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Hepatitis G Virus Infection in Patients with Hepatitis C

Heba S. Selim*, Mohamed A.El Barrawy*, Osama N. Mohamed*, Marwa S. Gamal El Din*.

Abstract: Hepatitis G virus (HGV) infection is a worldwide health problem causing acute and chronic non A- E hepatitis. Because HGV and hepatitis C virus (HCV) share same modes of transmission, co-infection with the two viruses is not uncommon especially among people at high risk of parenteral infection. The aim of this study was to determine the prevalence of HGV among HCV virus cases, and to determine the degree of concurrent association between HGV and other prevalent infections in Egypt as *Schistosoma*, and hepatitis B virus (HBV) infections. This study included 100 blood donors attending Alexandria University Blood Bank in EL Shatby, proved to be positive for HCV antibodies by enzyme linked immunosorbant assay (ELISA) technique. Blood samples were collected and tested for the detection of HBV surface antigen (HBsAg) and *Schistosoma* antibodies by ELISA technique and HGV RNA by nested polymerase chain reaction (PCR) technique. Out of 100 anti-HCV positive blood donors, 39(39%) had HGV RNA in their serum, of them 10 (25.6%) were positive for HBsAg, on the other hand 34(87.2%) were positive for *Schistosoma* antibodies. From this study it could be concluded that HGV is a common co-infection in HCV cases, however there was no significant statistical relation between the presence of HGV RNA and the presence of HBsAg and /or *Schistosoma* antibodies. Screening for HGV among blood donors in addition to the routinely screened HBV and HCV may have a beneficial effect in reducing its transmission among the population.

Key words: HGV, HCV, HBV, *Schistosoma*

INTRODUCTION

Viral hepatitis is a major global public health problem. It is the most common cause of chronic hepatitis resulting in liver fibrosis, cirrhosis, and liver carcinoma.(1) Over the past 40 years, five hepatitis viruses have been identified and named A, B, C, D, and E. Acute or chronic hepatitis, in which both non viral causes and infection with any of the five known and characterized hepatitis viruses have been excluded, is described as non-A-E hepatitis. (2) Other hepatitis viruses have been reported. One of the blood borne hepatitis viruses is hepatitis G virus. (3)

* High Institute of Public Health Department of Microbiology Alexandria University

HGV is a single-stranded, positive sense RNA virus, belonging to the family *Flaviviridae*. It is a hepatotrophic virus discovered in 1995.[4] It is highly prevalent among volunteer blood donors and general population, as many cases can be chronic carriers for HGV for decads with no symptoms.[5] Although HGV infection alone rarely causes acute hepatitis with transient elevation of liver enzymes, about 10-20% of infections develop chronic hepatitis with elevated serum ALT levels.[6] HGV RNA was detected in patients with fulminant hepatic failure (FHF) of unknown etiology.[7] HGV also causes extra hepatic manifestations as hepatitis/ aplastic anaemia syndrome.[8] HGV co-infection with other hepatitis viruses as HCV and HBV will worsen the case of chronic hepatitis leading to more progression and faster hepatic damage and increases the HCC incidence.[9]

HGV is transmitted via exposure to blood and blood products; so infection is frequent among multiple transfused haemophiliacs, thalassaemia and haemodialysis patients.[10,11] Other routes of transmission such as sexual, vertical transmission, nosocomial, and intra-familial transmission have been documented.[12,13]

HGV infection is diagnosed by reverse transcriptase polymerase chain reaction (RT-PCR). Serological tests for antibodies to envelope E2 protein have been developed, however they indicate past infection only. [14,15]

In this study we aimed to determine the prevalence of HGV among HCV cases, and to determine the degree of concurrent association between HGV and other prevalent infections in Egypt as *Schistosoma*, and HBV infections.

SUBJECTS AND METHODS

This study was carried out through the period from August 2008 to February 2009. It included 100 blood donors attending Alexandria University Blood Bank

in El Shatby , proved to be positive for HCV antibodies by ELISA technique. They were asked about personal data including; sex, age, occupation, past history of blood transfusion, past history of surgical operations and past history of liver diseases or bilharzial infection. Informed consent was obtained from each patient.

Five ml blood were aseptically collected from each patient in sterile tubes, sera were separated by centrifugation, distributed into aliquots and stored by deep freezing at -70˚C until tested for the detection of HBsAg, *Schistosoma* antibodies and HGV RNA.

HBsAg was detected using DIMA diagnostic ELISA kit, while *Schistosoma* antibodies were detected using DRG IgG ELISA kit according to the manufacturer's instructions.

HGV RNA was detected using nested PCR technique.[16] RNA was extracted from 100 µl of serum samples by using Promega SV total RNA isolation system and resuspended in 100 µl of elution buffer. The extracted RNA was subjected to reverse transcription and DNA amplification, both were done together using the Promega Access Quick RT-PCR System. For first round of amplification outer primers were used:

G_{58} (outer; forward), 5`-CAGGGTTGGTCGTAAATCC-3`; **G_{75}** (outer; reverse), 5`-CCTATTGGTCAAGAGAGACAT-3`; The amplification protocol included 25 cycles of denaturation at 94˚C for 45 seconds, annealing at 55˚C for 45 seconds and extension at 72˚C for 90 seconds.

Reamplifications of the resulted amplicons were done using specific inner primers. **G_{134}** (inner; forward), 5`-GGTCAYCYTGGTAGCCACTATAGG-3`; **G_{131}** (inner; reverse), 5`-AAGAGAGACATTGWAGGGCGACGT-3`;

The second amplification was done using promega Go Taq Green Master mix with the same amplification protocol. All samples were pre-aliquoted to prevent degradation resulting

from repeated freezing and thawing. To avoid contamination, sample preparation, DNA extraction, and PCR amplification steps were performed in separate areas. Aerosol filter pipette tips were used for handling all liquids. All appliances, containers, and the work areas were cleaned and irradiated with UV light for at least 60 minutes. One nuclease-free water control was included per 6 samples, and results were negative in all cases.

PCR products resulting from the second round of amplification were loaded on 2% agarose in Tris-EDTA buffer (TBE) containing 0.5 µg of ethidium bromide per milliliter. After electrophoresis, The DNA bands were visualized on a 302 nm UV transilluminator and photographed. The gel was examined for specific bands of 208 bp as determined by the molecular weight markers run at the same time.

Statistical analysis

Data were analyzed using SPSS (Statistical Package for the Social Sciences) version 13.0. The association between the categorical variables were assessed by using the chi-square test. P value <0.05 was considered statistically significant.

RESULTS

Table1 shows that out of 100 anti-HCV positive blood donors, 39(39%) had HGV RNA in their serum, while the remaining 61(61%) were HGV RNA negative. Out of 100 anti-HCV positive blood donors, 54 had history of blood transfusion, among them 29 (53.7%) were HGV RNA positive, while out of 46 patients with no history of blood transfusion, only 10 (21.73%) patients were HGV RNA positive. The results were statistically significant (X^2 = 10.668, P=0.001).

Table 2 shows that out of the 39 HGV RNA positive cases, 10 (25.6%) had HBsAg in their serum, on the other hand 34(87.2%) were positive for *Schistosoma* antibodies. On the other hand, out of 61 HGV RNA negative cases, 24(39.3%) had HBsAg in their serum,

and 54 (88.5%) were positive for *Schistosoma* antibodies. Five (5%) of the studied patients had HGV RNA, HBsAg and *Schistosoma* antibodies in their serum, while 17(17%) patients had only HBsAg and *Schistosoma* antibodies in their serum in absence of HGV RNA .The results were statistically insignificant ($P > 0.05$).

DISCUSSION

HGV infection is a worldwide health problem causing acute and chronic non A-E hepatitis. Voluntary blood donors are the most dangerous group for the transportation of HGV infection, careful investigation for the taken blood is the only way to protect others who receive this blood. Several studies showed that there was a very large variation in the prevalence of HGV RNA in healthy blood donors ranging from 1% to 44%. [17- 20]

Because HGV and HCV share same modes of transmission, co-infection with the two viruses is not uncommon especially among people at high risk of parenteral infection. In the present study, HGV RNA was detected by nested PCR technique in 39 (39%) persons out of 100 anti-HCV positive blood donors. Similar results were reported by many studies as that by Wang *et al*, (1997) [21] who found that the percent of HGV infection in HCV positive blood donors was 29%. A study by Brojer *et al*, (1999) [22] estimated HGV co-infection in 22.2% of HCV chronic cases. Another study by Chu *et al*, (1999) [23] reported that HGV infection was detected in 17.3% of chronic HCV patients. Also Yan and Dennin, (2000) [24] found a high frequency (35%) of HGV co-infection in hepatitis C patients in Germany. Chu *et al*, (2001) [25] found that co-infection between HGV and HCV was proved in 22% of patients. Al-knawy *et al,* (2002) [26] reported co-infection between the two viruses in 31% of Saudi patients. In the United Arab Emirates, the percentage was 47.6 % as reported by Abou Odeh *et al*, (2005).[27] In 2008, Rajan *et al,* [20] found that co-infection of HGV and

HCV in chronic liver disease cases was 71.4%. While Salehi *et al*, (2009) [28] stated that the prevalence of HGV and HCV co-infection was 25%.

On the other hand, many studies reported a lower frequency of HGV co-infection among chronic HCV patients ranging from 5%-14%. [16, 29-33] The low prevalence of HGV infection in HCV chronic patients in some studies may be attributed to the use of interferon therapy that may lead to HGV RNA clearance in those patients as proved by a study conducted by Yu *et al,* (2001), [34] where the co-infection rate between HGV and HCV was 35.2 % while after the use of interferon, HGV RNA disappeared in 20.7% of cases.

In this study, there was significant statistical relation between the presence of HGV RNA and history of blood transfusion as HGV RNA was detected among 29 cases (53.7%) with positive history of blood transfusion. A study by Hinrichsen *et al,* (2002) [35] reported that repeated blood transfusions more than five times increases the risk of HGV infection significantly. Abad *et al*, [36] found that the prevalence of HGV RNA in multi-transfused thalassemic patients was 2.24%. In 2008, Samadi *et al* [37] reported that the number of transfusion of blood and blood products increased the risk of HGV infection.

In this study *Schistosoma* antibodies were detected in 88 (88%) of anti-HCV positive cases, Out of the 39 HGV RNA cases, 34 (87.2%) were positive for *Schistosoma* antibodies. Hassoba *et al,* (1997) [38] reported that the presence of GBV-C RNA was associated significantly with a history of schistosomiasis. Another work by Gallian *et al,* (1998)[39] studying the prevalence of HGV in rural area in Brazil, in which the prevalence of schistosomiasis was 80-90%, found that the percentage of HGV viremia was 16.4% and the anti-E_2 was 18.3% with a total exposure rate of 34.7%. These findings

were explained by the presence of *Schistosoma* infection which influence the immune response to HGV resulting in delayed viral clearance. Darwish *et al*, (1998)[1] who studied the prevalence of HGV in Egypt, documented that older age and history of schistosomiasis were significant factors in the wide spread of HGV in Egypt.

In the present study, HBsAg was detected among 34 (34%) of anti-HCV positive cases, and out of the 39 HGV RNA positive cases, 10 (25.6%) were positive for HBsAg. As the three hepatotropic viruses are mainly transmitted through blood and blood products, thus co-infection between them can occur in a substantial proportion of patients. A study by Brojer *et al,* (1999) [22] reported the presence of HGV infection in 16.6% of HBV patients. The prevalence of HGV in HBV cases was 14.3% according to the study carried out by Abu Odeh *et al*, (2005). [27] Nevertheless, Rajan *et al*, (2008) [20] documented that the prevalence of HGV RNA in chronic liver disease cases co-infected with HBV was 50%.

From this study it could be concluded that HGV is a common co-infection in HCV cases. There was significant statistical relation between the presence of HGV RNA and history of blood transfusion, however there was no significant statistical relation between the presence of HGV RNA and the presence of HBsAg and /or *Schistosoma* antibodies. Thus, screening for HGV among blood donors in addition to the routinely screened HBV and HCV may have a beneficial effect in reducing its transmission among the population. We should pay more attention to HGV when studying the possible pathogens of the so called “Hepatitis of unknown aetiology”

Table (1) Distribution of HGV RNA in 100 anti- HCV positive blood donors according to past history of blood transfusion:

History of blood transfusion	HGV RNA				Total	X^2-test
	Positive		Negative			
	No	%	No	%		
Yes	29	53.70	25	46.30	54	X^2= 10.668* P=0.001
No	10	21.73	36	78.27	46	
Total	39	39.00	61	61.00	100	

*Significant p< 0.05

TABLE (2): HGV RNA, HBsAg and *Schistosoma* antibodies among 100 anti-HCV positive blood donors:

		HGV RNA					X^2 – test	
		Positive N=39		Negative N=61		Total		
		No	%	No	%		P value	Chi square x^2
HBsAg	Positive	10	25.6	24	39.3	34	0.158	1.991
	Negative	29	74.4	37	60.7	66		
Schistosoma Ab	Positive	34	87.2	54	88.5	88	0.840	0.041
	Negative	5	12.8	7	11.5	12		

Insignificant (p>0.05)

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