

Diagnosis of HCV Infection in Renal Chronic Infection Patients by Using ELSA and RT- PCR in Tikrit City

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Abstract

Introduction: Viral hepatitis infection is an important cause of mortality and morbidity among patients treated hemodialysis and the spread of this disease varies from one region to another in the world. Chronic renal failure is the most common type of hepatitis C infection due to the need for blood transfusion and use of dialysis devices.

Methods: The study was conducted in the laboratories of the liver and digestive system. Hospital and in the city of Baghdad for the period from 1/1/2017 to 1/1/2018, which included the diagnosis of infection with viral hepatitis C of serum patients using the technique of Elisa and RT-PCR for patients who are deceased and incoming to Tikrit Hospital and clinics In the study, 100 samples were taken from 50 patients with renal failure and 50 healthy people aged 50-60 years, with clinical signs and according to the doctor's diagnosis, 5 ml of blood and serum were withdrawn into two parts (1 ml) For the purpose of isolating DNA and detecting the virus with RT-PCR technology and the second to purpose virus detection and enzyme Liver and kidney function using ELISA technique, SPSS version 16 was used in data analysis. The results were considered statistically significant at the level of $P \geq 0.05$.

Results: The study result appearance of the 50 people with chronic renal failure (CRF), 35(70%) had positive an anti-HCV, while 15 (30%) had negative an anti-HCV antigen. While the results of renal function and liver enzymes showed a significant difference at $P \geq 0.05$ level and between infection with the C virus except for the glutamic-pyruvic transaminase (GPT) enzyme did not show a significant difference at the level $P \geq 0.05$, and the results also showed relationship between age and infection with the virus, which affects most age groups between 40-50 Years. HCV-RNA levels were also determined in positive and negative serum samples for ELISA test using Real Time-PCR was positive for 2.9% (1) positive Hepatitis C virus patients via Real-time-PCR as well as negative from enzyme-linked immunosorbent assay, whilst 28.6% (10) are positive HCV via enzyme-linked immunosorbent assay, but Not via Polymerase chain reaction.

Abbreviations: CRF=chronic renal failure, GPT=glutamic-pyruvic transaminase, HCV=Hepatitis C Virus.

Keywords: HCV Infection, Renal Chronic infection, Patients, ELSA, RT- PCR.

1. INTRODUCTION

HCV disease is a main community health trouble with a global prevalence estimated at 3% HCV. There are about 180 million loaded and about Four million citizens a year are recently infected. (Flamm, 2013)

Hepatitis C virus is a 9.6-kb RNA virus that belongs to the virus (Flaviviridae) and Hepacivirus virus. Hepatitis C virus transmitted by blood, and includes known danger factor for spread of hepatitis C virus using injectable drugs, blood transfusion / blood produced. Implantation, chronic dialysis, working contact amongst health care staff, therapeutic injection, main / secondary surgery, dental cure, barber shops, unsafe sex, and vertical contact. (Yen et al., 2015 and Strader et al., 2004).

Patients with renal failure on dialysis at elevated risk for blood-borne infection due to long-term vascular contact and the possibility of contact to infection patients as well as polluted tools. disease as a result of HV are one of these infections, an chief cause of disease and death in dialysis People and a trouble in the organization of patients in renal dialysis unit. (Meyer's et al., 2003) In India, a broad vary of hepatitis virus frequency rates (4.3% - 45.2%) in the dialysis population were Reported (Abacioglu et al., 2015 and Jasuja et al., 2009) There is a lack of available information of Hepatitis C Virus disease in Punjab. Since a great numeral of acute chronic renal failure patients call our tertiary care hospital, this retrospective learning be conduct decide positive antibody Hepatitis C Virus Abs in person undergo first-time transfusion at Guru Go bind Singh medicinal

university, Hospital, Faridkot Punjab), its environmental plus region delivery.

In these patients, HCV disease is usually asymptomatic (Jaiswal et al., 2002) and be able to be diagnose by serological method as well as by amplification of HCV RNA (RT-PCR) (Yuki et al., 2000). Which differentiate among viraemic and non viraemic Hepatitis C Virus person in addition to use Hepatitis C Virus genotype (Young et al., 1993). HCV isolate have been categorize into six chief genotypes, numerous of which have a number of closely connected subtypes (Galan et al., 1998). New HCV variant from Vietnam, Jakarta and Indonesia have been explain as genotypes 7, 8, 9, 10 and 11 (Simmond et al., 1994 and Tokita et al., 1994). The delivery of Hepatitis C Virus genotypes vary into diverse country when documented in blood donor, and haemodialysis and chronic hepatitis citizens (Tokita et al. 1996, Dusheiko et al. 1994 and Bosmans et al. 1999

2. METHODS

100 blood samples were collected (50 patients with renal failure and 50 healthy people aged 50-60 years, with clinical signs and according to the doctor's diagnosis from hemodialysis unit in Tikrit Hospital and clinics from (1/1/2017 to 1/1/2018).

Blood was collected in the first hour before the blood-washing session. The samples were separated into two tubes for each patient, then frozen and stored immediately (-20 ° C) and -80 ° C respectively for viral and serological assays to reduce viral degradation. DNA, preventing mutual contamination is unnecessary. The third generation of Elisa Kits was used consistent with manufacturer instruction (ERBA Transasia, India). The group contains 100% sensitivity and specificity of $\geq 99\%$ according to manufacturer, for the following signs: HCV IgG antibody and enzyme Liver and kidney function.

2.1. RNA Extracted

HCV RNA was extract from 200 μ l of patient serum using sum viral DNA group according to the manufacturer instruction (Invitrogen, Carlsbad, CA, USA). The eluted genetic materials tore up at 70°C.

2.2. cDNA combination & Real Time PCR for 5'NC area

For HCV RNA discovery by RT-PCR, all serum samples were tested individually for the

presence of HCV RNA by specific RT-PCR (Sacace Biotechnologies, REF V-1-100R, and Italy). To allow the molecular examination of a great amount of seronegative sample, the collected plan was developed, like the method describe previous (McOmish et al., 1994). This involves the synthesis of four negative serum samples and study of the combination because of HCV RNA. Twenty-five μ l of every of the four samples were mix together, and then 100 μ l of the pond was use for the examine. The RT-PCR procedure is based on four main processes: isolation of HCV RNA from samples using RNA / DNA kit (Ribo-Sorb, Sacace Biotechnologies, REF K-2-1, Italy), reverse transcription of RNA using reverse primer, For M-MLV with the kit incubated in a cycler thermocouple at 37 ° C for 30 minutes, and then cDNA was amplified by PCR with special primers for the non-localized zone 5 of the viral genome. Amplification was performed as follow: 95 ° C for 5 minutes, then 42 cycles of 95 ° C for 30 seconds, 67 ° C for 30 seconds, and 72 ° C for 30 seconds, follows via a finishing extension at 72 ° C for a minute one. After the products have been amplified on the agarose gel. The set contains internal control that can be used to conduct insulation and acts as an amplification control for each individual treated sample Determining potential inhibition of interaction. Negative and positive controls, inverse versions, were extracted and amplified in each batch of samples tested by polymerase chain reaction. Serum samples have been shown to contain HCV RV by RT-PCR. This ready-to-use PCR group contains basic materials, solutions, PCR main mix, positive control, negative control, molecular marker and loading dye (Fig.1). The procedure was done in accordance with manufacturers' instructions. Statistical examination was made using SPSS report 16 using crosstabs and Chi-square test. P-value ≥ 0.05 was considered as statistically significant.

3. RESULT

In the present study, a total number of 100 patients (50 patients with renal failure and 50 healthy people aged 50-60 years), are enroll for hemodialysis in the two following years (1/1/2017 to 1/1/2018). Out of the sum 50 patients, 24 (48%) were establish to behave HCV infection. And the number of patients who give negative results for anti- HCV was 26 (52%) Table 1.

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Table1. Number and percentage anti-HCV SERUM hemodialysis patients using ELSA

Study groups	Total number	Anti-HCV	
		Positive	Negative
Patients groups	50	35(70%)	15(30%)
Control groups	50	0	0
Total groups	100	35	15

The maximum frequency was establishing in the 40-50 years of age group (26%) followed by 50-60 years (24%) as well a slowly frequency was experimental in the age grouping 30-40 years (20%) Table2.

Table2. Relationship between age groups and virus infection

Age groups	Infected		Total
	Infected with Anti- HVC	Non Infected with Anti- HVC	
30-40 years	10(20%)	5 (10%)	16
40-50 years	13(26%)	5(10%)	18
50-60 years	12(24%)	5(10%)	16
Total	35	15	35
Chi square	0.53		
Coefficient value	0.07		

In this study studied some biochemical variables related to kidney function and liver enzymes. The results showed a significant raise ($P < 0.05$) in serum creatinine, GOT and TSB concentration and lessen significant ($P < 0.05$) in GPT concentrated in patients with chronic renal failure Table3.

A significant variation was found among the study people and the two technique of the study. In the current study of a total of 50 patients, 35 (70%) of HD persons are positive Hepatitis C Virus, with ELISA. though, of the sum positive persons 2.9% (n=1) persons are positive for

HCV by Real-time-Polymerase chain reaction in addition to negative of enzyme-linked immunosorbent assay, as 28.6% (10) were positive cases of HCV via enzyme-linked immunosorbent assay but not via Polymerase chain reaction. Privacy and sensitivity The third age group of ELISA compare to PCR over lapping RT for Hepatitis C virus was 97.8% and 80%, in that order. Positive analytical rate was 71.4% while the negative analytical rates 84.3% for enzyme-linked immunosorbent assay Table4. Hepatitis C disease has reach outbreak proportions worldwide and linked by numerous additional liver manifestations.

Table3. Shows the links between the liver and kidney function and the infection with hepatitis

Kidney and	Liver Function test	HCV
urea	Pearson Correlation	.408**
	Sig. (2-tailed)	.007
	N	50
creatinine	Pearson Correlation	.493**
	Sig. (2-tailed)	.001
	N	50
GOT	Pearson Correlation	.335*
	Sig. (2-tailed)	.002
	N	50
GPT	Pearson Correlation	.201
	Sig. (2-tailed)	.196
	N	50
TSB	Pearson Correlation	.664**
	Sig. (2-tailed)	.000
	N	50

Table4. The percentage of negative and positive lesion of the HCV-Ab enzyme-linked immunosorbent assay test compare with Real Time-Polymerase chain reaction.

	NO. of ELISA Negative	PCR Positive person from Negative ELISA		NO. ELISA Positive	PCR Negative persons from Positive ELISA
sum Persons n=50	15	1	sum Patients n=50	35	10
% fake negative ELISA		6.7	% fake positive ELISA		28.6
Negative Predictive rate		84.3	Predictive positive rate		71.4



Fig1. Agarose gel electrophoresis patterns (RT-PCR) harvest of hepatitis C persons. Well 1 to 9 HCV positive samples (256 bp); positive Control: DNA size marker 100 bp; Negative control.

4. DISCUSSION

Viral hepatitis is a major health problem worldwide, especially in tropical and subtropical regions. It is among the causes of morbidity and has already headed the list of infectious diseases reported in many countries. Chronic liver disease, especially due to HCV disease, is a main difficulty among patient with dialysis (HD) patients (Schneeberger et al., 1998). The high occurrence of HCV infection between patients treat with HD preservation has been credited to transfusion supplies in this group of risk (Chan et al., 1991). The frequency of viral hepatitis is larger in patients with HD than in general groups that affect value of life and death among patients. According to our results as shown in Table1, the total number of patients 100 (50 patients with renal failure and 50 healthy people aged 50-60 years) enrolled in this study; 35 (70%) were positive for hepatitis C virus and 15 (30) % of patients were negative for the hepatitis C virus. This finding was in agreement with the results of the study conducted by (Zeldis et al., 1991) to evaluate the hepatitis C virus in regular dialysis patients in BeniSuef between 70% of the study sample and a retrospective study conducted by (Senosy et al., 2016) on 186 patients in the HD unit in Casablanca, reported a high prevalence of HCV infection (76%) and the prevalence of HBV infection was reported at 2% (Boulaajaj et al., 2005). In contrast to the results of other studies, such as in Gaza, the prevalence of hepatitis B virus in patients with HD patients was 8.1%, 22% with HCV (Omaretal.,2003), in Basra - Iraq of 122 patients with HBV infection were positive (50%), While HCV seropositivity (42.6%) was 5 in Kosovo out of 583 HCV prevalence (12%) (El-Ottol et al., 2010). In the case of Tocantins, Brazil of HCV antibodies was detected in 13% of patients (Shihab et al., 2014). In Amman, Jordan, the prevalence of HCV in HD patients was 5.9% (Telaku et al.,

2005). The variance in the ratios in the different studies may be due to the difference in the size of the samples, the sensitivity of the methods and the specificity of the methods used in the detection of the anti-HCV Abs. In relation to age and type C infection among chronic renal failure patients, the results shown in Table.2 showed that with age, infection rate with HCV is increased (26%).this rate was establish in the age group of 40-60 years. The results were consistent with (Al Hijazat, and Ajlouni2008) in Jordan, who reported that the age factor had a significant effect on type C infection with respect to HCV infection, the current rate was low (26%) compared to the number of countries in the world, in Libya, Palestine, Jordan and Turkey 20.2% ,28%, 24%, 31.1% respectively, (Ghazzawi et al.,2015,Alasheketal.,2012 and Al. Jamal ,2009) While the results of the virus type C high compared to Recorded some studies of the doses in Sudan, Bahrain and Saudi Arabia with92%, 59%, 85% Respectively (Yakaryilmaz et al., 2006, Gasim, et al., 2012 and Reddy et al., 2005).

The results in Table.3 showed a significant increase ($P > 0.05$) in the concentration of urea, creatinine, bilirubin, GOT and GPT. The same table showed a significant decrease ($P < 0.05$) in the GPT concentration among patients with dialysis Compared with the control group. These results were consistent with the studies conducted by (Qadi et al., 2004 Mehdi et al., 2012 and Merzah et al., 2015) in both Baghdad, Waist and Sudan respectively.

Both urea and creatinine are considered nitrogenous substances in the blood, and doctors depended on the concentration of these nitrogenous wastes to determine whether the patient has kidney disease, as these tests help determine the efficiency of kidneys in the clearance of blood from these wastes or toxins (Mehdi et al., 2012). Patients with chronic kidney failure are a dangerous factor in the collection of waste or nitrogenous toxins, so treatment of renal failure in the hemodialysis leads to rid the body of these uremic toxins, depending on the effect of the efficiency process of dialysis and fluid structure used in the process of dialysis (Merzah et al., 2015)

The significant increase in the concentration of urea, creatinine and bilirubin in the serum of chronic renal failure patients treated with hemodialysis in the present study compared to the control group may be due to the incomplete filtration of these substances by dialysis or due

to the stimulation of the internal structure or deterioration during the dialysis session.

The researcher's study (Ahmed et al., 2016) confirmed that the high level of creatinine indicates a decrease in glomerular filtration rate thus reducing the efficiency of kidneys in detoxification.

The high efficiency of liver enzymes may be due to the effect of liver cell membranes and change their effectiveness and destruction, which leads to the disruption of the transfer of metabolites and the leakage of these enzymes into the bloodstream and high serum levels of patients, or may be due to oxidative stress and the formation of free radicals that lead to harmful structural and functional changes in the liver cell such as, Peroxide lipid in the cell membrane, GOT and GPT enzymes are present in both the liver and the kidney so any damage to the kidney or liver or their tissues results in an increase in these enzymes in patients' serum (Shahbazian et al., 2009 and Damera et al., 2011).

In this study, a few negative persons were detected falsely by ELISA, using RT-PCR (1, 6.7%). This shows that PCR-based assay is capable to verify accurate amount of HCV RNA in serum, as previously report (Benouda et al., 2009). PCR specifically help to resolve weak ELISA-positive results in the presence of clinical markers consistent with HCV infection and / or danger factor though, the outcome of the two methods must be interpret by care when the discovery of Hepatitis C Virus RNA typically precede discovery Abreaction in serum following acute introduction. Hepatitis C Virus RNA can be recognized as early as two weeks after introduction, while HCV Abs are not usually detect 8-12 weeks ago (Tashkandy et al., 2007). On the other hand, throughout the path of disease when virus is clean, just the antibodies Remain positive, as well as RNA are usually not detect. Thus, PCR discovery rate was lesser when ELISA was use as a gold normal (Umar, 2011). In this study, 70% of Hepatitis c virus persons are positive by both Real-time–Polymerase chain and enzyme-linked immunosorbent assay indicate that Hepatitis c virus disease are acute or chronic by scientific context. In 6.7% of Hepatitis c virus samples, the results are positive via Real-time–Polymerase chain and negative interlaced by Real Time – Polymerase chain enzyme-linked immunosorbent assay. This may point to an early on Hepatitis c virus disease, chronic

Hepatitis c virus in patients with chronic immunodeficiency or HCV RNA positive false examination. PCR consequences were report as negative interleaved RT and positive in 10 (28.6%) which may indicate HCV solution, acute HCV during a low period, or anti-viral C. The kindness and privacy of in enzyme-linked immunosorbent assay the present study are 80% and 97.8%, respectively; there are good sufficient to perform an analytic examination. equally, the specificity of RT-PCR was total at elevated kindness (100%) noting to it was not just appropriate for experimental analysis but also appropriate for show to Hepatitis c virus disease stops pread of the sickness (Wang et al., 2004 and Ghany et al., 2009).

5. CONCLUSION

ELISA tests have several advantages in investigative preparation include cease mechanization, case of utilize, relation rise efficiency, and low down changeability. Though, as with all immunohistochemistry assays, the false positive outcomes are sometimes a trouble through the third age group of enzyme-linked immunosorbent assay, or further or assertive. A test such as PCR overlapping RT is often useful. Additional studies are suggested to study the genetic patterns of Hepatitis C to help enhanced scientific outcome and epidemiological study and to supply in formation with significant implication for the scientific organization of hepatitis C and vaccine advance.

Ethical Approval

The ethical committee of the concerned institute approved the research protocol. The purpose and procedures of the study were to be explained to all the study subjects, and informed consent was to be obtained from them.

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