ORIGINAL ARTICLE

DETECTION OF TORQUE TENO VIRUS ANTIGEN AND ASSOCIATED RISK FACTORS AMONG HEMODIALYSIS PATIENTS

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ABSTRACT

The aim: To determine the prevalence of TTV in patients undergoing hemodialysis and to evaluate the possible risk factors.

Materials and methods: This study was conducted in 93 patients, attending hemodialysis unit at AL-Imammain AL-Kadhimain Medical City Hospital for a period from November 2020 to March 2021. The demographic and clinical characteristics including age, sex, underling medical condition, hepatitis B and C status and laboratory tests such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline Phosphatase (ALP) and total serum bilirubin (TSB) were obtained from the record of the patients in hemodialysis unit in the hospital. Direct detection of TTV-Ag was done by enzyme linked immunosorbent assay (ELISA).

Results: TTV-Ag was detected in 38 out of 93 (40.9%) hemodialysis patients. Demographic, clinical and risk factors i.e. sex, age, history of diabetes, history of hypertension, history of blood transfusion, number of blood transfusion, the hemodialysis duration, history of surgery and liver enzymes levels did not show significant relation (P>0.05).

Conclusions: This study showed high prevalence of Torque Teno virus in hemodialysis patients, however, TTV did not play a role in liver injury among these patients.

KEY WORDS: Torque Teno Virus, risk factors, hemodialysis

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INTRODUCTION

The use of hemodialysis for end-stage renal disease has increased patient life span significantly. It also makes these patients more susceptible to infections, particularly those caused by blood-borne viruses, which play an important role in morbidity and mortality in hemodialysis patients [1]. Some of the most common viral infections are caused by hepatotropic or other hepatitis-associated viruses such as hepatitis C virus (HCV), hepatitis B virus (HBV), SEN Virus (SENV), and torque Teno virus (TTV) [2-7]. TTV is a non-enveloped, single stranded DNA virus, classified as a part of Anelloviridae family [8,9]. TTV is thought to be transmitted by blood transfusion, and its frequency is mainly associated with populations with a history of blood transfusion [10], but there are another ways of transmission due to it is presence in wide range of human samples, including breast milk, synovial fluid, feces, bile juices, and saliva [11]. TTV is associated with some clinical conditions. For example, TTV prevalence is ranging from 30% to 42.9% in hemodialysis patients [12,13], 20% in intravenous drug abusers [14], 46.7% in hepatocellular carcinoma patients, 40 % in cirrhotic patients [15], 75% in hemophiliacs, 46% in non A-G viral liver infections, 48%in Fulminant hepatitis patients, and 84.2 % in HIV-infected persons [13]. In addition, TTV is detected in 23.3% of healthy blood donor [5].

THE AIM

In the current study, we aimed to investigate TTV prevalence by using ELISA technique among hemodialysis patients, to evaluate the relation between TTV infection and demographical, clinical characteristics, risk factors and liver enzymes.

MATERIALS AND METHODS

SUBJECTS

This cross-sectional study included 93 patients undergoing hemodialysis in AL-Imammain AL-Kadhimain Medical City Hospital, from November 2020 to March. The ethical approval was obtained by the Institutional Review Board (IRB) at AL-Nahrain University on 20th September 2020 (No. 20200977).

SPECIMEN'S COLLECTION

Five (5) ml blood was drawn from each patient prior to starting hemodialysis in sterile gel tubes and allowed to clot at room temperature 25˚C for one hour, then centrifuged at 3000 rpm for 10 minutes. Serum samples were divided into aliquots in sterile Eppendorf tubes then stored at -20°C until being used.

IMMUNOASSAY

Ninety-three serum samples have been tested for TTV Ag presence using Human Transfusion Transmitted Virus (TTV) ELISA Kit (Abbexa, England) which is a sandwich enzyme-linked immune-sorbent assay for qualitative de-

tection of TTV Ag in serum. An antibody specific to TTV covered the wells of the microtiter plate. All the samples were diluted (1:5) with the dilution buffer, by adding 10µl of serum to 50µl sample diluents. Measurement of optical density is done at 450nm. The cut-off value is equivalent to 0.15. The test is considered positive if the absorbance of the test is the same or higher than 0.15; otherwise it is considered negative.

STATISTICAL ANALYSIS

Statistical Package for Science Services (SPSS) version-19 has been used to computerize statistical analysis. Comparison is obtained by using of Chi-square (χ^2 - test) for the categorical data, represented as count and percentage. In contrast, the differences in mean were examined by using t-test for the numerical data, presented as mean and standard deviation. The P-value same as or below 0.05 has been considered statistically significant, and below 0.01 has been considered highly significant, while, P value above 0.05 was considered non-significant.

RESULTS

In this study, 93 sera sample were screened by ELISA for TTV-Ag. Results showed that 38 (40.9%) out of 93 samples were positive to TTV-Ag, as shown in the table I. Several studies on the epidemiology of TTV showed that the infection is found worldwide. The frequency of TTV infection may differ, depending on the genomic region tested and the place where the study is undertaken (geographical location). It also depends on the group of dialysis patients [16]. In the present study, the prevalence of TTV-Ag was 40.9% in hemodialysis patients, this result is close to what's mentioned by Wahid & Saadoon (2019), who detected that TTV prevalence was 38.7% in hemodialysis patients [17].

DISCUSSION

The current study showed that there was no significant association between TTV infection and any of demographic, clinical characteristics and risk factors such as age, sex, diabetes (DM), hypertension (HT), blood transfusion, number of blood transfusions, duration of hemodialysis, history of previous surgery, liver enzymes levels and viral hepatitis, as shown in the table I. Considering age of studied population, TTV-Ag positive individuals were with mean age of (51.68±13.77) years according to table I, there was no significant difference in age between TTV positive and TTV negative in hemodialysis patients, which is in agreement with Irshad et al., (2010) [18].

In addition, when comparing the rate of TTV-prevalence between males and females, the results showed that male had a higher rate (42.6%) than female (38.5%) with no significant differences between them. This result is in accordance with Takemoto et al., (2015)including the Torque teno virus (TTV, who showed that there is no significantly difference between gender and TTV infection and also found that the majority of dialysis patients were males (55%) with a mean age of (53.8) years [16]. This variation in number between females and males may be due to the differences in pathophysiology, physiological nature, the type of work and nutrition which may lead to males being more possible than female to have kidney disease or kidney failure [15,7]. Considering the underling medical condition, 31.0% of TTV positive patients were suffering from diabetes (DM) and 40.2% of them had hypertension (HT). There was no significant statistical association between TTV positivity and diabetes mellitus or hypertension according to the table I, this results are in agreement with Akbari et al., (2018), who observed that there is no significant relation between TTV infection and hypertension [20].

Another study also in accordance with the current findings done by Gallian et al., (1999)we tested 150 attendees of two hemodialysis (HD found that the TTV prevalence in hemodialysis diabetic patients was not significantly higher than that detected in a diabetic patients without renal disease [21]. Interestingly, 61% of hemodialysis patients have a history of blood transfusions. However, there were no significant relation between the TTV infection and blood transfusion or the number of blood transfusions in hemodialysis patients which is in accordance with Irshad et al., (2010) study of comparative analysis, conducted among patients with a history of blood transfusion [18].

The use of erythropoietin to treat renal anemia resulted in a significant reduction in blood transfusions; however, infections in hemodialysis units can still occur in the absence of other parenteral risk factors [22-23]. This suggested other route of viral transmission since the virus present in healthy individual and can transmit by different mechanism. Regarding the hemodialysis duration, the current study showed no significant difference between the TTV infection and the period of hemodialysis. This is near to other study done by Akbari et al., (2018), who indicated that viral infection was not significantly associated with duration of hemodialysis [20]. Although nosocomial infection still may play an important role, but we can't be ruled out that TTV had other transmission routes such as fecal-oral route, salivary droplet, sexual transition, breastfeeding [24-25], as well as, through presence of TTV in environment and many animal reservoirs [26]. In addition, the present study showed that there was no noticeable significant association between the positivity of TTV infection and people who undergone surgery, in agreement with Khudair et al., (2019) [27]. These findings disagree with Spandole-dinu et al., (2018) who found a significant difference between the two variables, TTV and surgery. Also, it was showed that human anelloviral DNA was higher in healthy women who had at least one surgical procedure such as abortion or cesarean section [28]. This could be explained by the differences in the study population, sample size, geographical region, the percentage of virus in the normal people in the community. In this study, 46 (49.5%) of hemodialysis patients were tested negative to HBV&/or HCV and 19 (41.3%) of them had TTV infection.

Table I. Relation of TTV Ag and demographic, clinical characteristic and risk factors in studied population (n=93)

Parameter		Total No (%)	TTV Ag		P value
			Negative No (%)	Positive No (%)	
Age (mean ± S.D.)		93 (100%)	48.98±16.03	51.68±13.77	0.400NS
Sex	Male	54 (58.1%)	31 (57.4%)	23 (42.6%)	0.689NS
	Female	39 (41.9%)	24 (61.5%)	15 (38.5%)	
	Total	93 (100%)	55 (59.1%)	38 (40.9)	
DM	No	64 (68.8%)	35 (54.7%)	29 (45.3%)	0.194NS
	Yes	29 (31.2%)	20 (69.0%)	9 (31.0%)	
	Total	93 (100%)	55 (59.1%)	38 (40.9%)	
HT	No	11 (11.8%)	6 (54.5%)	5 (45.5%)	0.741NS
	Yes	82 (88.2%)	49 (59.8%)	33 (40.2%)	
	Total	93 (100%)	55 (59.1)	38 (40.9%)	
History of Blood Transfusion	No	32 (34.4%)	15 (46.9%)	17 (53.1%)	0.081NS
	Yes	61 (65.6%)	40 (65.6%)	21 (34.4%)	
	Total	93 (100%)	55 (59.1%)	38 (40.7%)	
No. of blood transfusions (time)	< 1	41 (44.1%)	21 (51.2%)	20 (48.8%)	0.348NS
	1-4	34 (36.6%)	23 (67.6%)	11 (32.4%)	
	≥4	18 (19.4%)	11 (61.1%)	7 (38.9%)	
	Total	93 (100%)	55 (59.1%)	38 (40.9%)	
History of Previous Surgery	No	49 (52.7%)	30 (61.2%)	19 (38.8%)	0.666NS
	Yes	44 (47.3%)	25 (56.8%)	19 (43.2%)	
	Total	93 (100%)	55 (59.1%)	38 (40.9%)	
Hemodialysis duration (year)	< 1	3 (3.2%)	3 (100%)	0 (0.0%)	0.129NS
	1-3	26 (27.9%)	18 (69.2%)	8 (30.8%)	
	3-5	22 (23.7%)	14 (63.6%)	8 (36.4%)	
	≥5	42 (45.2%)	20 (47.6%)	22 (52.4%)	
	Total	93 (100%)	55 (59.1%)	38 (40.9%)	
HBV &/or HCV Status	No	46 (49.5%)	27 (58.7%)	19 (41.3%)	0.986NS
	HBV	2 (2.1%)	1 (50.0%)	1 (50.0%)	
	HCV	42 (45.2%)	25 (59.5%)	17 (40.5%)	
	HBV& HCV	3 (3.2%)	2 (66.7%)	1 (33.3%)	
	Total	93 (100%)	55 (59.1%)	38 (40.9%)	

While the remaining 47 (50.5%) out of 93 of hemodialysis patients had HCV&/or HBV.

TTV was detected in 1 (50%) out of 2 of hemodialysis patients who had co-infected with HBV and 17 (40.5%) out of 42 of hemodialysis patients who had co-infected with HCV. In addition, one patient had triple infection with HBV, HCV and TTV. This is logical since these viruses share the parenteral route of transmission. However, current study showed that there was no significant association between these viruses and TTV status, as shown in the table I. In addition, there was no significant difference between the level of liver function tests between TTV-positive and TTV-negative on in hemodialysis patients, as shown in table II.

Table II. Liver enzymes level in relation to TTV Ag status in studied population

Parameters	TTV-Status		P-value
	Negative Mean ± S.D.	Positive Mean ± S.D.	
ALT mg/dl	19.24±13.06	18.05±18.72	0.719 NS
AST mg/dl	18.37±12.37	21.42±18.19	0.338 NS
TSB mg/dl	0.37±0.22	0.35±0.15	0.613 NS
ALP mg/dl	193.36±192.64	203.26±186.51	0.806 NS

This suggests that TTV presence in the liver did not cause severe damages to it. This is similar to study done by Irshad et al., (2006)a novel agent, in relation to its molecular characteristics, epidemiological features, modes of transmission, tissue tropism, pathogenesis, role in various diseases and its eradication from the body. TTV, a DNA virus, is a single stranded, non-enveloped, 3.8 kb long DNA virus with a small and covalently closed circular genome comprising 3852 bases. It was tentatively designated Circinoviridae virus. TTV genome sequence is heterogeneous and reveals the existence of six different genotypes and several subtypes. TTV has been reported to transmit not only via parenteral routes, but also via alternate routes. This virus has been detected in different non-human primates as well. At present, TTV is detected by polymerase chain reaction (PCR who stated that the hemodialysis patients with TTV infection did not necessarily have liver dysfunction [29]. Another study by Akbari et al., (2018) showed that there was no association found between a variety of epidemiological and laboratory variables including liver enzymes and TTV infection[20]. Also, this could be similar to the fact that HCV related liver damage in hemodialysis patients is mild due to immunosuppressant. Hemodialysis patients decreased secretion of Th-1 associated cytokine (INF-γ), but increased secretion of Th-2 associated cytokine (IL-10), resulting in immunological deficiency [30]. However, the pathogenicity of TTV is not clear; several experiments have been conducted to determine its target organs. Initial study suggests hepatotropic tropism and its replication in liver cells [21,32]. Torque Teno virus infection was found to be very common in people who suffered from idiopathic Fulminant hepatitis and those suffering from cryptogenic chronic liver diseases including liver cirrhosis, chronic hepatitis, and hepatocellular carcinoma [33]. In addition, researchers discovered that TTV frequency was the same in patients suffering from liver disease and in assorted cases of non-B and non-C liver diseases, and also in few healthy individuals. They settled that TTV does not cause harm to the liver [32]. Lemon et al. (2000), observed that there are harmless viruses referred to as orphan viruses and are beneficial to the body in a way that they maintain homeostasis [34]. These viruses were isolated but not yet associated with any infection, so they are considered "simple guests". Although it may be difficult to attribute the term "guest" or "endosymbiont" to viral agents, but they have a characteristic responsible for altering the normal functioning of cells [35]. It is thought that the factor that might cause the difference in infection is the immunity of an individual or viral load. The finding that TTV is most likely to be found in serum of HIV-infected abusing drugs is an indication that there is a possibility of a high viral titers due to immunosuppressant. It has also been confirmed because of it is high capability of genetic diversity; only specific TTV strains are clinically important and can cause hepatitis [36].

CONCLUSIONS

This study concluded that the prevalence of TTV Ag was high (40.9%) among hemodialysis patients. However, TTV did not seem to be associated with any of the risk factors; therefore, it may transmit by different routes rather than blood transfusion. Also, TTV have no role in increasing the level of liver enzyme or the severity of HCV infection in hemodialysis patients.

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