Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2020; Vol. 18(2)

The Possible Role of Torque teno Virus in Kidney Allograft Recipients in a Sample of Iraqi Patients

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Abstract

Background The use of immunosuppressive medications remains the most important challenge in renal transplantation because of the activation of many infections mainly viruses. The study was designed to evaluate the frequency of Torque teno virus (TTV) viremia among renal transplant recipients (RTR).

Objective To detect TTV in a sample of Iraqi RTR, and its association with renal functions.

Methods This cross-sectional study included 80 serum samples collected from RTR and subjected for TTV detection by real-time polymerase chain reaction (RT-PCR).

Results Qualitative RT-PCR run gave positive results for TTV in 45 out of 80 (56.25%) RTR, the results showed non-significant association between TTV and allograft rejection (p=0.26).

Conclusion TTV seems not associated with post transplantation renal impairment and/or kidney rejection.

Keywords Torque teno virus, renal transplantation, RTR

Citation Taher NM, Hussein MR, Al-Obaidi AB, Kadhim HS. The possible role of Torque teno virus in kidney allograft recipients in a sample of Iraqi patients. Iraqi JMS. 2020; 18(2): 130-137. doi: 10.22578/IJMS.18.2.7

List of abbreviations: CYC = Cyclosporine A, IS = Immunosuppressive drugs, MMF = Mycophenolate, ORF = Open reading frame, PTP = Post transplantation period, RT-PCR = Real-time polymerase chain reaction, RTR = Renal transplant recipients, TAC = Tacrolimus, Tm=melting temperatures, TTV = Torque teno virus

Introduction

Kidney transplantation, the most effective treatment for end stage renal diseases and is strongly increasing all over the world. Aside from the side effects of life long immunosuppress therapy, However, infection remains one of the greatest causes of morbidity and mortality of patients after solid organ transplantation [1,2].

Recently, a lot of studies shown that peripheral blood levels of the ubiquitous and apathogenic Torque teno virus (TTV) mirror whole strength of the immune system or could be a predictive biomarker for risk of infection in renal transplant recipients (RTR) [1,3]. Though, replication of TTV is closely linked to immune status modifications and viral load is now considered as a potential tool for the follow-up of immune status in post transplantation patients [4].

TTV belong to the group of Anello viruses that compose a large fraction of the human total blood virome. The virus is abundantly prevalent in the regular population with reported

infection rates of >90% [5]. This high prevalence of the virus makes it almost ubiquitous in the human population and able to evade clearance by the host immune response thereby establishing long-term persistent infections [3].
TTV is a small, nonenveloped, single-stranded virus with a circular DNA genome of negative sense [6]. The virus was first isolated in 1997 from a Japanese patient with post transfusion hepatitis by Nishizawa et al. [7]. Moreover, it's not known to cause any clinical manifestations in human body, but has gained attention as a possible marker of the immune status of its host, with increased levels of TTV DNA found in various states of immune deficiency [5].
It has been suggested that TTV infection is associated with many diseases, however there is no direct evidence of links between infection and specific clinical diseases, and many questions remain to be clarified for example, how can TTV interfere in many pathological processes and in the dysregulation of the immune system? These questions undoubted lyre present rich fields for research on TTV [8].
The present study was designed to evaluate the rate of occurrence of TTV among RTR and to ascertain whether TTV have a role in renal impairment, rejection or any other morbidity among RTR.

Methods

Study design

The current cross-sectional study conducted from April 2019 to September 2019, eighty RTR including 59 males and 21 females aged from 15 to 65 years, who had undergone their first or second kidney transplantation from living donor in the Center of Kidney Diseases and Transplantation in the Medical City of Baghdad, patients' informed consent was taken before sampling. This study was approved by Institution Review Board of the College of Medicine Al-Nahrin University (Approval code: No.20181257 at the date of 23/3/2019).

Criteria

Key inclusion criteria were a functioning graft at 6 month or longer post-transplantation. Key exclusion criteria were acute rejection less than 6 month before screening and acute deterioration of graft function suspicious of acute or hyperacute rejection.

Clinical parameters

Immunosuppressive regimens, acute rejection episodes, transplant renal function, any signs and symptoms, and late complications obtained from patient's medical records. Two main standard immunosuppressive regimens were mainly followed in RTR; either the cyclosporine (CYC), mycophenolate (MMF), and prednisolone, or the regimen that included tacrolimus (TAC) instead of CYC, in addition to MMF and prednisolone, and induction with monoclonal anti-CD25 antibodies (Basilixibam/Daclizumab).

Samples

A total of 80 blood specimens were collected from the RTR during the period of the study. From All 80 patients 3 ml blood were collected by gel tube and centrifuged at 1,000 x g for 20 min, and supernatant serum aliquots were collected and stored at ‑20 ˚C until the testing was performed. Serum creatinine was determined utilizing a ready-made laboratory kit from Linear company (Spain). It had been determined based on the reaction of creatinine in alkaline solution, with picrate to form a colored complex (Jaffe reaction).

DNA extraction

QIAamp® DNA Mini Kit (QIAGEN, Germany) was used for viral DNA extraction from the serum samples, and the extraction process has been done according to the kit instructions. After extraction of viral DNA from serum samples, the purity of the DNA yield and concentration measured by using a µlite biodrop (England), by applying. Five µl of the extracted DNA in the instrument cuvette. Extracts with purity in between (1.7-1.9) at absorption wavelength 260/280 were included in the study, otherwise; DNA extraction of the sample was repeated.

Real time polymerase chain reaction (RT-PCR) for determining TTV viremia

For the qualitative detection of TTV; Bosphore® TTV Detection Kit v1, which is a qualitative test used to detect TTV encompassing all subtypes of TTV. Polymerase chain reaction master mix contains the specific primers required to detect TTV DNA with SYBR green filter. The monitored samples are confirmed by melting curve protocol. So, 15 µl of PCR Master Mix was added into PCR tubes, and 10 µl of the (sample DNA or Negative/Positive controls) were added to the master mix. The final reaction volume was 25 µl. RT-PCR instrument used in this work was Mic, which developed and manufactured by Bio Molecular Systems (BMS) and depended on kit thermal profile. For RT-PCR the following amplification protocol was used: 1 cycle at 95 °C for 14:30 min to initial denaturation followed by 45 cycles consisting of 30 s at 95 °C, 01:30 at 55 °C, and 45 s at 72 °C for denaturation, annealing and synthesis (Fluorescent detection) respectively, and melting curve analysis at 60 °C to 90 °C (0.5 drop in each cycle).

Melting curve analysis applied after PCR is to characterize the amplifications. Samples of DNA obtained after amplification have their specific melting temperatures (Tm). The positive results of the test are confirmed by comparing Tm of amplicons obtained from samples versus positive control. Non-specific PCR products are eliminated by considering their low Tm values.

Statistical analysis

Data were analyzed via statistical package for social sciences (SPSS) version 21.0 (SPSS Inc., Chicago, IL, USA). Assemblage of results was depending on variables involved in the questionnaire. Fisher exact test was used to describe the association of these data. Numerical data were described as mean, standard deviation of mean. P ≤0.05 was considered statistically significant.

Results

This cross-sectional study enrolled 80 RTR, among these 80 RTR, 59 (73.8%) were males, and 21 (26.2%) were females; their mean age was 38.35±13.15 years, ranging between 15 and 64 years, and the mean post-transplantation period (PTP) was 35.42±41.57 months. Thirty one out of 80, have more than two years transplantation period (38.8%) while 27 (33.8%) and 22 (227.5%) of 80, which between 1 to 2 and less than 1 year respectively.

The mean serum creatinine value was 1.46±0.84 mg/dl, and the mean of their creatinine clearance was 81.93±36.56 ml/min (Table 1), which calculated from the standard Cockcroft–Gault formula using the corresponding serum creatinine and patient body weight [9]. The number of patients with serum creatinine more than 1.2 are:

Creatinine Clearance (ml/min) = [[140-age]*weight] / [72*serum Cr] (And multiplied by 0.85 for females)

Table 1. Mean of post transplantation period (PTP), age and creatinine clearance among renal transplant recipients (RTR)

	N	Minimum	Maximum	Mean	Std. Deviation
PTP (month)	80	6.00	180.00	35.42	41.57
Age (year)	80	15.00	64.00	38.35	13.15
Creatinine clearance (ml/min)	80	1.80	165.59	81.93	36.56

All RTR (80) received their allografts from living donors, and out of the 80 RTR; 25 (31.25%) received their allograft kidney from living related donors, while the remaining 55

(68.75%) received their kidney allograft from living unrelated donors, and the majority of patients had their 1st transplantation 78 of 80 (97.5%), while just two patients had their transplantation for second time (2.5%). Among these 80 RTR, 37.5% had received CSA regimen, and 62.5% had received TAC regimen as shown in Figure 1. On relating with the type of immunosuppression drugs used, 14 of 20 kidney rejected patients were on TAC, and 4 patients were on CSA regimen which is not significant correlation with rejection.

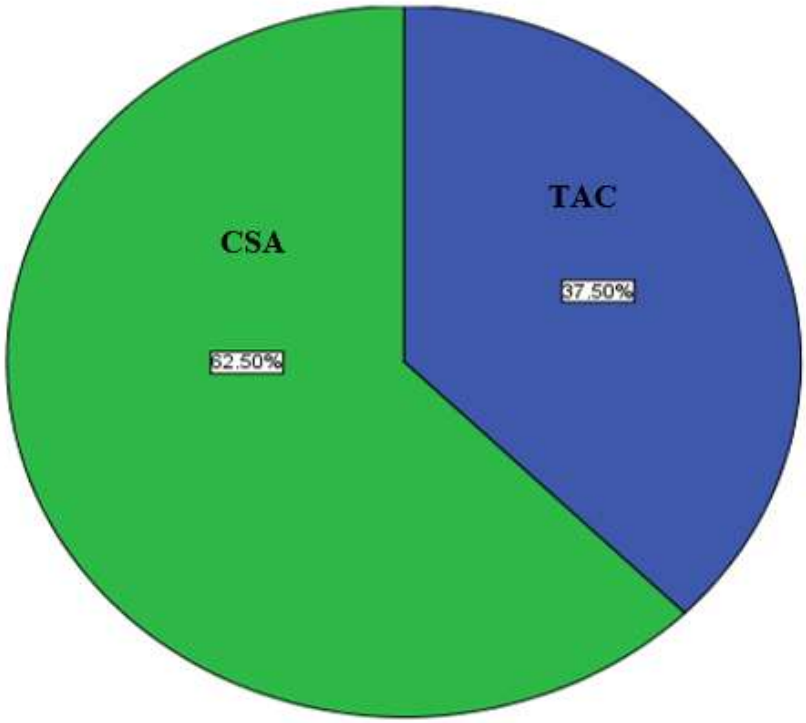

Figure 1. Two main immunosuppressive regimes for 80 RTR

Qualitative RT-PCR run gave positive for TTV in 45 out of 80 (56.25%) RTR as shown in figure 2. In this study, the results showed non-significant association between TTV and age (p=0.22), PTP (p=0.51), creatinine clearance (p=0.68) and serum creatinine (p=0.71) (Table 2).

The frequency of TTV in RTR serum and rejection is shown in Table 3. The virus was detected in 65 % of the rejection samples (13 out of 20), while 35% of the rejection samples (7 out of 20) were negative to TTV with no significant difference (p=0.26).

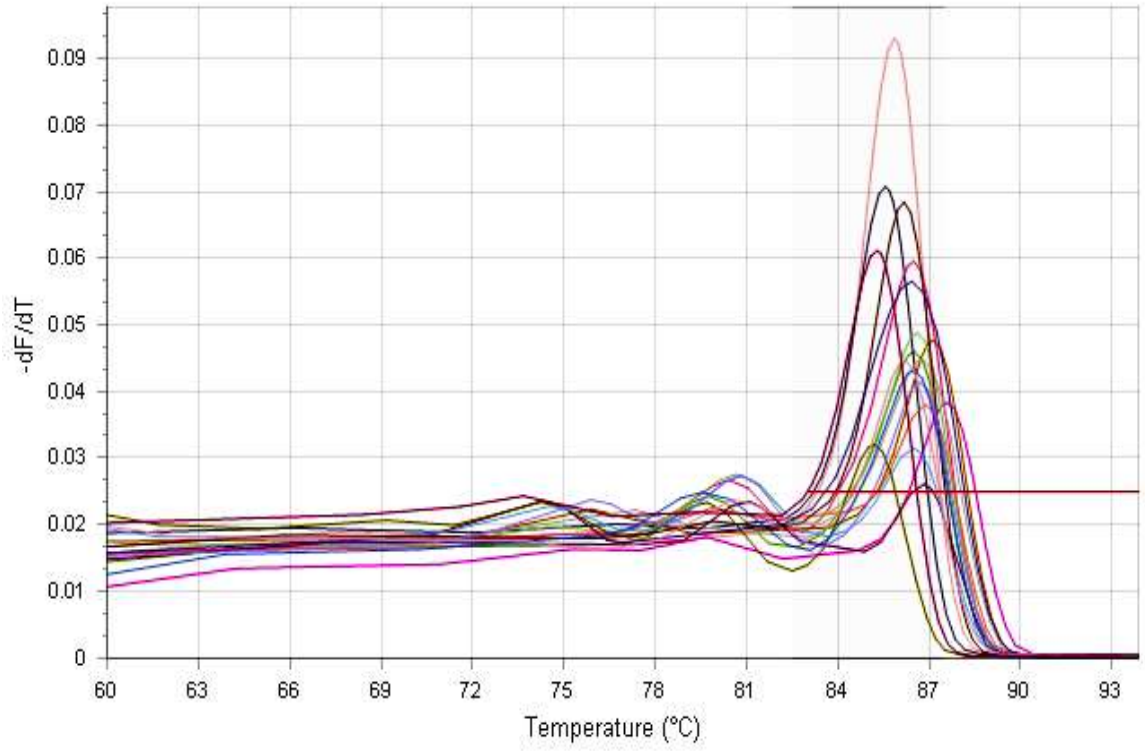

Figure 2. Torque teno virus (TTV) RT-PCR Melting curve analysis

Table 2. Association of Torque teno virus (TTV) viremia with patients' descriptive data

TTV		N	Mean	Std. Deviation	p value
RTP (month)	Negative	35	31.96	35.37	0.51
	Positive	45	38.12	46.042	
Age (year)	Negative	35	36.31	12.41	0.22
	Positive	45	39.93	13.62	
Creatinine clearance (ml/min)	Negative	35	83.82	41.93	0.68
	Positive	45	80.46	32.21	
Serum creatinine (mg/dl)	Negative	35	1.48	1.07	0.71
	Positive	45	1.41	0.56	

Table 3. Relationship between rejection and Torque teno virus (TTV)

TTV		Rejection		Total	p value
		Negative	Positive		
Negative	Count	28	7	35	0.26
	% within TTV	80.0%	20.0%	100.0%	
	% within Rejection	46.7%	35.0%	43.8%	
Positive	Count	32	13	45	
	% within TTV	71.1%	28.9%	100.0%	
	% within Rejection	53.3%	65.0%	56.2%	
Total	Count	60	20	80	
	% within TTV	75.0%	25.0%	100.0%	
	% within Rejection	100.0%	100.0%	100.0%	

Discussion

TTV infection is a benign infection with high prevalence in large number of healthy populations reported worldwide, based on reports from several studies conducted world over, it appeared as TTV was simply a by-stander virus without causing a significant damage of tissue in human body [10]. However, TTV viral load routine use will only be possible after further evaluation in clinical studies and with the availability of a standardized test. The extremely high seroprevalence of TTV worldwide, and its reactivation in almost all immunocompromised patients, makes TTV a good candidate for a biomarker for immune status [4].

The incidence of TTV in RTR in this study was 56.25%, which was near to study done in Brazil with percentage 53.8% [11]. Though, there are other studies demonstrating higher prevalence of TTV among RTR like Iranian study done in 2018 found that prevalence of TTV in RTR was 34.6% [12]. Other study done by Takemoto and her colleges found that the incidence of TTV in the RTR was 10% (5/50) [13].

All studies before are low compared with kidney transplantation report done in the United States, where nearly 75% of RTR underwent immunosuppression induction in 2016 [14] and Japanese study reported a 66% prevalence of TTV [15]. Such differences may be due to higher prevalence of TTV in their general population. Actually, there are different patterns of virus, and different genotypes.

Study conducted in Italy presented a significant increase in TTV frequency in solid organ transplant recipients with huge rate 92% done by Maggi et al. in 2018 [16]. The high mutation rate is unexpected of DNA viruses, since they lack their own replicative equipment and therefore use the host's DNA polymerases, which have a high level of proofreading ability [17].

Many factors like the type of specimen (plasma, serum or whole blood) and PCR method or the primer which used can affect the frequency of detectable TTV. For example, the prevalence of transplanted patients with detectable TTV in RTR by nested-PCR is around 33% when using primers precise for Open Reading Frame 1 (ORF1) region in virus genome while the rate increasing to 92% among the same patients when using primers specific for non-coding region of the TTV genome [18]. SYBR Green-based PCR with primers annealing to more conserved regions may be preferable method, using SYBR Green-based q-PCR assay combines simplicity with satisfactory sensitivity and may be suitable for monitoring the

immune status of transplant recipients, where TTV loads over time may serve as a marker for immune reconstitution in human samples [19].

This mean that the prevalence of TTV in population depending on the identification method that used in study, therefore; TTV prevalence may vary from 94.0% in Russia [20], 75% in USA [14], 66% in Japan [15], 34% in Iran [12] and 10% in Brazil [2].

One of the most central goals in solid organ transplantation is to tailor immunosuppressive therapy to the individual needs of the patient, avoiding both, rejection episodes caused by insufficient immunosuppression, and opportunistic infections and malignancies, which are consequences of over-immunosuppression and remain a significant cause of death after transplantation [21].

Finally, the present statistics propose independent negative association of TTV and rejection because of type of immunosuppression. Close to studies done by Spanish [22] and French groups [23] investigative accuracy present of TTV in our research does not allow for accurate diagnosis of subsequent rejection [24], but rather defines patients at risk. Therefore, TTV is not up to serve as a diagnostic parameter for rejection.

As a conclusion of this study, the findings of 56.25% positive TTV viremia among RTR and 65% of them who had rejection signs, so even though the high rate of TTV prevalence in RTR and the ubiquitous natural surroundings of this virus, the study found there is no obvious statistically significant risk factor for TTV viremia in RTR and specifically rejection patients.

Acknowledgement
None.

Author contribution
Dr. Taher: Collection of specimens, DNA extraction, and RT-PCR, writing of the references. Dr. Hussein: Consultant nephrologist help in providing all patients' data. Dr. Al-Obaidi: Supervision and performance of viral DNA extraction and real time-PCR run, writing of the manuscript. Dr. Kadhim: Final editing of the manuscript.

Conflict of interest
Authors declare no conflict of interest.

Funding
There is no funding source for this research.

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Received Aug. 9st 2020
Accepted Oct. 6th 2020