Original Article

Hepatitis G virus infection among Iraqi patients on maintenance hemodialysis

Mohammad A. Al-Karkhy* **MBChB**
Shatha F. Abdullah** **FICM**
Ali A. Allawi*** **FICM**

Summary:

Background: Patients on maintenance hemodialysis are at increased risk of infection with parentally transmitted viral agents. In recent years a high prevalence of hepatitis G virus infection among end stage renal diseases and chronic hemodialysis patients has been well documented.

Objectives: To assess the percentage and risk factors of HGV in hemodialysis patients, and to evaluate the clinical consequences of HGV in this population.

Patients and methods: Fifty (50) patients with chronic renal failure who underwent maintenance hemodialysis. Patients were currently attending hemodialysis department of Baghdad teaching hospital during the period of October 2011 to January 2012, compared to forty one (41) healthy blood donors who underwent a full blood screening tests collected from blood bank. Aged matched as a control group. Anti-Hepatitis G antibodies (IgM and IgG) were detected using enzyme linked immunosorbant assay and HGV-RNA was determined by RT-PCR.

Results: Hepatitis G virus -IgM and HGV-IgG were detected in 26 patients (52%) and in 36 patients (72%) respectively. HGV-RNA was detected in 16 patients (32%). Furthermore, nine (18%) patients revealed HGV-RNA bands and gave a positive HGV-IgM. Hepatitis G virus was significantly associated with the history and numbers of blood pints intake among hemodialysis patients, while there were no association with the hemodialysis duration, history of renal transplant nor with raised liver enzymes.

Conclusions: Our results showed that hemodialysis patients carry the risk for HGV infection as a major possibility of parenteral transmission, especially by transfusion of blood and blood components. Decisions to screen blood supplies for a transfusion-transmitted infection agent should be based on sufficient benefits for recipients.

J Fac Med Baghdad 2014; Vol.56, No. 1
Received May.2013
Accepted Oct.2013

Key words: HGV, maintenance hemodialysis, blood donors.

Introduction:

Hepatitis G virus (HGV) is a recently described member of flaviviruses. The genome of the virus consists of single-stranded RNA and has a positive polarity. Similar to other members of the flaviviridae, it contains a single open reading frame that encodes the viral polyprotein (1). Although HGV is able to persist in humans, so far a chronic hepatitis due to HGV infection has not been reported (1, 2, 3).HGV is highly prevalent among population groups at risk of parenterally transmitted viral agents, but it has also a worldwide distribution in other non-risk population groups. Unexplained sporadic outbreaks of hepatitis by the mid-1990s prompted the discovery of hepatitis G virus and hepatitisGB virus C in 1995, Although epidemiologic analyses
revealed high prevalence rates of such virus in the hemodialysis population, their exact role in liver disease has yet to be determined (4) However,
little is known about other modes of transmission that could explain the high prevalence and worldwide distribution of this virus (4).Patients with chronic renal failure are at high risk of acquiring this virus because they require frequent blood transfusions and undergo medical procedures that accompany bleeding (5, 6).

**Ministry of health/AL-Numan general hospital.*
*** Dept. Of Microbiology/College Of Medicine/ University Of Baghdad.*
****Dept. of Medicine/Baghdad teaching hospital/Baghdad College of medicine.*

Patients and Methods:

A cross-sectional study involved fifty patients with chronic renal failure who underwent hemodialysis for at least six months and above. Patients were currently attending hemodialysis department of Baghdad teaching hospital during the period of October 2011 to January 2012. Their ages ranged from 15 to 72 years with mean ±SD was equal to 48.52±14.77 years. Compared to Forty one healthy blood donors who were already under screening test for HBsAg, anti-HCV antibodies, HIV-antigen and anti-HIV antibodies and they were attending Iraqi blood bank center age and sex matched as a control group.Five ml blood samples were harvested from all study groups, centrifuged for 10 min X 3000 rpm, then the serum were divided into several 0.5 ml aliquots in Eppendorf tubes. All sera were immediately frozen at -20 C°.The sera were tested for HGV-IgM and HGV-IgG using Enzyme-Linked Immunosorbent Assay (ELISA) in

Post graduate Laboratory of Department of Microbiology/ Baghdad collage of medicine. Technique used human IgM and IgG as the antigen coated the micro wells plate. Sample Diluents and samples were added to the appropriate microtiter plate wells and incubated, and then Horseradish Peroxidase (HRP)-conjugated anti-human IgM was added and incubated. After that substrate solution was added to each well. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of human hepatitis G virus antibody (IgM) and (IgG) in the samples was then determined by comparing the O.D. of the samples to the standard curve (Cusabio China).HGV RNA in serum was determined by reverse transcription (RT) and amplification by PCR was carried in Iraqi Central Health Laboratory. RNA was extracted from 150µl using RNA extraction kit (Ribo-virus extraction kit, REF: K-2/C, Sacace Biotechnologies, Italy). Complementary DNA(cDNA) was synthesized by added 10 µl of Reaction Mix to 10 µl of extracted RNA then 30 minutes incubation at 37ºC finally 20 ml of TE-buffer was added. PCR amplification was carried out using a commercial kit (HGV 340/625 IC kit, REF V-2-50R, Sacace Biotechnologies, Italy). According to manufacturer's instructions, one positive control was included in each run. Samples were considered positive for HGV RNA if a band of 340bp could seen on 2% agarose gels with ethidium bromide. Other data included in this study (biochemical liver function tests TSB, SAST and SALT) collected from Laboratory reports for each patient during the follow up period.

Statistical Analysis:

The chi-square and t- test (analysis of variances) were used to detect the significances between variables of our study. The SPSS program (version- 17 package program) was done for statistical analysis. Data are presented as means ± standard deviation or, when indicated, as absolute numbers and percentages. A P - value of less than 0.05 was considered to be statistically significant. Odd ratio was used as a measure relative risk.

Results:

Serodiagnosis of Hepatitis G virus among study groups using ELISA:

HGV-IgM was detected in 26 out of 50 patients tested (52%) and of 41 apparently healthy blood donors tested with anti-HGV IgM antibodies against HGV infection were detected in 11 (26.83%) persons whom belong to control group.Statistical significant difference was clearly noticed (P-value=0.000). The risk of HGV infection in patients on maintenance hemodialysis was 2.95 times (odds ratio) as showed in table-1.

HGV IgG was detected in 36 out of 50 patients (72%) compared to 6 out of 41 (14.63%) of apparently healthy blood donors. Statistical significant difference was clearly noticed (P-value < 0.05) as showed in table-1.

Table-1 Serodiagnosis of HGV infection

Study groups		*Patient* No. (%)*	*Controls** No. (%)*	*Total No=91*	*P-value*
HGV-IgM	Positive	26 (52)	11 (26.83)	37	0.015
	Negative	24 (48)	30 (73.17)	54	
HGV-IgG	Positive	36 (72)	6 (14.63)	42	0.000
	Negative	14 (28)	35 (85.37)	49	

* Total number of patients = 50.

** Total number of controls = 41.

Detection of HGV-RNA by RT-PCR among study groups:

Hepatitis G virus -RNA was detected in 16 (32%). Nine (18%) of HGV-RNA positive cases had a positive HGV-IgM with no statistical difference with regard to control group, as illustrated with Table-2.

Table-2: Correlation between HGV IgM and HGV-RNA among study cases

HGV-RNA	HGV IgM No. (%)		Total No. (%)
	Positive	Negative	
Positive	9 (18)	7 (14)	16 (32)
Negative	17 (34)	17 (34)	34 (68)
Total	26 (52)	24 (48)	50 (100)

$X^2 = 0.170$ DF = 1 P-value = 0.680

Figure-1 demonstrates that cDNA complementary to HGV-RNA bands were migrated toward the 340 bp by gel electrophoresis at the same level with positive control.

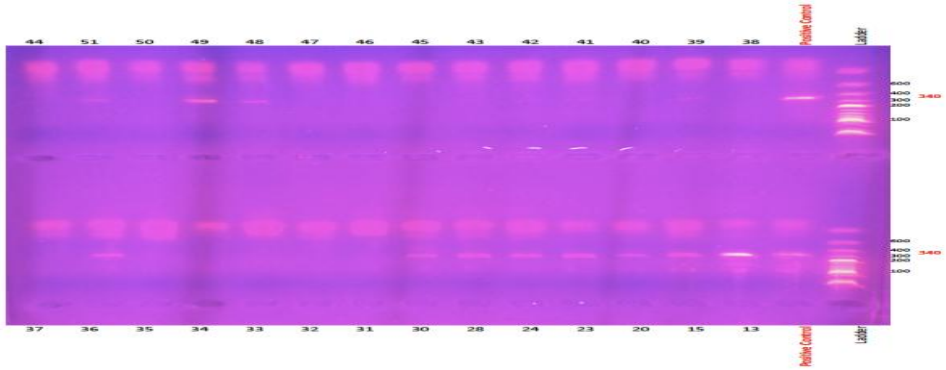

Figure-1: Image of HGV-cDNA that was appeared on agaros gel

HGV-RNA bands were appeared as cDNA.
From the *(*left): *Ladder* started from 0 to > 600 bp.
Positive control
Positive bands: No. 13,15,20,23,24,28,30,36,48,49 and 51
Faint bands: No. 39, 41, 42 and 43

Risk factors associated with HGV infection:

In order to assess the potential risk of HGV infection in hemodialysis patients, risk factors were divided in to three categories as illustrated in table- 3.

Table- 3 Risk factors and clinical relevance associated with HGV infection

Risk Factors		*HGV-IgM*		*P-value*
		Positive* No. (%)	Negative** No. (%)	
History of blood transfusion	Nill <5 Pints ≥5 Pints	10 (38.4) 4 (15.4) 12 (46.2)	8 (33.33) 14 (58.33) 2 (8.34)	0.040
History of renal transplantation	Yes No	2 (7.7) 24 (92.3)	1 (4.2) 23 (95.8)	0.600
Duration of Hemodialysis	6-12 13-24 >24	11(42) 5(19) 10(39)	10(42) 6(25) 8(33)	0.408
liver enzymes (mean ± SD)	TSB (1.41±0.39) SALT(43.875 ±29.7) SAST(43.625±27.02)	8(30.8) 5(19.2) 3(11.5)	5(20.8) 3(12.5) 5(20.8)	0.580

* Total number of positive HGV IgM cases = 26.
**Total number of negative HGV IgM cases = 24.

Notes:

A- Patients with HGV infection may have one or more than one elevated liver enzymes.

B- Normal liver enzymes among patients group (mean ± SD):

TSB = (0.7 ± 0.18)
SAST = (12.6±3.31)
SALT = (11.5±3.16)

Furthermore, patients were subdivided into three subgroups according to the number of blood pints whom received.Significant difference were observed between the history and numbers of blood pints intake among hemodialysis patients with positive HGV IgM compared to control (P-value <0.05) as the later group denied take blood.Twelve (46.2%) of cases received more than 5 blood pints during the preceding years.There was no significant correlation between renal transplant and HGV infection as demonstrated in table-3 that shows 2 out of 26 (7.7%) cases proved to have HGV infection and gave a history of renal transplant whereas only1 case out of 24 cases (4.2%) had a history of renal transplant whom HGV infection could not traced. The relative risk of HGV infection was nearly 2 times in patients with history of renal transplantation by measuring the odds ratio which was equal to 1.92. Table-3 shows that 11 out of 26(42%) cases gave a positive HGV-IgM who underwent hemodialysis for 6-12 months and 10 out of 26 (39%) HGV- IgM positive cases on maintenance hemodialysis for more than 2 years whereas, only 5(19%) infected cases with HGV on hemodialysis for 13-24 months duration.No statistical significance was observed between

HGV infected cases and the duration of hemodialysis as P- value = 0.408.

Hepatitis G Virus infection and clinical relevance:

In this study, there were 8 patients had clinical evidence of liver diseases who were presented with jaundice when comparing the mean serum alanine amino transferase enzymes levels (TSB, SALT and SAST) of 50 patients at time of their test for HGV, table-3 shows that from all hemodialysis patients with positive HGV-IgM, 8 cases (30.8%) had elevated TSB mean ± SD equal to (1.41 ± 0.39), 3 cases (11.5%) had raised SAST with mean ± SD equal to (43.63 ± 27.02) and 5 (19.2%) of cases had elevated SALT with mean ± SD equal to (43.88 ± 29.7). No statistical differences were found among hemodialysis patients and HGV infection (P-value >0.05) compared to hemodialysis patients with negative HGV-IgM and raised liver enzymes and to the control group.

Discussion:

Hemodialysis patients are uniquely vulnerable to the development of health care associated infections because of multiple factors including exposure to

invasive devices, the high prevalence of diabetes mellitus, malnutrition state intrinsic to end stage renal disease, immune suppression, the lack of physical barriers between patients in the hemodialysis environment, frequent hospitalization and frequent contact with health care workers during procedures and care (7).Liu *et al*. proposed that serodiagnosis of HGV infection by ELISA method was sensitive, specific, rapid and stable for detecting anti-HGV IgM and is useful in early diagnosis of the HGV infection (8). In this study, the presence of anti HGV-IgG seems to indicate past HGV exposure and association with immunity and protection for new infection (9).Unfortunately there is no report on the prevalence of HGV infection in hemodialysis patients, central Iraq yet. Besides one study in Iraq by Al-Obeidy and her colleagues which was conducted to investigate Iraqi patients with chronic liver diseases (10). Until recently HGV/GBV-C could only be detected by very sensitive RT-PCR assays (11, 12). Poor laboratory detection of HGV–Ag might be due to low level of HGV antigenemia, underlying hepatitis C or B viruses co infection, immune suppression or other host factors (13).HGV-RNA was detected in 16 (32%) of 50 serum samples .However, the invisibility of RNA bands by gel electrophoresis might due to many factors either the virus might be cleared with the appearance of HGV-IgG.The frequency of HGV infection in our patients was relatively near the higher rates that were reported in hemodialysis patients from other countries, which ranged from 3.1% in Japan (5) and up to 57.5% which was from France (6). The seroconversion to HGV antibodies is often associated with less detectable HGV viremia and detection of anti-E2 antibodies may be useful for diagnosing recovery for HGV infection (9, 14). Non-optimal choice of PCR primers can be another reason for differences in the reported rates of the HGV-RNA detection. Most recent finding has indicated that some parts of the GBV-C/HGV genome are more variable than others (15).20 (25%) (10). Furthermore, it was recorded 2% of blood donors in KSA had HGV infection (16), 16.1 % in Egypt (17) and 32.6% in Iran (18).These discrepancies in the rate of viral hepatitis G infection in dialysis patients may reflect the diverse prevalence of country and within different dialysis units, different length of time on hemodialysis of the different population, also might due to variations in the transfusion practices and hygienic standards as had been shown per HCV infection, socioeconomic status, sample size and composition of the study groups.In our cases, multiple risk factors were found to be present in these patients of hemodialysis. The percentage of HGV infection seems to be higher (46.2%) in hemodialysis patients who had received five or more blood pints during their admission to dialysis unit. Long duration of dialysis was noted to be associated with increased presence of anti-HGV (5, 6, 9) and among patients repeatedly exposed to blood products (19).Although a positive correlation between the prevalence of HGV infection and the history and/or numbers of blood transfusion have been reported in some studies (5, 20). Moreover, nosocomial spread had been important and patient to patient exposure was a significant factor in patients with renal failure and dialysis (2).In this study,The risk of acquired HGV infection is not associated with increasing duration of hemodialysis. The mean duration of patients on hemodialysis is largely different from other study (21), It is probably related to higher mortality rates in our patients, the effect of political and safety status of Baghdad in the last few years with higher immigration rate could not be excluded (21).Renal Transplant recipients are at increased risk of infection from transmitted viral agents. The significant of HGV infection among kidney transplant patients was observed by many studies who had received transfusion of intravenous immunoglobulin and cellular components during organ transplantation as reported to be 27.5% by Dussol (22).Our findings agree with previous studies reporting that biochemical evidence of liver inflammation is uncommon in patients with HGV infection (10, 23). Halarsz *et al*. noted that HGV infection might infect as well as replicate in hepatocytes and might lead to some persistently unknown complications (24). Other reports confirmed that HGV infection to be associated with acute (25) up to fulminant hepatitis (26). Despite the ongoing conflicts in HGV pathogencity, our possible explanation might be due to the immune deficient state in hemodialysis patients and that was explain the low effect of HGV on hepatocytes cell by decreasing the invasion of the immune system against liver tissue (27). Furthermore, it was shown that HGV had the most pronounced interferon-inducing activity (28). Our results are consistent with previous studies which appear that patients on hemodialysis are at increased risk of HGV infection. As well as a pronounced frequency of HGV infection among blood donors was found, thus further investigations are necessary to clarify the role of HGV infection in the development of liver disease in this clinical setting.

Author contributions:

The study conception was originally done and data were analyzed for this study by Dr Mohammad A. Al-Karkhy, the study design and data interpretation as well as drafting of manuscript was done by Dr.Shatha F A. Informations were analyzed and critical revision of manuscript was done by Dr. Ali A. Allawi.

References:

1. Linnen J, Wages J J, Zhang K Z Y, et al. Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. Science. 1996; 271:505-508.

2. Kao J H, Huang C H, Chen W, et al. GB virus C infection in hemodialysis patients: Molecular evidence for nosocomial transmission. J Infect Dis. 1999; 180:191-194.

3. Ozdarendeli A, Toroman Z A, Kalkan A, Kilic S S, Ozden M, and Doymaz M Z. Prevalence and genotypes of hepatitis G virus among hemodialysis patients in Eastern Anatolia, Turkey. 2005; 14(2):102-6.

4. Tang S and Lai K N. Chronic viral hepatitis in hemodialysis patients. Hemodialysis International. 2005; 9(2): 169-179. DOI: 10.1111/j.1492-7535.2005.01129.x.ISSN: 14927535. Blackwell Publishing (IVSL).

5. Masuko K, Mitsui T, Iwano K, et al. Infection with hepatitis GB virus C in patients on maintenance hemodialysis. N Engl J Med. 1996; 334:1485-1490.

6. De Lamballerie X, Charrel RN and Dussol B. Hepatitis GB virus C in patients on hemodialysis. N Engl J Med. 1996; 334:1549.

7. CDC. Recommendations for preventing transmission of infections among chronic hemodialysis patients. MMWR 2001; 50(RR05):1–43.

8. Liu C J, Kao J H, Lai M Y, et al. Minimal role of GB virus C/ hepatitis G virus in fulminant and subfulminant hepatitis in Taiwan. J Gastroenterol Hepatol. 1999; 14:352-357.

9. Desassis J, Laperche S, Girault A, et al. Prevalence of present and past hepatitis G virus infection in a French hemodialysis centre. Nephrol Dial Transplant. 1999; 14:2692-7.

10. Al-Obeidy E Sh, Abdullah S F and Mukhlis F A. Hepatitis G virus infection among Iraqi patients with Chronic liver diseases. J Fac Med Baghdad. 2010; 52(2).

11. Martin P, Fabrizi F, Dixit V, et al. Epidemiology and Natural History of Hepatitis G Virus Infection in Chronic Hemodialysis Patients. American Journal of Nephrology. 1999; 19(5): 535-540. ISSN: 02508095. Karger (IVSL).

12. Barril G, Castillo I, Arenas M D, et al. Occult Hepatitis C Virus Infection among Hemodialysis Patients. J Am Soc Nephrol. 2008; 19: 2288–2292.

13. Eslamifar A, Hamker R, Ramezani A, et al. Hepatitis G virus exposure in dialysis patients. Int Urol Nephrol. 2007; 39:1257–1263.

14. Tacke M, Kiyosawa K, Stark K, et al. Detection of antibodies to a putative hepatitis G virus envelope protein. Lancet. 1997; 349:318-320.

15. Okamoto H, Nakano H, Inoue T, et al. The entire nucleotide sequence of two GB virus C/hepatitis G virus isolates of distinct genotypes from Japan. J Gen Viro. 1997; l78:737-745.

16. Al-Ahdal M N, Rezeig M A, Kessie G, et al. GB virus C/hepatitis G virus infection in Saudi Arabian blood donors and patients with cryptogenic hepatitis. Arch Virol. 2000; 145(1):73-84.

17. El-Zayadi AR, Selim O, Naito H, Hess G and Ahdy A. Prevalence of GBV-C/hepatitis G virus viraemia among blood donors, health care personnel, chronic non-B non-C hepatitis, chronic hepatitis C and hemodialysis patients in Egypt. J Virol Methods. 1999; 80:53-58.

18. Kafi-Abad S A, Samiei S, Talebian A, et al. Hepatitis G Virus Infection in Iranian Blood Donors and High-Risk Groups. Hepatitis Monthly. 2009; 9(4): 282-286. ISSN: 1735143X. Tehran Hepatitis Center (IVSL).

19. Karayiannis R T. Hepatitis G virus: identification, prevalence, and un answered questions. Gut. 1997; 40:294-296

20. Watanabe T, Ishiguro M, Kametani M, et al. GB virus C and hepatitis C virus infections in hemodialysis patients in eight Japanese centers. Nephron. 1997; 76:171-175.

21. Khattab O S. Prevalence and risk factors for hepatitis C virus infection in hemodialysis patients in an Iraqi renal Transplant center. Saudi J Kidney Transpl. 2008; 19(1):110-115.

22. Dussol B, Charrel R, De Lamballerie X, et al. Prevalence of hepatitis G virus infection in kidney transplant recipients. Transplantation. 1997; 64:537-539.

23. Alter H J, Nakatsuji Y, Melpolder J, Wesley R, Shih J W K and Kim J P. The incidence of transfusion-associated hepatitis G virus infection and its relation to liver disease. N Engl J Med. 1997; 336:747-754.

24. Halarsz R, Weiland O and Sallberg M. GB virus C/hepatitis G virus. Scand J Infect Dis. 2001; 33: 572-580.

25. Ling B, Zhuang H, Cui Y, et al. A cross-sectional study on HGV infection in a rural population. World J Gastroenterol. 1998; 4:489-92.

26. Sheng L, Soumillion A, Beckers N, et al. Hepatitis G virus infection in acute fulminant hepatitis: prevalence of HGV infection and sequence analysis of a specific viral strain. J Viral Hepat. 1998; 5:301-6.

27. Chan TM, Wu PC, Lau JY, Lok AS, Lai CL & Cheng IK. Interferon treatment for hepatitis C virus infection in patients on haemodialysis. Nephrol Dial Transplant. 1997; 12:1414-1419.

28. Dzhumagaliyeva A, Shuratov I, Omarova M, Orakbay L and Aspetov D. Effect of HGV-infection on clinical course of hepatitis C and the possible role of endogenous interferon. Medical and Health Science Journal. 2012; 12: 40-43.