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Evaluation of Prevalence and Risk Factors of Hepatitis G Virus Infection Among Hemodialysis Patients Referred to Iranian Army Hospitals in Tehran During 2012-2013

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Background: GB virus C (GBV-C) or hepatitis G virus (HGV) is a newly discovered and enveloped RNA positive-stranded flavivirus-like particle, which has not yet been proven to have major negative effects on liver.
Objectives: Increasing the risk of blood-borne infections in hemodialysis patients is a main health care concern in different countries. Therefore, it is important to estimate the prevalence and risk factors of hepatitis G virus infection in Iranian hemodialysis patients to design standard prevention and treatment plans.
Patients and Methods: In this multicenter observational or epidemiologic study, 138 patients who underwent hemodialysis in Iranian Army hospitals in Tehran were included. Serum HIV antibody (Ab), HCV antibody and HBS antigen (Ag) were assessed. Demographic data such as gender, age, blood group, cause of renal failure, dialysis onset and duration were collected from medical files. GBV-C/HGV was evaluated by nested reverse transcription polymerase chain reaction (RT-PCR) method. Then, all data were analyzed by SPSS ver. 13.
Results: In total, 81 males and 57 females were included. The mean age of patients was 62.16 ± 14.86 years. Six (4.3%) had positive results for GBV-C/HGV by RT-PCR. Except gender ($P = 0.045$) and duration of dialysis in a week ($P < 0.001$), other demographic factors revealed no significant difference ($P > 0.05$). All patients had negative results for HIV Ab, HCV Ab and HBS Ag.
Conclusions: Overall, 4.3% of patients had positive results for GBV-C/HGV and all negative for HIV, HCV and HBV. Further studies are needed to elucidate real prevalence, risk factors and characteristics of HGV infection in Iranian hemodialysis patients.

*Keywords:*GB virus C; Prevalence; Risk Factors; Renal Dialysis; Polymerase Chain Reaction

1. Background

Patients receiving chronic hemodialysis (CHD) are at a high risk of infectious complications. Prior to developing screening system and vaccines for hepatitis B virus (HBV), the most common etiologic agent of hepatitis in chronic hemodialysis patients was HBV. Afterwards, hepatitis C virus (HCV) was a main problem in CHD (1). From 1995 to 1996, two independent laboratories in the USA isolated a new enveloped RNA virus similar to flaviviruses. The first laboratory named it GB virus C/GBV-C and the second as hepatitis G virus (HGV) (2). HGV is a virus in the flaviviridae family and known to be infectious for human, but it has not been established to cause human disease with certainly (3). However, there is a suspicious link between HGV infection and acute or fulminant hepatitis, chronic hepatitis and hepatic fibrosis (4, 5). HGV infection has a worldwide distribution. Until now, five major genotypes of HGV are known as genotype 1 is the most common in the west Africa, genotype 2 known in the US and Europe, genotype 3 in parts of Asia, genotype 4 is specific for Myanmar, Vietnam and Indonesia and finally genotype 5 is frequently observed in south Africa (6, 7).

High prevalence is observed among subjects with risk of parenteral exposure including those with exposure to blood and blood products, such as CHD patients and intravenous drug users (8). CHD patients and other kinds of chronic renal failure (CRF) patients usually require blood transfusion. It is one of main risk factors of HGV transmission (9-11). Some studies suggested links between HGV and transfusion requirement, dialysis duration, renal transplantation and other kinds of viral hepatitis in CHD patients (10-12). Approximately, 2% of healthy United States blood donors had viremia with HGV and up to 13% of blood

donors had antibodies against E2 protein, indicating a possible prior infection (13). Sexual contact and vertical transmission could be another route of HGV transmission.

Furthermore, HCV and HIV-1 (Human Immunodeficiency virus-1) infected patients have evidence of higher rate of HGV infection (14, 15). Recently, several studies revealed that HGV could decrease progression of HIV virus and prolong the duration between HIV infection and AIDS (16).

Increased chronic disorders such as diabetes (DM), renal failure and end stage renal disease (ESRD) have become important issues in health care policies. Therefore, CHD and its complications are major hospital concerns. However, none of the studies indicated that HGV infection can cause any liver enzyme elevation or hepatic failure certainly, but coinfection with other hepatitis viremia can increase morbidity and mortality rates (17). Different surveys indicated prevalence of HGV in CHD patients between 3.1% in Japan and 57.5% in France (10, 11).

2. Objectives

Therefore, estimating HGV infection in dialysis patients of different countries seems to be reasonable and applicable in health care system to design standard prevention and treatment plans. The aim of the present study was to determine the prevalence and risk factors of HGV in Iranian ESRD patients undergoing routine CHD.

3. Patients and Methods

3.1. Study Population

This was a descriptive cross-sectional and multicentric study conducted in four major centers of hemodialysis in Iranian army hospitals in Tehran, Iran from February 2012 to March 2013. This survey covered 138 patients of 4 dialysis units, 81 males and 57 females, respectively. West, north, city center and east as well as southeast of Tehran were covered by units of 1, 2, 3, and 4, respectively. In these hospitals, all dialysis patients are examined for detection of HIV-1, HBV and HCV every six months, and patients with positive blood samples referred to one specific unit of hemodialysis. Therefore, all known cases of other types of hepatitis and HIV-1 were referred to one specific hospital based on routine hospitals regulations.

The following epidemiological data were obtained in all patients; (I) history of previous blood transfusion, (II) length of time on dialysis, (III) history of major surgery, (IV) blood group, (V) household contact with hepatitis, (VI) family history of hepatitis, (VII) reasons of dialysis (DM, Hypertension, infection, renal stone, polycystic kidney and others), (VIII) age and (IX) gender. Prior to initiation, the survey was approved by AJA Medical University Ethics Committee and an informed consent was obtained from all patients.

3.2. Sampling and Extraction of RNA

Two milliliters of blood was sampled from every patient and centrifuged immediately, then plasma was separated stored at -20°C and transferred to laboratory. Immediately, HGV-RNA was extracted from 100 μL plasma samples using the guanidine isothiocyanate-phenolchloroform method and reverse transcribed using random primer and the Moloney murine leukemia Virus (MMLV) reverse transcriptase (Fermentas, Lithuanian).

3.3. Detection of HGV

The oligonucleotide primers for amplification of c-DNA by PCR detected HGV RNA based on highly conserved domains of the 5´ non-coding regions. The nucleotide sequences of the primers were 5´-CACTATAGGTGGGTCTTAAG-3´ (150-169 nt and 5´- GCCTATTGGTCAAGAGAGAC-3´ (352-333 nt) for the first round of PCR and 5´- GCGCACGGTCCACAGGTGTT-3´ (207-226 nt) and 5´ GGGCGACGTGGACCGTACGT-3´ (326-307 nt) for the second round of PCR. PCR amplification was performed for 30 cycles (94°C for 30 seconds; 55°C for 90 seconds; 72°C for 90 seconds) in the first round of PCR and 35 cycles with the same time temperature conditions in the second round of PCR. Amplified products were separated with agarose gel (2%) electrophoresis and visualized by ethidium bromide staining under UV-detector system. The limit of detection was 1000 copy/mL using the samples with determined HGV viral load (determined with a homemade kit based on RNA in vitro transcription).

3.4. Statistical Analysis

Obtained data was analyzed by SPSS software ver. 13 (SPSS Inc., Chicago Ill., USA). The data was expressed and mean ± SD and No. (%) were calculated. Mann-WhitneyU and chi-square tests were used. $P < 0.05$ was considered significant.

4. Results

The study population consisted of 81 males (59%) and 57 females (41%). The mean age of patients was 62.16 ± 14.86 years. Moreover, the mean dialysis onset of patients was 24.75 ± 30.82 months and the mean weekly dialysis duration was 10.78 ± 18.25 hours. Only 2.5% of all patients had a positive family history of hepatitis diseases. In six of 138 patients, HGV was detected by nested RT-PCR and HGV RNA positive and shown 120 bp bands length in 1.2% agarose gel electrophoresis. Therefore, an overall prevalence of 4.3% was found for HGV among hemodialysis patients in our study. There was a significant difference between the two genders (HGV positive and HGV negative) ($P = 0.045$). Overall, 83.34% of HGV positive patients were female and 16.66% male.

Furthermore, duration of dialysis in a week, had a substantial difference between HGV positive and HGV negative patients ($P < 0.001$), indicating that increasing dialysis duration could increase the risk of HGV infection in patients (Tables 1 and 2).

Additionally, there was noother association between HGV and age, blood transfusion, major surgery, dialysis onset, DM and blood groups (Tables 1, 2 and 3). The secondary information of this study revealed the prevalence of cause of hemodialysis in Tehran (Table 4).

5. Discussion

Here, we investigated the prevalence and risk factors of HGV infection in hemodialysis patients in Tehran army hospitals. The results indicated a prevalence of 4.30% in those hemodialysis patients who all had negative results for HBV, HCV and HIV.

As patients of the present study refer to hospitals three days a week, the risk of nosocomial transmission is increasing. Several reports showed a high prevalence of HGV viremia (1-4%) in Europe and north American healthy population and widespread prevalence (10-33%) among south American as well as African people (3, 4, 8, 9). A high rate (55%) was reported in Indonesia (18).

HGV is a RNA virus and a member of Flaviviridae family. Several studies showed that it would not cause major liver damage, whereas some others showed it might infect and replicate in hepatocytes (15, 17, 19).

There are numerous reports about HGV prevalence worldwide. There is a polish report comparable to our study evaluating HGV among dialysis patients with

Table 1. Comparison of Variables Between HGV Positive and Negative Patients [a]

Variable	HGV Positive	HGV Negative	P Value
Ratio, male/female, No.	1/5	72/47	0.045
Age, mean ± SD, y	55.00 ± 18.56	62.52 ± 14.65	0.236
Dialysis onset, mean ± SD, mo	38.17 ± 33.92	24.07 ± 30.65	0.18
Dialysis duration, mean ± SD, h	12.00 ± 0.01	10.72 ± 1.85	0.001 >
DM, %	16.66	50.42	0.115
Familial history positive of viral hepatitis, %	0	2.58	0.858

[a] Abbreviations: DM, diabetes; HGV, hepatitis G virus.

Table 2. Comparing the Prevalence of Risk Factors Between HGV Positive and HGV Negative Patients [a]

Risk Factors, %	HGV Positive	HGV Negative
Blood transfusion	20	48.15
Major surgery	40	12.04
Major surgery + blood transfusion	40	12.96
Negative	0	26.85
Total	100	100

[a] Abbreviation: HGV, hepatitis G virus.

Table 3. Characteristics of Six Patients With Positive Results for HGV RNA [a]

Patient	Age	Gender	Familial History	Blood Transfusion	Major Surgery	Dialysis Onset,mo	Duration in a Week, h	Reason of HD	Blood Group	DM
1	64	Female	Negative	Positive	Negative	4	12	HTN	B+	Negative
2	27	Female	Negative	Negative	Positive	9	12	-	B+	Negative
3	47	Female	Negative	Negative	Positive	96	12	HTN	A+	Negative
4	63	Female	Negative	Positive	Positive	48	12	DM	O+	Positive
5	81	Male	Negative	Positive	Positive	48	12	HTN	A+	Negative
6	48	Female	Negative	Suspicious	Negative	24	12	HTN	O+	Negative

[a] Abbreviations: DM, diabetes ; HD, hemodialysis; HTN, hypertension.

Table 4. Causes of Hemodialysis in Our Study

Reasons	No. (%)	Valid
Diabetes	61 (44.2)	50.4
Hypertension	45 (32.6)	37.2
Infection	6 (4.3)	5
Renal stone	2 (1.4)	1.7
Polycystic kidney	2 (1.4)	1.7
Other	5 (3.6)	4.1
Total	121 (87.7)	100

negative results for anti HCV antibody. In their survey, prevalence of HGV was 6.7% among 215 patients (19). In a study in Turkey, Iran's neighboring country, prevalence of HGV was 14% among hemodialysis patients and 2% in blood donors (20). In an Iranian investigation, 3.89% of hemodialysis patients had positive results for anti E2 antibody, but none of their samples had positive results for HGV RNA (17). Ramos Filho et al. in Brazil found that 14.6% (95% CI: 9.2-21.7) of samples had positive results HGV RNA. A high positivity for HGV RNA was observed in patients who received kidney transplant (16.7%) followed by those on hemodialysis (15.3%) and peritoneal dialysis (7.7%) (15). However, none of these studies showed any significant difference in demographic factors, but our study showed substantial difference between gender and duration of dialysis in a week and HGV positivity.

There are some concerns in our study, which could cause misunderstanding of real prevalence of HGV. For instance, initially, HIV, HCV and HBV in our study had negative results, because patients with positive blood samples for HIV, HCV and HBV viruses referred to one specific unit of hemodialysis, based on routine hospitals regulations. Therefore, results of this study probably showed a smaller prevalence than other studies. Secondly, a significant difference between gender and HGV positivity in the present study could be due to low percentage of HGV positive patients.

Here, we used RT-PCR technique for detecting HGV with an acceptable sensitivity (21-24). The limit of detection was 1000 copy/mL using samples with determined HGV viral load (determined with a home-made kit based on RNA in vitro transcription). Four hospital centers were included in this investigation, so this could be considered a multicenter study.

In conclusion, patients on maintenance hemodialysis treatment are at high risk of acquiring parenterally transmitted viral infections. This study evaluated the prevalence of HGV among ESRD and under hemodialysis patients in Iranian army hospitals in Tehran. The current results showed that 4.30% of hemodialysis patients in these hospitals had positive results for HGV RNA. Females had higher percentage for HGV RNA. Duration of dialysis in a week is important to increase the risk of HGV transmission. Further studies are needed to elucidate real prevalence, risk factors and characteristics of HGV infection in Iranian hemodialysis patients.

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Authors' Contributions

Study concept and design: Mohammad Hosseinzadeh and Maryam Dadmanesh. Analysis and interpretation of data: Mohammad Hosseinzadeh, Maryam Rahimi, Mehdi Hosseinzadeh and Maryam Dadmanesh. Drafting of the manuscript: Mohammad Hosseinzadeh. Critical revision of the manuscript for important intellectual content: Mohammad Hosseinzadeh, Maryam Dadmanesh, Hossein Keyvani, Khodayar Ghorban, Maryam Rahimi and Mehdi Hosseinzadeh. Statistical analysis: Mohammad Hosseinzadeh and Maryam Dadmanesh.

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