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Full Length Research Paper

Molecular evaluation of hepatitis G virus and hepatitis C virus in patients with chronic renal failure in Iran

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Prevalence rates of hepatitis G virus (HGV) and hepatitis C virus (HCV) are high in people who have received regular blood transfusion. Patients with chronic renal failure carries are susceptible to acquiring blood borne virus infections, especially viral hepatitis. The aim of this study was to define the actual prevalence of HCV and HGV in Iranian HD patients. This cross sectional-descriptive study the samples were collected from 62 Patients with chronic renal failures and 100 healthy controls. The presence of HGV-RNA and HCV-RNA in serum or plasma and the prevalence of antibodies against an HGV envelope protein (E2) and anti-HCV antibodies are investigated in patients undergoing chronic hemodialysis and controls by multiplex-nested-RT-PCR protocol and an enzyme-linked immuno sorbent assay, respectively. HGV-RNA were detected in 17.74% of the patients and in 3 of 100 blood donors, also anti-E2 antibodies and anti-HCV antibodies were detected in 24.19 and 19.35% of the patients, and six and five of controls, respectively. HGV viremia was diagnosed in 11 and in 3 of patients and controls, respectively. HCV-RNA was diagnosed in 9 of 62 patients and 1 of 100 healthy controls. The data revealed a high frequency of infection with HGV and HCV in patients with chronic renal failures that have received multiple blood transfusions.

Key words: Chronic renal failure, hepatitis G virus, hepatitis C virus, blood transfusions.

INTRODUCTION

Hepatitis C virus and hepatitis G virus are RNA viruses and are members of Flaviviridae family (Kato, 2001; Alavian et al., 2005). Patients with chronic renal failure carries are susceptible to acquiring blood borne virus infections, especially viral hepatitis (Albertl and Benvegnu, 2003; Nakatsuji et al., 1996). Co-infection with HGV was seen in 10 to 20% of HCV infected subjects (Choo et al., 1989; Pereira and Levey, 1997). Patients with chronic renal failure usually require frequent blood transfusions which make them more vulnerable to infections (Willems et al., 1991; Masuko et al., 1996; De Lamballerie et al., 1996). One of the important causes of morbidity and mortality in patients with end stage renal failure are infections (Choo et al., 1989).

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Chronic hepatitis is a major complication of chronic haemodialysis (HD). After hepatitis B virus (HBV), HCV emerged as a new problem. In USA, rates of positive anti-HCV reached up to 36% in HD patients in 1990s. Hepatitis C virus was isolated in 1989; this virus is a RNA virus and a member of Flaviviridae (Kato, 2001; Alavian et al., 2005). Major HCV infections lead to chronic hepatitis, which results in progressive fibrosis ultimately resulting in cirrhosis, liver failure and an increased risk of hepatocellular carcinoma (Farhana et al., 2009; Shepard et al., 2005). In 1995 and 1996, researchers isolated a new flavivirus like-RNA virus and named it the "hepatitis G virus "(HGV). The GB virus C (GBV-C)/hepatitis G virus (HGV) is a member of the Flaviviridae family. HGV is an RNA virus and its genome encodes a polyprotein of approximately 2900 amino acids. The genomic organization of HGV is similar to HCV. HGV infection has been reported to be associated with fulminant

Table 1. Oligonucleotide primers used for polymerase chain reaction and sequence HVC and HGV nucleic acids.

Primers	Target	Sequence (5′ – 3′)	Annealing temp (°C)	Expected size (bp)
Forward- R1	HGV	GGTCGTAAATCCCGGTCACC	55	180
Reverse-RI	HGV	CCCACTGGTCCTTGTCAACT		
Sense-R2	HGV	TAGCCACTAGAGGTGGGTCT	64	
Anti-sense-R2	HGV	ATTGAAGGGCGACGTGGACC		
Forward-R1	HCV	CAG GCA GAA AGC GTC TAG CCA TG	55	220
Reverse-R1	HCV	TCG CAA GCA CCC TAT CAG GCA G		
Sense-R2	HCV	CCC CTG TGA GGA ACT ACT GTC	64	
Anti-sense-R2	HCV	TGC ACG GTC TAC GAG ACC TC		

hepatic failure, post transfusion acute or chronic hepatitis autoimmune hepatitis, and cryptogenic chronic hepatitis (Choo et al., 1989; Yoshiba et al., 1995; Heringlake et al., 1996; Heringlake et al., 1996). High prevalence rates of HGV and HCV are observed in people who received regular blood transfusion. Patients with chronic renal failure carries a definite risk of acquisition of blood borne virus infections, especially viral hepatitis (Albertl and Benvegnu, 2003; Nakatsuji et al., 1996). In the present study, we investigated the presence of HGV-RNA and HCV-RNA in serum or plasma and antibodies to HGV envelope protein (E2) and Anti-HCV antibodies in patients undergoing chronic hemodialysis.

Possible relationships to medical, biological, and epidemiological markers were investigated. We also examined the possibility of viral transmission within HD units and some features concerning the natural history of hepatitis infection.

MATERIAL AND METHODS

Patients

62 patients with chronic renal failure (42 men and 20 women) and 100 (53 men and 47 women) healthy control group, who clinically and laboratory rule out any hematological abnormalities, were included in this study .The serum from each patient was collected and kept at -70°C. Demographic data were obtained from patient records.

Serological testing

Anti-E2 antibodies in the serum were assayed with a commercial Enzyme linked Immunosorbent Assay (ELISA) (Dade Behring Murburg, Germany). Results were evaluated by optical density and were compared to the cut off value, in proportion to the manufacturer's instructions. Anti-HCV was performed using a second-generation anti-HCV ELISA.

Sera from all patients were tested for serological markers of hepatitis C virus (HCV) by ELISA test system (Dade Behring Murburg, Germany). They were also tested for the presence of E2 antigen of hepatitis G virus (HGV) using commercial enzyme-linked immunosorbent assay (ELISA) kits.

RNA extraction and cDNA synthesis

Total RNA was extracted from 100 µl of plasma samples by RNX plus extraction procedure as previously described by Kao and Sun (1996). The isolated RNA was used for reverse transcription (RT). First, cDNA was sensitized from HGV and HCV genomes according to instruction as follows: 3 µl of viral extracted RNA was incubated at 25°C for 1 h and at 72°C for 10 min after treating with random hexamer and Moloney murine leukemia virus reverse transcriptase (M-Mulv-RT). 20 µl RT-master mix contained 0.2 mmol of dNTPs, 0.01 mg/ml of random hexamer, 7.5 U/ml of M-Mulv-RT, 1 U/ml of ribonuclease inhibitor, and 4 µl of 5 µl RT- buffers.

Detection of HGV and HCV nested-PCR

The first PCR amplification was performed in 25 µl reaction mixtures containing 2 µl of cDNA, 0.1 pmol/µl of primers, 0.2 mmol of dNTPs, 2.5 U of Taq DNA polymerase, 2.5 µl of 10X PCR buffer, and 1.5 mmol of $MgCl_2$, according to following conditions: denaturation at 95°C for 5 min, followed by 25 cycles at 95°C for 50 s, 55°C for 40 s and 72°C for 50 s. In single round, HGV-RNA and HCV-RNA were detected with primers specific to the nonstructural (NS5) region F and R. Second round cycling was carried out under the following conditions: denaturation at 95°C for 4 min, followed by 35 cycles at 95°C for 40 s, 64°C for 60 s and 72°C for 40 s, and a final extension step at 72°C for 5 min, and primer pairs used were sense and anti-sense for HGV and HCV in this step. The PCR products were detected by electrophoresis on 2% agarose gel and visualized under UV light using a 100-bp molecular marker as standard. The expected band sizes were 225 and 180 bp for HCV and HGV in second-round PCR, respectively. The primer pairs and annealing temperatures of the nested PCR primer sets used are shown in Table 1.

Statistical analysis

Significant differences of serological and molecular diagnostic markers of studied HGV and HCV between chronic renal failure patients and controls, and also statistical correlations between viral hepatitis diagnostic indices and possible risk factors of chronic renal failure were analyzed by use of parametric and non parametric analyses with SPSS for Windows (version 15, Chicago, IL, USA). A level of $P \leq 0.05$ was accepted as statistically significant.

Table 2. Frequency of HGVRNA. Anti E2 and the relationship between transfusion duration with prevalence of HGV-RNA and anti-E2 in HD patient.

Characteristics	Patients with		Patients with	
	HGV RNA (+)	HGV RNA (-)	Anti-E2 (+)	Anti-E2 (-)
Gender (M/F)	9/2	3/18	12/3	30/17
Age	58.90 ± 18.25	56.58 ± 16.14	52.60 ± 19.75	58.38 ± 15.15
Transfusion duration (months)	41.00 ± 15.86	22.19 ± 16.49	31.00 ± 18.22	23.78 ± 17.48
HCV RNA (+)	2	7	1	8

Table 3. The prevalence of HCV-RNA and HCV-antibodies among HD patients.

Characteristics	Patients with		Patients with	
	HCV RNA (+)	HCV RNA (-)	Anti-HCV (+)	Anti-HCV (-)
Gender (M/F)	8/1		7/5	
Age	58.22 ± 16.59	57.69 ± 16.51	51.46 ± 18.46	59.19 ± 15.42
Transfusion duration (months)	22.00 ± 6.91	25.73 ± 19.08	31.26 ± 18.73	23.95 ± 17.67
HGV RNA (+)	2	9	1	10

RESULTS

Sixty two HD patients and 100 normal individuals were included in our study. 42 of 62 (67.72%) patients with chronic renal failure are males and 20 of 62 (32.28%) of patients were females. Also 53 of 100 (53%) of controls were males and rest of them 47 of 100 (47%) were females with a mean age of 38.80 ± 16.40 years. The duration of dialysis in HD patients ranged between 1 and 74 months in HD patients (mean, 25.72 ± 18.06 months).

Prevalence of HGV infection and anti-E2

Of the 62 HD patients and 100 normal individuals, HGV RNA was detected in 11 (17.74%) and 3 (3%), respectively. The mean age of patients with positive HGV RNA was 58.90 ± 12.25 years. And, 15 (24.19%) and 6 (6%) tested positive for anti-E2, HD patients and normal individuals, respectively. The mean age of patients with positive anti-E2 was 52.60 ± 19.75 years, there was no significant difference between age and positive HGV RNA or with positive anti-E2 ($p = 0.413$ and 0.098, respectively). But there was significant difference between sex and positive anti-E2 ($p = 0.014$). The mean duration of hemodialysis in anti-E2 positive and HGV-RNA patients was 31.00 ± 18.22 and 41.00 ± 15.86 months, respectively. There was significant difference between positive HGV-RNA/duration of hemodialysis and positive anti-E2/duration of hemodialysis ($p = 0.00$).

Prevalence of HCV infection and HCV-antibodies

HCV-RNA was found in 14.51% (9/62) of patients and HCV-RNA was found in 1% (1/100) of normal individuals. Also, HCV-antibody was diagnosed in 19.35% (12/62) and 5 of 100 (5%) of HD patients and normal individuals, respectively. The mean age of patients with positive HCV RNA and positive HCV-antibody were 58.22 ± 16.59 and 51.46 ± 18.46 years, respectively. The mean duration of hemodialysis in HCV-antibody positive and HCV-RNA patients was 25.78 ± 19.03 and 22.00 ± 6.91 months, respectively. There was significant difference between positive HCV-RNA/duration of hemodialysis and HCV-antibody/duration of hemodialysis ($p = 0.00$). HCV RNA and HCV-antibody were not found simultaneously in any patients.

Prevalence of co-infection of HGV and HCV

Of the 62 HD patients (11 positive HGV RNA, 9 positive HCV RNA), two patients were simultaneously positive HGV RNA and positive HCV RNA (Table 2), and only one patient was positive for both anti-E2 and HCV RNA. The prevalence of HGV infection in HD patients was higher than that of HCV infection ($p = 0.00$), also found was the number of individual who have positive anti-E2 over from anti-HCV ($p = 0.00$) (Tables 2 to 4).

DISCUSSION

Patients on maintenance hemodialysis (HD) are at a great risk of acquiring HGV and HCV infections because of receiving regular blood for a long time, and potential for exposure to contaminated equipment (Sheng et al., 1998). Hepatitis C virus (HCV) infection is the major cause of acute or chronic hepatitis in patients on hemodialysis (HD). HGV and HGV prevalence in HD patients has been studied in many countries with somehow different results.

The world wide prevalence of HCV viral markers among HD patients has been shown to range from 2.6 to

Table 4. Frrequency of HGV RNA, anti-E2 and HCV-RNA and HCV-antibodies in blood donors.

Characteristics	Control group N = 100		Control group N = 100	
	HCV RNA (+)	HGV RNA (+)	Anti-E2 (+)	Anti-HCV (+)
N (%)	1 (3)	3 (3)	6 (6)	5 (5)
Gender (M/F)	1/0	2/1	2/4	3/2
Age	33.60 ± 10.70	50.80 ± 4.80	37.40 ± 10.30	33.40 ± 10.70

54% (Linnen et al., 1996; Fissell et al., 2004). HGV virus is known to be transmitted parenterally, and a high frequency of infection with HGV has been reported in chronic carriers of hepatitis C virus, haemodialysis patient and haemophiliacs (Orii et al., 2000; Mederacke et al., 2011). Some recent studies reported that HGV infection could be detected in 3.1 to 26% of patients receiving hemodialysis (Hammad and Zaghloul, 2009). The study of the prevalence of HGV and HGV in HD patients has different results in different countries. As reported by Ikeuchi et al. (1999), of the 240 patients, 67.91% were positive for HCV and 32.08% negative for anti-HCV. Also, 19.8 and 14.8% were positive for anti-HCV antibody and HCV-RNA marker, respectively (Gallian et al., 1999).

The aim of this study was to evaluate the prevalence of infection of HCV and HGV in HD patients in south of Iran. To define the actual prevalence of HGV and HCV infections in HD patients, the investigators of this study determined not only HCV-RNA and HGV-RNA but also HCV antibodies and anti-E2. The prevalence of HCV-RNA and HCV antibodies in HD patients was 14.51 and 19.35%, respectively. The mean of hemodialysis duration in HCV-antibody positive and HCV-RNA patients were 25.78 ± 19.03 and 22.00 ± 6.91 months, respectively. 11 (17.74%) in HGV-RNA and 15 (24.19%) in anti-E2 patients were positive, and the mean durations of hemodialysis in anti-E2 positive and HGV-RNA patients were 31.00 ± 18.22 and 41.00 ± 15.86 months, respectively. The mean duration of hemodialysis in HGV-RNA and anti-E2 positive patients was higher in comparison with negative patients. We observed that in the positive HCV antibodies, the mean duration of hemodialysis was further in proportion to the negative samples, but we did not see this increasing in the mean duration of hemodialysis in HCV-RNA, however, in patients infected with other parenterally transmitted viruses, such as HCV and GBV-C/HGV, despite the fact that there was a significant correlation between HBV and HCV infections.

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