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Article

Detection of Torque Teno virus in IRAQI hemodialysispatients by ELISA technique

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ABSTRACT

Background: Much evidence points to the presence of new factors that affect the liver in addition to the well-known hepatitis A-E viruses, and of these viruses is the Torque Teno virus, which was found to be more prevalent in the liver. 0bjecte: Detection of Torque Teno virus infection in patients undergoing dialysis with hepatitis C virus(HCV) patients and non-infected patients, comparing them with healthy people by ELISA technique to determine the extent of the virus spread in patients undergoing dialysis for the first time in the Iraqi community and how to affect the severity of infection. Methods: The study was conducted from 2021 until the end of 2022. Blood samples were collected from 35 patients infected with HCV undergoing hemodialysis, 35 patients not infected with HCV undergoing hemodialysis, and 20 healthy people. Clinical information and tests for hepatitis were obtained from the patients' registry. Chemical and hematological tests were done, and the results were recorded. A test for the detection of the Torque Teno virus was done by ELISA technology. Results: Torque Teno virus was detected in serum samples of patients using the ELISA technique. The infection rate was 0.0% in healthy people, (14.29%) in patients undergoing dialysis without HCV infection (5 out of 35) and (22.86%) in patients undergoing dialysis with HCV infection (8 out of 35), the novel result of this study showed that there is a higher prevalence of Torque Teno virus in HCV patients than in patients undergoing dialysis without HCV infection in Iraq. Also, this study found non-significant differences between Torque Teno virus infectionand liver function enzymes, neither with WBCs nor HB concentrations. Conclusion: Hepatitis may be caused by TTV, which was shown to be connected with biochemicalindicators of liver damage and persistent HBV or HCV infection.

Keywords: Torque Teno virus, hemodialysis, ELISA technique

INTRODUCTION

The Torque Teno virus (TTV) was initially found in a Japanese patient who had post-transfusion non-A-G hepatitis after receiving blood transfusions [1]. TTV is a non-enveloped, circular, negative single-stranded DNA. It was assigned to the Anelloviridae family for classification purposes [2]. Hepatotropic viruses are

considered to have a wide range of transmission routes [3]. TTV is divided into seven genogroups, each containing several genotypes, with genotype 1 being the most common [4,5]. The virus is found worldwide, but the prevalence of antibodies against it varies greatly depending on where you are from[6,7,8]. Bloodtransfusion patients, including thalassemia, hemodialysis patients, and intravenous drug users, are frequent carriers of TTV [9]. Though TTV genotypes have a wide range of genetic diversity, a few of these genotypes may cause illness 10. Hemodialysis, despite its many benefits for patients with chronic renal failure, has been linked to several complications; it has been cited as a high-risk environment for blood-borne transmitted infections, which could be spread by a variety of means in hemodialysis units, including equipment, surfaces, and personnel staff [11]. TTV is one of the most often transmitted viruses among hemodialysis patients, along with HCV and HBV. However, the incidence of transmission varies greatly depending on virological, demographic, and clinical parameters [12].

MATERIALS AND METHODS

The study consisted of 90 samples, 20 samples as a control and 70 samples from patients who had dialysis, including (35) patients without HCV and (35) patients with HCV (46 males and females) aged (18 to 55). Blood samples were collected randomly between November 2021 and January 2022 from the Medical Hospital, Al-Kindi Hospital in Baghdad, and Baquba Teaching Hospital in Diyala.

Specimens collection

Five ml of blood was drowned from each patient via vein puncture using disposable syringes. The blood sample was divided into 2 ml and collected in the tube containing ethylene diamine tetra acidic acid (EDTA) as an anticoagulant with alow mix for hematological study. The second part(3 ml)of the blood sample was collected in a gel tube left about one hour to clot in room temperature then centrifuged for 15 min at 3000 rpm to separate serum, then divides the serum into three aliquots: the first one for biochemical test, the second for Immunological test, a and third one for viral DNA extraction, all store in the -20 °C.

Biochemical testing

Alanine Aminotransferase (ALT) and Aspartate Transferase levels were assayed on the Kinetic (Biosystem bts350) according to the manufacturer's kit from the linearenzymatic method (Spain) [13].

Abnormal values were considered when ≥45 IU/L for ALT and ≥40 IU/L for AST

Hematology Tests

Hematology is the study of blood and blood disorders according to blood samples (whole blood in EDTA tubes) of patients and control were assigned for hematologicaltest: Complete blood picture (CPC) to assess the changes in the HB and WBC values ifrelated to the infection with TTV. The test was performed by Spincell 3.

The normal value of Hb (Hemoglobin) is 12.2-16.1 g/dL. The Normal value of WBC (white blood cell) is 4.7-10.2 K/µLImmunoassay

Enzyme-linked Immunosorbent Assay (ELISA) was used as the primary diagnosis for detecting human TTV by ELISA kit (BT LAB China). Ninety samples were tested by ELISA technology according to the instructions on the kit (BT LAB Chin Cat.NO ED0591HU)

Work was carried out according to the manufacturer's Prosser. Standard sweeteners were prepared at room temperature, and the number of the well was

selected. 50 µl of control negative and control positive were added, and 10 µl sample was added in a 40 µl diluent. The diluted sample was added to each well and then incubated for 30 min at 37°C and washed 5 times. Then, HRP solution was added to each well, placed in the incubator for 30 minutes at 37°C, and washed 5 times; then substrate solution A and substrate solution B were added and incubated in the dark for 10 minutes at 37°C. Then, read the OD value within 15 min at 450 nm. The calculated patient and control serum absorptions were compared with the cut-off value. The cut-off value = absorbance of negative control + 0.15. If the absorbance of the sample is equal to or higher than the cut-off value, the test sample is considered positive. Otherwise, the test sample is considered negative.

Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effectof different factors on study parameters. The least significant difference – the LSD test (Analysis of Variation-ANOVA) was used to significantly compare between means.

RESULTS

This study included 90 samples, including 20 samples for healthy people, 35 for hepatitis C patients, and 35 non-infected patients with an average age (18-55) years, who were randomly selected. Regarding the sex distribution, the percentage ofmales was 47 (52%), while the percentage of females was 43 (47%), as shown in **(Table 1**). Also, the Table illustrated that there was no significant difference in age among dialysis patients with HCV, without HCV, and the control group (39.68 %, 39.4%,

38.45 %) respectively.

Group		**Control (No=20)**	**H.NO virus (No=35)**	**H.C.V (No=35)**
Gender	**Male**	**8 (40.00%)**	**20 (57.14%)**	**19 (54.29%)**
	Female	**12 (60.00%)**	**15 (42.86%)**	**16 (45.71%)**
Age (year)	**Mean ± SE**	**37.45 ±3.07**	**39.40 ±1.48**	**39.68 ±1.43**

Table1. Distribution of sample study according to Gender and Age in differentgroups

Biochemical test

Table 2 shows differences in the level of AST and ALT enzymes between thehealthy group and the group of patients undergoing dialysis.

Group	Mean ± SE	
	AST NV (0-40 U/L)	**ALT NV (0-45 U/L)**
Control	5.95 ±0.87 c	**14.65 ±1.04 b**
H.NO virus	13.91 ±2.63 b	**29.11 ±3.37 a**
HCV	21.94 ±2.59 a	**37.80 ±3.14 a**
LSD value	7.332 **	**9.122 ** **
P-value	0.0003	**0.0001**

Data expressed as mean±SE. LSD test was used to calculate the significant differences between the tested mean, the letters (a, b, and c) LSD for columns represented the levels ofsignificant, highly significant starting from the letter (a) and decreasing with the last one.
Similar letters mean there are no significant differences between tested ** (P≤0.01).

Table 2. Comparative statistical analysis between AST and ALT mean valuefor all tested groups

Hematology Tests

This study was conducted to find out the difference in white blood cell ratio between patients and controls, as shown in figure (1), which shows significant differences in white blood cells between the control group, patients undergoing dialysis without HCV infection, and patients with HCV infection, with means (8.00), (5.34), (4.27) respectively.

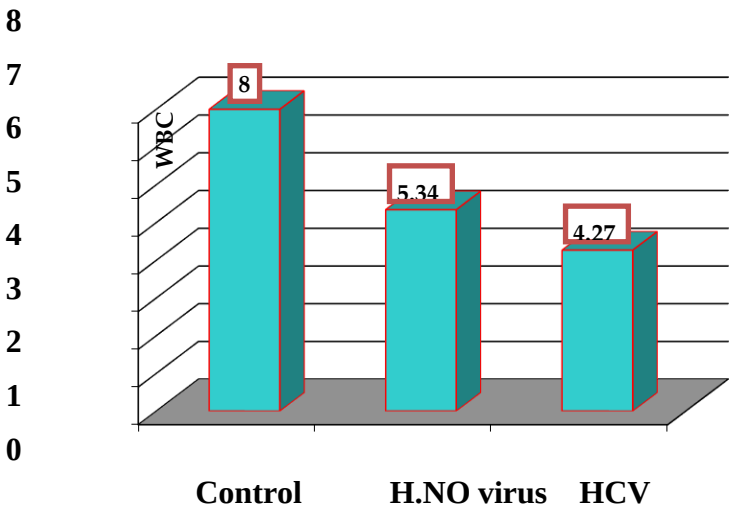

Figure1. Comparison between groups in WBC

Figure 2 showed small differences in hemoglobin ratios between the healthy group and the group of patients undergoing dialysis with HCV infection and without HCV infection.

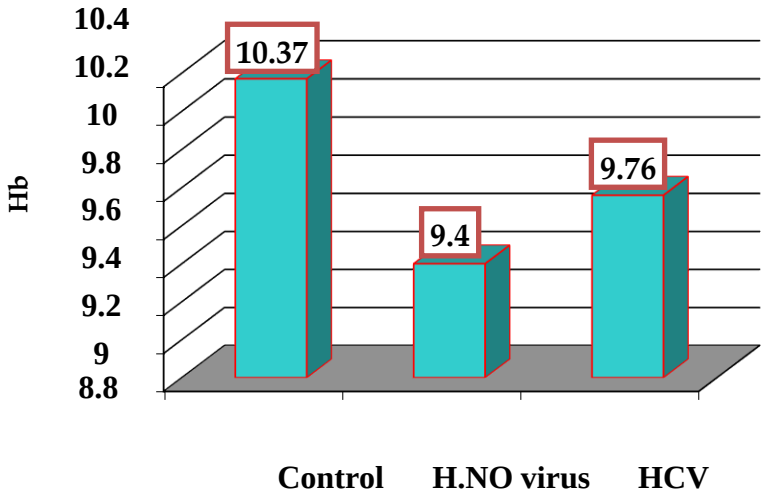

Figure 2. Comparison between groups in Hb

Immunoassay

TTV was detected in 5 (14.28%) of patients not infected with viral hepatitis, the number of males and females was 2 (5.71%) and 3 (8.57%), respectively, as well as 8 (22.86%) of patients infected with viral hepatitis, the number of males and females was 5 (14.28). %), (3) (8.57%) respectively. Results showed that there was a significant difference in sex between TTV-positive and negative individuals. The percentage of infection in males was higher than in females, shown in Table (3).

TTV by ELISA		Control (No=20)	H.NO virus (No=35)	HCV (No=35)
Positive	Total	0 (0.00%)	5 (14.28%)	**8 (22.86 %)**
	Female	0 (0.00%)	3(8.57%)	**3(8.57%)**
	Male	0 (0.00%)	2(5.71%)	**5(14.28 %)**
Negative	Total	20 (100%)	30 (85.71%)	**27 (77.14%)**
	Female	12(60%)	12(34.2%)	**11(31.4%)**
	Male	**8(40%)**	**18(51.4%)**	**16(45.71)**

Table 3. Distribution of positive and negative results of TTV according to ELISAamong study groups

Relationships between all parameters under study and TTV infection

The results in this study pointed to variants in age and levels of each liver enzyme AST, ALT, WBC, and Hb among TTV-positive individuals, as shown in Figure 4.

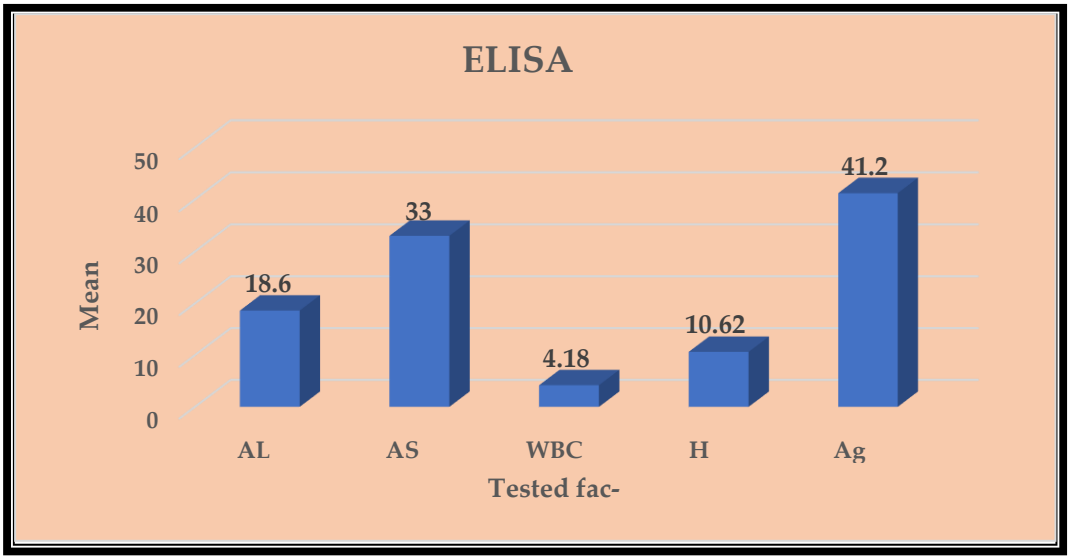

Figure 3. Relationship between the positivity of TTV and the parameter understudy

DISCUSSION

Despite that, the pathogenicity of TTV is not fully clear, but undoubtedly, TTV can infect patients already infected with other viruses previously [15]. Since TTV is considered a post-transfusion virus that causes hepatitis, the most important high-risk persons are those infected with HBV and HCV [16]. The existence of TTV DNA in different organs indicates the presence of a wide affinity to the host cell. The TTV history indicates a potential hepatitis virus, so the main target organ (liver) has also been studied extensively. By in situ methods, TTV is found in the nucleus and/or the cytoplasm of hepatocytes in patients with liver damage [17,18]. However, with no cytopathogenic effect (CPE) in liver cells [19,20] The prevalence of TTV infection is changing due to the development of new variants or new detection methods [21,22].

The results in Table 1 showed differences in proportions between patients and healthy people regarding gender. The number of males is more than the number of females in dialysis patients, with percentages of 52.22% and 47.77%, respectively. This is consistent with a study conducted on hemodialysis patients in Libya in 2012, where the percentage of males was the highest, reaching 58% of patients [23]. In addition to another study, among 83 chronic HD patients, the proportion of males is higher than females, (44 male and 39 female) respectively [24] Table 1 also showed that there are no differences in ages between the healthy group and the other groups and this agreed with the results of a study showed that there were no significant differences between hemodialysis patients infected with viral hepatitis and those who were not infected [24].

AST (Aspartate Aminotransferase) and ALT (Alanine Aminotransferase) enzymes are present in the kidneys and the liver, so any damage to the kidneys or liver will increase the effectiveness of these enzymes in the serum [25]. Table (2) shows differences in the level of ALT enzyme between the healthy group and the group of patients undergoing dialysis in general. There are no differences in the level of ALT enzyme between the group of patients undergoing dialysis with or without HCV infection. This agreed with [26], who indicated that the level of ALT enzyme cannot act as a factor for assessing the liver in patients with hepatitis. On the other hand, Table (2) shows that there are significant differences in the AST enzyme Between the healthy group(5.95) and the group of dialysis patients infected with HCV(21.94) and between the group of dialysis patients without HCV infection(13.91), with the highest significant difference in enzyme levels among the group of people with HCV (21.94), this is what agreed with the study

that showed that ALT, AST values were higher in hepatitis than in healthy blood donors, [14]

There are statistically significant differences in white blood cells between the control group and patients undergoing dialysis without and with HCV infection, with

averages of (8.00), (5.34), (4.27) respectively, as shown in figure (1), and this agrees with a study in 2016 which pointed to the effect of the kidneys on decreasing the number of white blood cells [27]

While there are differences in hemoglobin ratios between the healthy group and the group of patients undergoing dialysis with HCV infection and without HCV infection, as cleared in figure(2), because of anemia associated with renal failure due to relative deficiency in erythropoietin as a result of a defect in its production, severedeficiency in iron and foliate, shortening the lifespan of red blood cells with an increase in the rate of Its breakdown and a decrease in the rate of production in the bone marrow, as well as blood loss due to hemorrhage through the gastrointestinal tract and blood in the urine as a result of poisoning resulting from the accumulation of nitrogenous waste in the blood, and poisoning with heavy metals [28]

The results of the ELISA test showed that 14% of dialysis patients had TTV, whichis similar to the results of a study conducted in Iran, which found that the percentage was 16.7% in dialysis patients)24) while comparing another study showed a higher prevalence of TTV virus in Saudi Arabia with 42.9%. [29], and TTV DNA was found in dialysis patients with (41.7%) [in Italy 30].

In this study, TTV was detected at a rate of 22.68% and 14.29% in patients undergoing dialysis with HCV and without HCV, respectively, while the rate of infection was in healthy subjects 0 (0.00%), which is opposed to studies of [29,30] which they noted that the rate of TTV infection in the control group was 19% and 10.7% respectively.

The results of the study in Table 3 showed that patients undergoing dialysis with HCV [8 (22.86 %)] had a higher incidence of TTV than patients without HCV [5 (14.28%)] with no significant differences, and this agrees with other studies [31,32] they found no biochemical evidence of a link between liver disease and TTV infection in the TTV-DNA-positive HD patients.

Also, the results showed that the incidence of TTV in patients with HCV [8 (22.86%)], including [2 males & 3 females]and patients without infection HCV [5 (14.28%)], including [5 males & 3 females] means that the infection in males is higher than in females, which is same to the results of the study showed a higher incidence of males (42.6%) than females (38.5%), with no significant difference [33]

In comparison with the in vitro enzymes for liver injury, no significant correlation was observed in the levels of AST and ALT in healthy subjects and hemodialysis patients with positive and negative TTV, and this is in agreement with astudy that showed no significant association between liver enzymes and TTV positivity [33], and this is on the same line with another study that showed no association between TTV positivity and laboratory factors such as liver enzymes AST,

ALT. [34] In addition, another study showed no significant difference was detected ineither AST or ALT levels in any of the groups [35], which concurs with the postulation that TTV is a commensal virus and only certain genotypes and Genogroups areassociated with liver pathology [36]

Interestingly, there are no significant differences in age between TTV-infected hemodialysis patients with HCV and non-HCV infection, as shown in Figure 3.

The results of our study proved that there are no significant differences between TTV positivity and age, and this agreed with the results of a study that there is no correlation between age and TTV infection(37) and this does not agree with a study showed significant differences between age and TTV infection [38]Non-significant association of age in this study could be explained by the fact that this kind of viral infection could occur in people with any age [37] In addition, the results of our study showed that there is no relationship between TTV infection and hemoglobin, as well as the number of white blood cells. But in general, WBC and granulocyte counts were higher in patients with bacterial infection than in those with viral infection, as mentioned by [39].

CONCLUSION

This study showed a higher prevalence of the Torque Teno virus in HCV patients than in patients undergoing dialysis without HCV infection in Iraq. Also, this study found non-significant differences between Torque Teno virus infection and liver function enzymes, neither with WBCs nor HB concentrations. Hepatitis may be caused by TTV, which was shown to be connected with biochemical indicators of liver damage and persistent HBV or HCVinfection.

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