out of 351 among blood donors, with no significant association between sex and TTV infection, as shown in Table 3. These findings agree with studies that showed the positive rate of TTV DNA was not significantly associated with males and females.[32,33]

According to risk factors, there was no significant association between TTV prevalence rate and risk factors, as shown in Table 3. These results agree with several studies that observed no significant relation between TTV infection and risk factors.[34-37] According to underlying medical conditions, there was no significant association between hypertension and diabetes with TTV infection rate. These results agree with studies that had similar results.[38-40] Another study found a significantly higher level of anellovirade DNA in type 2 diabetes mellitus (T2DM patients) than healthy.[19]

Regarding the history of (surgical procedures, tattoos, and cupping practices) the present study showed no noticeable significant association between the positivity of TTV infections and participants; these results agree with other studies.[41,42] According to (marital status, multiple sexual partnerships, traveling, and occupations) there was no significant association between these parameters and the TTV infection rate. These findings, in agreement with other studies observed that TTV infection was not associated with these risk factors among blood donors.[43,44] TTV could have other transmission routes rather than sexual transmissions, such as the fecal-oral route, salivary or air droplet, blood transfusion, and even zoonotic transmission.[45] According

to hepatitis HCV and HBV co-infection, there was no significant association between hepatitis and TTV status in the present study. This result agrees with other studies that reported no significant association between (HCV and HBV) co-infection and TTV infection rate.[46,47] However, other results disagree, showing a high TTV viremic rate (90.75% – 100.0%) in positive hepatitis patients,[48] due to these viruses generally share a similar transmission route.[49] According to animal husbandry, the results showed a non-significant association between animal husbandry and TTV positive rate; this finding agrees with studies that reported no association between domestic animals and TTV infections.[18,50]

Conclusion

The present study showed a high rate of TTV among Iraqi blood donation with no significant association with any of the demographic and risk factors. These results support that TTV could transmitted by different route and therefore present normally in the majority of populations and this highlights the possibility of its use as biomarker.

List of Abbreviations

Items	Meaning
TTV	Torque Teno Virus
HTTV	Human Torque Teno Virus
UTR	Untranslated Region
PCR	Polymerase Chain Reaction.

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Author' Contribution

All authors contributed to this manuscript. Prof. Dr. Arwa Mujahid. Al-Shuwaikh design, interpreted and arranged this manuscript, Maha Haidar Salman performed all the laboratory work and implementation of this study as a part of her MSc study.

Conflict of Interest

There is no conflict of interest.

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