Prevalence of Hepatitis B virus in hemodialysis patients infected with Hepatitis C virus in Mosul district / Iraq

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Abstract

Background:

Prevalence of HBV DNA and HCV RNA viral load, HBSAg and anti-HBC IgM was studied in hemodialysis patients in Mosul district/Iraq.

Patients and Methods:

Between August 2010 until April 2011, a total of (60) blood samples were collected from hemodialysis patients. The samples tested againstanti-HCV, HBSAg anti-HBS and anti-HBC IgM, using ELISA technique and determination HBV, DNA, HCV, RNA viral load by Real time polymerase chain reaction.

Results:

The overall prevalence HCV RNA was 12(20%) out of 60 patients were RT-PCR positive for HCV RNA with viral load. Up to 100,000 Copy/ml and 10(16.690) out of 60 patients were RT-PCR positive for HBV DNA in both positive and negative HCV RNA patients group with average viral load more than 100,000 copy/ml.3 Patients out of 12 (25%) HCV RNA positive patients were also positive for HBV DNA, while 40 (66%) out of 60 patients were anti-HBSAg positive, while the positivity of anti-HBCoIgM was 5 (50%).

Conclusion:

Prevalence of hepatitis B and C confection was high in hemodialysis patients in Mosul district/Iraq

Introduction

Although dialysis is the treatment of choice for end-stage renal failure, dialysis patient are at risk for contracting blood-borne infection, including hepatitis viruses (HBV and HCV)[1]: viral hepatitis among dialysis patients is associated with significant severity and poor prognosis[2] and both HBV and HCV synergize in accelerating the progression to hepatic anomalies[3], although some dispute any effect of liver disease[4].

Previous screening for HBV and HCV infection relied on serologic tool (HBsAg and anti-HCV for HCV), and change the liver enzymes[5]. In view of the false-negatives inherent in serology testing[5,6], this highlighted the need for accurate means of detecting HBV/HCV including polymerase chain reaction (PCR)[6,7]

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Determination of the virus type and viral load serve an important diagnostic tool in the patients follow up and treatment, [2,7]

. There are very few reports on the prevalence of such dual infections in hemodialysis patients. The present stuey was undertaken to estimate the prevalence of HBV and HCV dual infection among hemodialysis patients.

Patients and Methods

We collect blood samples of 60 chronic hemodialysis patients in Mosul-Iraq to detect HCV and HBV infection in them, there were 40 (66.6%) males and 20 (33.3%) females and the average age of patients was 20-60 years.**Enzyme linked immu**

nosurbent assay (ELISA)

Anti HCV and HBSAg screening test were performed using Anti-HVC ELISA kit (plasmatic UK) Anti-HBC and Anti-HBS test were also performed using ELISA kit (Bio kit, Spain).

Polymerase chain reaction test

RNA Extraction

HCV RNA extraction test were performed using Eazy nucleic acid isolation kit (Omega Bio-tek).

- 200 ìl of serum had been add in to 2 ml micro center fuge tube
- 210 ìl VRS Buffer, 8ìl carrier RNA and zoul proteinase k solution (20 mg/ml) had been add in to micro centrifuge tube. Mix toughly by vortexing for 30 seconds.
- Incubate at 37
- Centrifuge briefly to collect any liquid drop from the tube cap.
- 220µl absolute ethanol (96-100%) and mix the sample throughiy by overtaxing for 20 seconds
- Incubate at room temperature for 5 minutes.
- Apply entire sample to micro elute ® RNA column assembled in a 2 ml collection tube, centrifuge at 6000xg for 1 minute. Discard flow and collection tube.
- Place the columns in to a new collection tube 500 µl RWE buffer had bean add to the column. Centrifuge at 10.000xg for 30 seconds, discard the flow-through.
- Place the column in to a new collection tube. 700 µl RWB buffer had been add to the column. Centrifuge at 10.000xg for 30 seconds, discard the flow-through and re-use the collection tube.
- With the collection tube empty- insert and center fuge the spin column for 1 min at full speed to completely dry the hibind ® matrix.
- Elution of RNA: transfer the column to aclean 1.5ml microfuge tube and apply 15-20 µl of DEPC-treated water. Centrifuge 1 mine the concentration and purity of RNA, measure absorbency at 260 nm and 280 nm in spectrophotometer. 10.0 unit measured at 260 nm corresponds to 40 µl of RNA per mel. The ratio of A 260/A280 of pure nucleic acids is 2-0. While for pure protein it is approximately 0.6. aeration of 1.8-2.0 cores ponds to 90-100% pure nucleic acid.

- DNA extraction.

  HBV DNA extraction test were preformed using instant virus DNA kit (analytic jean, Germany).

- 200 µl lysis solution TLS, 200µl of serum and 25µl proteinase k had been add, mix vigorously by pulsed vortexing for 10 second, incubate at 50C for 15 minutes

- 400µl binding solution TBS had been add to the lysed sample, mix by vortexing several times.

- Apply the sample to the spin filter located in a 2.0µl receiver tube. Close the cap and centrifuge at 10.000xg for 1 minute. Discard the receiver tube with the filtrate, place the spin filter into a new 2.0 ml.

- Open the spin filter and add 500 µl washing solution HS, close the cap and centerfuge at 10,000xg for I minute, discard the receiver tube with filtrate. Place the spin filter in to a new 2.0ml reciver tube.

- Open the spin filter and add 650µl washing solution MS, close the cap and center fuge at 10.000xg for 1 minute. Discard the receiver tube with the filtrate. Place the spin filter in to a new 20.ml receiver tube.

- Center fuge at max speed for 2 minutes to remove all traces of ethanol. Discard the 2.0j ml receiver tube.

- Place the spin filter into a 1.5 ml elution tube. And open the can of the spin filter and add 100 µl pre-heat elution buffer (50C).

- Incubate at room temperature for 2 minutes. Centrifuge at 6,000xg for 1 minute.

- Real time RCR tests

- Quantitative of HBV DNA.

  HBV DNA quantitation test were preformed using HBV DNA (primer design kit) and used real times PCR detection (9 tower for analytic gene company, Germany)

- Prepare a reaction mix according to the table below:

Component	Volume
2x precision™ Master Mix	5 µl
pathogen primes probe Mix	0.5 µl
Eternal extraction control primer/probe Mix	0.5 µl
RNAse/DNA free water	1.5 µl
Final volume	7.5 µl

- Pipette 7.5 µl of this mix into each well.
- Pipette 2.5 µl of DNA template and standard in to each well and negative control for negative control weels use 2.5 µl of RNA se/DNA free water.
- Preparation of standard curve dilution series.
- Pipette 900 µl of RNA se/DNAse free water into 5 tube and lable 2-6.
- Pipette 100 µl of positive control template in to tube 2.
- Vortex thoroughly.
- Chang pipette tip and pipette 100 µl from tube 2 into tube 3.
- Vortex thoroughly
- Repeat steps 4 and 5 to complete the dilution series.Amplification protocol

	Step	Time	Tempreture
	Enzyme activation	10 mints	95 °C
50 cycles	Denaturation	5 sc	95°C
	Denaturation	25 sc	60°C

Interpretation results according the instruction manual.

- Quantitative of HCV RNA

Results:

Table (1): The percent of HVB serology marker in hemodialysis patients by ELISA

	Step	Time	Tempreture
	Enzyme activation	10 mints	95 °C
50 cycles	Denaturation	5 sc	95°C
	Denaturation	25 sc	60°C

Table(2): The percent of positive of HCV RNA and HBV DNA in Hemodialysis patients in real time PCR.

Total Number	HCV RNA		HBV DNA	
	Positive	Negative	Positive	Negative
60	12(20%)	48(8%)	10(16.6%)	50(83.3%)

Table (3): The percent of HCV RNA and HBV confection among hemodialysis

Total number of HCV RNA positive	Confection HCV RNA positive and HBV DNA positive
12	3(25%)

Anti-HCV was positive in 12 out of 60 (20%), while patients, HBSAg was positive in 10 out of 60(16.6%) patients. But other HBV serological marks was positive anti-HBs, 40 out of 60(66%), anti-HBC 5 out of 10(50%). When examination by ELISA assay shown in table (1).

This result is compatible when examination by real time technique, HCV RNA was positive in 12 out of 60(20%). HBV DNA was positive in 10 out of 60(16.6%) patients.

The viral load in HCV RNA and HBV DNA greater than 100000 copy ml. shown in table (2) coinfection HCV and HBV was positive in 3 out of 12 (25%) patients was shown in table (3). Our results also revealed no significant difference between the HCV RNA positive male (66.6%) and female hemodialysis patients (33.3%).

Furthermore, there was no significant difference in the duration of receiving blood and their components between the positive and negative patients as shown in table (4). The average

age of HCV patients was 45.5 years and 46.51 years in HCV negative patients, there for no significant difference was observed prevalence of HCV infection and the age of patients as shown in table (4).

Table (4): Average age and frequency of transfusions in hemodialysis patents with hepatitis C virus infection

HCV	Number of samples	Average ± SD age (years)	Average ± SD duration since last blood transfusion month
negative	48	45.5 ± 20.44	13.5 ± 8.23
positive	12	46.16 ± 14.52	12.5 ± 8.92
total	60	46.5± 20.1	12.9 ± 8.89

Our result showed that HBV DNA 10 out of 60(16.6%) was detected through the PCR study in both patients groups with positive and negative HCV RNA. But 3 out of 12 (25%) HCV RNA positive were HBV DNA positive, and 4-out of 60 (66%) patients were positive for anti-HBS. While 5 out of 10 (50%) patients and HBV DNA were positive for anti-HBC.

Discussion

As they are similar transmission models, HCV and HBV co-infection is prevalent, in our study, HBV DNA and HBSAg were detect in hemodialysis patients with chronic HCV hepatitis, viral interference has been described in patients with dual HBV and HCV infection [8]. In the present study, a higher frequency of HCV RNA positivity was found a many HBV-negative patients than HBV-positive ones, although this difference was not significant. More preside studies, such as quantitation of both viral genomes and gene type are needed to evaluate the interference of replication between these two viruses (9,10). The mechanism of viral interference is not known, but the host immune response could be involved in mediating the suppressive effect of one virus on the other[8], if this is the case, viral interference between HBV and HCV might not occur in immune compromised hosts like hemodialyzed patients.The possible routes of transmission of HCV in hemodialysis patients are multiple and some of them are still controversial. The frequent blood transfusions in this group of patients have been an important route of infection before blood testing become available. There is, haw ever, increasing evidence of the nosocomial transmission of HCV, as described previously for HBV. The sharing of equipment as awode of HCV transmission is still controversial. Recent studies argue against HCV transmission through the hemodialysis ultrafiltration[11].On the other hand the beneficial effect of isolating equipment for HCV positive patients has been described [12,13,14]

In fact, lower prevalence of HCV positivity have been found in other Venezuelan hemodialysis unit more strict aseptic norms, such as isolation of equipment, are enforced[14]

.The significantly higher frequency of blood recipient among HCV positive patients compared with that among the uninfected group table (4) suggests that blood transfusion remains an important mode of exposure to HCV[15]. However, blood transfusion alone cannot account for the high prevalence and incidence of HCV infection that was observed and nosocomial transmission of HCV map play a role in the spread of HCV in this group as described by others [16, 17, 18] we found that the duration of hemodialysis correlates with HCV positivity, nosocomial transmission among hemodialysis patients has recently been documented by molecular analysis[19, 20].

The hemodialysis machine used in the unit studied might also play a role in HCV dissemination because of accidental contamination of the device for pressure testing and inadequate subsequent disinfection. On the other hand, even if no disposable equipment of syringes were shared in these units, the multiple parental exposure and the sharing of drugs (heparin) among different patients could be involved in HCV transmission, on the other hand, even if transfusion has been the main mode of HBV and HCV transmission in the past (testing for HCV in blood bank was implemented in Iraq in 1992).Nosocomial transmission now seems to play arole in the dissemination of HCV among these patients.

Recent studies have shown that strict aseptic measures can virtually eliminate HCV contamination, even in unit with high prevalence of HCV infection 21, preventing the consequences of infection not only by HCV but by other non-A, non-B and non-C viruses that could circulating in these venally compromised patients[22, 23]. With those, our results are an comparable of [27] from Iran, the may be the difference in number of sample or genic factor or immune state in the patients, and the our results are un comparable with those of [26]

from Turkey. They were unable to detect HBV DNA in hemodialysis HCV infected patient, it is probably due to several factors such as the intermediate prevalence of HBV in your region and safety of blood and it is component.Most studies disclosed the existence HBV DNA genome in 22%-87%, 24.25 of patients with negative HBSAg and positive HCV RNA. HBV DNA is observed in 46% of anti-HBC positives, and in 20% of anti-HCB negative patients[25].

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