

# Impact of Hepatitis G Virus Infection on Chronic Hepatitis C Egyptian Patients: Clinical, Virological and Ultrastructural Aspects

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**Abstract:** Hepatitis G virus (HGV) coinfection in chronic hepatitis C patients has recently been an active area of research as the impact of HGV infection on HCV chronic liver disease is still controversial. This study was conducted to investigate the prevalence of HGV infection in chronic HCV patients and to clarify its clinical, virological and histopathological impact at the ultrastructural level on chronic HCV liver disease. One hundred chronic HCV patients and 80 healthy blood donors were subjected to clinical, laboratory and ultrasonographic examination. Blood samples were examined for HCV and HBV markers, HCV serotyping, HCV quantitation of viral load and HGV RNA detection by nested Rt-PCR. Liver biopsy specimens were obtained from 25 patients and processed for light and electron microscopic (EM) examination. Chronic HCV patients were classified into 4 groups: chronic hepatitis (CH= 45); compensated cirrhosis (CC= 11); decompensated cirrhosis (DC= 22); and hepatocellular carcinoma (HCC= 22). The prevalence of HGV infection was significantly higher in chronic HCV patients (19%) versus blood donors (5%)  $P < 0.001$ . HGV viraemia was significantly more common in patients with mild liver disease (CH+CC) than in patients with severe liver disease (DC+HCC) (23.2% versus 13.6%)  $P < 0.05$ . No significant difference was detected between HGV-infected and non-infected patients regarding mean age, sex, liver biochemical tests, virologic markers and HCV serotype distribution. Decompensated cirrhosis was significantly less common in HGV coinfecting persons (5.2%), than in those with isolated HCV infection (26%)  $P < 0.01$ . Also the HCV RNA viral load in the former group was lower (median  $2.1 \times 10^5 \pm 0.4$ ) than in the latter group (median  $2.9 \times 10^5 \pm 0.5$ ) but the difference was statistically insignificant ( $P > 0.05$ ). Histopathologic examination of liver biopsy specimens by light and EM revealed no significant difference in the grade of periportal, portal and intralobular necroinflammation and in the stage of fibrosis. No virus particles or any characteristic morphological discrimination were detected between HCV patients with and without HGV infection. [Life Science Journal. 2006;3(1):9-17] (ISSN: 1097-8135).

**Keywords:** hepatitis G virus; clinical; virological and ultrastructural aspects; hepatitis G virus infection on chronic hepatitis C Egyptian patients

## 1 Introduction

Hepatitis G virus (HGV) and GB virus type C (GBV-C) were independently discovered as putative blood-borne causative viruses of non-A-E hepatitis (Simons, 1995; Linnen, 1996). Molecular characterization demonstrated that they were different isolates of the same virus and they represent a new genus in the family Flaviviridae (Alter, 1996). HGV is not only phylogenetically closely related to hepatitis C virus (HCV), but it also has similar modes of transmission. It may infect the liv-

er as an independent virus or as a coinfection with HCV (Abraham, 2003; Lisukova, 2003). It appears that it is even more efficiently transmitted by sexual and vertical exposure than is HCV (Stapleton, 2003).

Evidence of HGV infection is also found among people who have no acknowledged risk of blood-borne infection. The distribution of the virus varies geographically and information worldwide is incomplete. Infection rates among eligible blood donors range from 1% - 5% in developed countries (Chams, 2003)

Although HGV has been initially associated with fulminant hepatic failure, acute and chronic hepatitis (Abraham, 2003; Yoshida, 1995), numerous studies failed to demonstrate its direct involvement in induction of significant hepatitis (Alter, 1997).

Coinfection with more than one virus may contribute to changes in the evolution of liver disease either negatively or favorably (Chams, 2003). The influence of HGV infection on HCV chronic liver disease is controversial. There was a growing consensus that coinfection has no apparent effect on the course or severity of chronic HCV liver disease and it does not alter the pathogenicity or replication of the virus (Shang, 2000; Petrova, 1999).

However, other investigators showed that acute and chronic hepatitis could be induced by HGV, and that coinfection worsens the liver histology of patients with chronic HCV (Moriyama, 2000; Xu, 2001).

Recent studies on the pathogenesis of HGV in human immunodeficiency virus (HIV)-infected patients yielded surprising results. Several studies found that HGV coinfection in HIV-positive people was associated with either a decrease in mortality or improved clinical outcome, compared to those without HGV infection (Stapleton, 2003; Williams, 2004). Moreover, it has been demonstrated in an *in vitro* model of HGV and HIV coinfection, using interleukin-2 stimulated human peripheral blood mononuclear cells (PBMCs), that GBV-C/HGV led to inhibition of HIV replication by inducing cellular chemokines (RANTES, MIP-1, SDF-1) that inhibit HIV, and also by down-regulating the cellular expression of the HIV co-receptors CCR5 and CXCR4 (Xiang, 2005).

These findings plus the capability of both HCV and HGV to replicate in PBMCs (Stapleton, 2003; Mazur, 2001), raise the speculation of possible viral interference and claims re-evaluation of the effect of interaction of two closely-related viruses on their host. So the aim of the study was to determine the prevalence of HGV infection in chronic hepatitis C patients and to clarify its clinical, virological and histopathological impact at the ultrastructural level on chronic HCV liver disease.

## 2 Patients and Methods

Two groups were enrolled in the study: Group 1 included 100 chronic hepatitis C patients attending the Gastroenterology Unit of Theodore Bilharz Research Institute (TBRI) Giza, Egypt, during the period from August, 2002 until September, 2003. Chronic hepatitis was diagnosed on the basis of: el-

evated serum ALT and AST for more than 6 months, ultrasonographic and/or histopathologic evidence of chronic hepatitis or cirrhosis. Hepatitis C was diagnosed by HCV antibody and/or HCV RNA testing. Group 2 included 80 healthy, age-matched, volunteer blood donors from the Blood Bank of TBRI.

Patients' characteristics including age, sex, clinical examination with special stress on manifestations and decompensation of liver disease were recorded. Patients were examined by ultrasonography and upper endoscopy.

Liver biopsy specimens were obtained from 25 patients who were feasible for biopsy and were processed for light and electron microscopic (EM) histopathologic examination.

Patients were further classified based on clinical data and available histopathology into 4 subgroups: chronic hepatitis (CH = 45); compensated cirrhosis (CC = 11); decompensated cirrhosis (DC = 22); and hepatocellular carcinoma (HCC = 22). Patients with clinical features of portal hypertension were assumed to have cirrhosis even if a liver biopsy was not done. Decompensated cirrhosis was defined as the presence of complications related to portal hypertension such as ascites, encephalopathy, decreased synthetic functions reflected by decreased albumin concentration and prolonged prothrombin time. HCC was either based on histopathological diagnosis or on the presence of a hepatic focal lesion by imaging associated with elevated alpha-fetoprotein.

Blood samples collected from patients and blood donors were subjected to: complete blood picture, serum bilirubin, ALT, AST, alkaline phosphatase, albumin, globulins, prothrombin time and concentration and alpha-fetoprotein.

Serum samples were stored in several aliquots at  $-70^{\circ}\text{C}$  until tested for viral markers of HCV, HBV and HGV.

### 2.1 Serologic assays

Assay for HCV antibody was performed by third generation enzyme immunoassay (EIA) (anti-HCV version 4 Murex-Biotech Ltd., UK). Serum HBs antigen (HBsAg) and HBc antibody (HBcAb) were tested by EIA (Murex version 3, Murex-Biotech Ltd., UK). HCV serotyping was performed by Murex HCV serotyping 1-6 EIA (Murex-Biotech Ltd., UK).

### 2.2 Detection of HCV RNA viral load by PCR

RNA extraction was performed by the acid guanidinium thiocyanate and phenol-chloroform single-step method (Chomezynski, 1987). Nested RT-PCR was used for quantitation of HCV RNA viral load using 2 sets of primers within the 5' non-

coding region. Amplification products were analyzed using 2% agarose gel electrophoresis (Van Doorn, 1994).

### 2.3 Detection of HGV RNA by PCR

RNA extraction was performed by the acid guanidinium thiocyanate and phenol-chloroform method (Chomezynski, 1987). Reverse transcriptase reaction and PCR were carried out using PTC-200 from MJ Research Inc. according to Schauder, (1995). All experiments included HGV positive and negative control. The oligonucleotide primer pairs used were: 5'-CGG CCA GGT GGA TG-3' (position 100 sense), 5'-CGA CGA GCC TGA CGT CGGG-3' (position 285 antisense).

The RT reaction and PCR were performed in 100 µL reaction volume containing 50 µL RNA dilution, 2.5U recombinant Taq DNA polymerase (Promega, Madison, WI), 3U avian myeloblastosis virus (AMV) reverse transcriptase (Promega, Madison, WI), 1.5U RNase, 100 µM each of 4 deoxy-ribonucleoside triphosphate, 0.2 mM each of primer, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.01% gelatin and 10 mM Tris-HCl pH 8.3. The RT reaction was performed at 42°C for 45 min followed by 5 min at 94°C. The PCR was subjected for 30 cycles each of 94°C (denaturing) for 1 min, 55°C (annealing) for 1 min and 72°C (extension) for 1 min and finally one cycle at 72°C for 10 min. Amplification products were analyzed using 2% agarose gel electrophoresis according to the method described by Van Doorn (1994).

### 2.4 Light and electron microscopic processing of liver biopsy specimens

For light microscopic examination, liver biopsy specimens were fixed in 10% buffered formalin and processed for the preparation of 4 µm thick paraffin sections that were stained by haematoxylin and eosin and Masson trichrome stains. The specimens were graded and staged semiquantitatively from 0–4 according to Desmet et al (1994), assuming that grade 1 activity is scored (1–3), grade 2 (4–8), grade 3 (9–12) and grade 4 (13–18) of the Knodell score Knodell et al (1981).

For EM examination, a small piece of liver biopsy about 3 mm<sup>3</sup> was divided into 1 mm<sup>3</sup> pieces and fixed in 4% glutaraldehyde buffered with 0.2 M sodium cacodylate, washed twice in equal volumes of sodium cacodylate 0.2 M and sucrose 0.4 M at 4 °C, postfixed in 2% osmium tetroxide for 1 hour then washed in distilled water and dehydrated in ascending alcohol concentration, embedded in Epon and polymerized at 60°C for 48 hours. Semithin sections stained with methylene blue azur II and ultrathin sections double stained with uranyl acetate and lead citrate were performed using an

Ultracut R ultramicrotome. Examination of the stained ultrathin sections was done using a Philips EM 208 S electron microscope.

### 2.5 Statistical Analysis

Analyses were conducted using Student's t-test and test of proportion. The level of statistical significance was set at  $P = 0.05$ . Histological variables were analysed according to Wilcoxon variance analysis.

## 3 Results

The prevalence rate of HGV infection was significantly higher in chronic HCV patients (19%) versus blood donors (5%)  $P < 0.001$ . Four of the 19 HGV-positive patients were also coinfecting with HBV. The overall prevalence rate of HGV infection was 12.7% (23/180). Among the 23 HGV-positive cases, isolated HGV infection was detected in 2 (8.7%) while coinfection with HCV was found in 21 (91.3%)  $P < 0.0001$  (Table 1).

The distribution of HGV infection in chronic HCV patients according to severity of liver disease is shown in Table 2. CH and CC were categorized as mild liver disease, while DC and HCC were considered as severe liver disease. HGV viraemia was significantly more common among patients with mild liver disease (23.2%) than among those with severe liver disease (13.6%)  $P < 0.05$ .

Demographic, virologic and clinical data of chronic HCV patients were compared according to the presence or absence of HGV infection (Table 3). There was no significant difference between HGV-infected and non-infected patients regarding mean age, sex distribution, ALT serum levels, virologic markers, or HCV serotype distribution. HCV RNA viral load was lower in patients with than without HGV infection, however the difference was statistically insignificant ( $P > 0.05$ ).

Analysis of disease categories denoting severity of liver disease showed that decompensated cirrhosis was significantly less common in HGV coinfecting persons (5.2%), than in those with isolated HCV infection (26%)  $P < 0.01$ . HCV serotyping showed that serotype 4 was the most prevalent (96%), 71% of patients had single type 4 and 25% had mixed serotypes (4 + 1/4 + 2), while 4% had serotype 1.

Liver histopathologic examination of 25 cases (8 HGV positive and 17 HGV negative) by light microscopy disclosed the presence of 22 (88%) cases of chronic hepatitis and 3 (12%) well differentiated cases of HCC of the trabecular pattern. Grading and staging of chronic hepatitis liver biopsies from HGV coinfecting patients compared to isolated

HCV infection showed no statistically significant difference in periportal, portal and intralobular necroinflammation and in the stage of fibrosis

(Table 4). Also there was no difference between the two groups regarding the presence of steatosis, bile duct damage and lymphoid aggregations.

**Table 1.** Prevalence rates of HGV infection in chronic hepatitis C patients and blood donors: isolated infection, coinfection with HCV

Group	No Tested	HGV RNA Positive N (%)	Isolated HGV infection	Coinfection of HGV&HCV
Group1(Chronic HCV)	100	19(19)**	—	19
Group 2(Blood donors)	80	4 (5)	2	2
Total N (%)	180	23(12.7)	2(8.7)	21(91.3)***

\*  $P < 0.001$ ; HGV prevalence in group 1 versus group 2

\*\*  $P < 0.0001$ ; HGV coinfection with HCV versus isolated HGV infection

**Table 2.** Distribution of HGV-positive viraemia in chronic HCV patients according to severity of liver disease

Character	Mild Liver Disease (n = 56)		Severe Liver Disease (n = 44)	
	CH (45)	CC (11)	DC (22)	HCC (22)
HGV-RNA positive	10	3	1	5
Total N (%)	13 (23.2)*		6 (13.6)	

\*  $P < 0.05$  for mild versus severe liver disease in HGV/HCV coinfection.

CH:Chronic hepatitis; CC:Compensated cirrhosis; DC:Decompensated cirrhosis; HCC:Hepatocellular carcinoma.

**Table 3.** Demographic, virological and clinical data of chronic hepatitis C patients according to the presence or absence of HGV RNA

Characteristic	HGV + ve (n = 19)		HGV - ve (n = 81)		P value
Age (mean(SD))	44 ± 8.5		43 ± 6.2		NS
Sex (M:F)	13:6		63:18		NS
Mean ALT level (IU/L)	60 ± 31		65 ± 34		NS
<b>Virological features</b>					
HCV RNA (copies/ml)					
Mean × 10 <sup>5</sup> (SD)	19	2.1 (0.4)	81	2.9(0.5)	NS
HCV serotype 4	13	68.4%	58	71.6%	NS
Mixed serotype (1 + 4/2 + 4)	5	26.3%	20	24.6%	NS
HCV serotype1	1	5.3%	3	3.7%	NS
HBs antigen + ve	4	21.0%	0	—	—
HBc antibody + ve	11	57.9%	38	46.9%	NS
<b>Disease categories</b>					
Chronic hepatitis	10	52.6%	35	43.2%	NS
Compensated cirrhosis	3	15.7%	8	9.9%	NS
Decompenstated cirrhosis	1	5.2%	21	26.0%	0.01
Hepatocellular carcinoma	5	26.3%	17	20.9%	NS

**Table 4.** Grading and staging of chronic hepatitis liver biopsies in HGV coinfection compared to isolated HCV infection

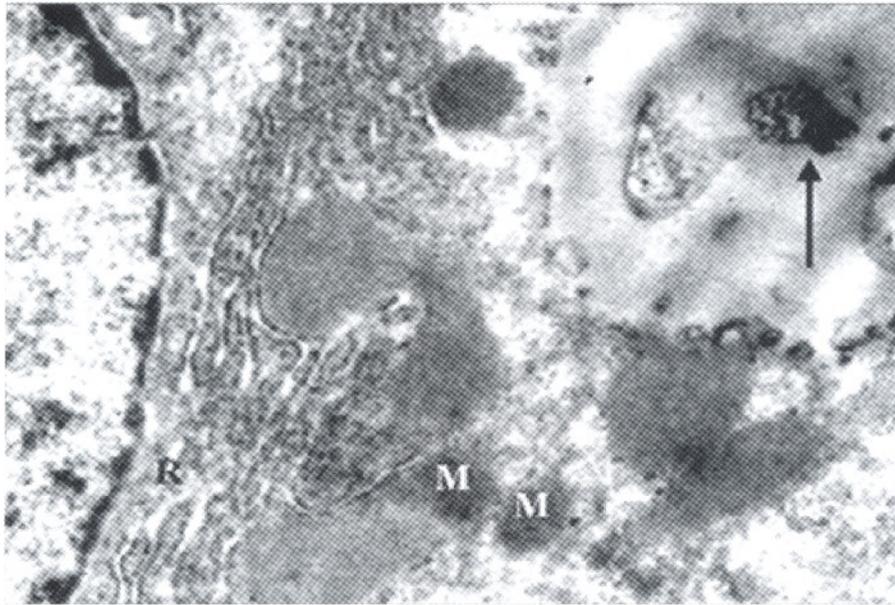
Characteristic	1	2	3	4	Mean ± SD	P value
<b>Grading</b>						
HGV Coinfection	0	4	2	2	9.75 ± 4.65	NS
HCV	0	7	4	3	9.5 ± 4.128	
<b>Staging</b>						
HGV Coinfection	3	2	1	2	3.25 ± 0.70	NS
HCV	6	3	3	2	3.14 ± 0.663	

EM examination of the ultrathin liver sections revealed no virus or virus-like particles in the nuclei

or in the cytoplasm of hepatocytes. There was evident proliferation of the smooth and rough endo-

plasmic reticulum associated with distended cisternae, as well as electron dense opaque mitochondria of different sizes (Figure 1). Moderate sized collagen fibrils were observed intercellularly and in perisinusoidal spaces and collagen like fibrils were seen extended in the cytoplasm between the organelles of the hepatocytes. Extravasation of RBCs together with infiltration by macrophages and lymphocytes were disclosed between hepatocytes. Many large and moderate-sized fat locules were observed

filling the hepatocytes, pushing the nuclei aside and compressing the neighbouring cells (Figures 2,3). Apoptotic cells were detectable showing either peripheral chromatin condensation beneath the nuclear membrane or dense chromatin aggregates in the nucleus, together with condensed cytoplasmic organelles. No characteristic morphological discrimination could be found between HCV infected specimens and those coinfecting with HGV (Figures 1 – 3).



**Figure 1.** Electronmicrograph from a case of HCV showing a hepatocyte with proliferated endoplasmic reticulum (R) and opaque mitochondria (M) (A degenerated hepatocyte with apoptotic nucleus (arrow) is observed) ( $\times 10\ 000$ )

#### 4 Discussion

To determine the prevalence of HGV viraemia and its impact on chronic HCV patients in our region, we studied 100 chronic HCV patients and 80 volunteer blood donors. The prevalence of HGV in chronic hepatitis C patients was significantly higher (19%) compared to blood donors (5%). This was comparative with the infection rates reported in researches on Egyptian HCV patients where prevalence rates were 18.5%, 14% and 11.5% (Heiba, 1999; El-Zayadi, 1999; Hassoba, 1997). These rates were also not markedly different from other studies from different geographic areas that reported HGV RNA viraemia among chronic HCV patients in the range from 17% to 23% (Martinot, 1996; Wang, 1998; Handajani, 2000; Li, 2001; Bjorkman, 2001).

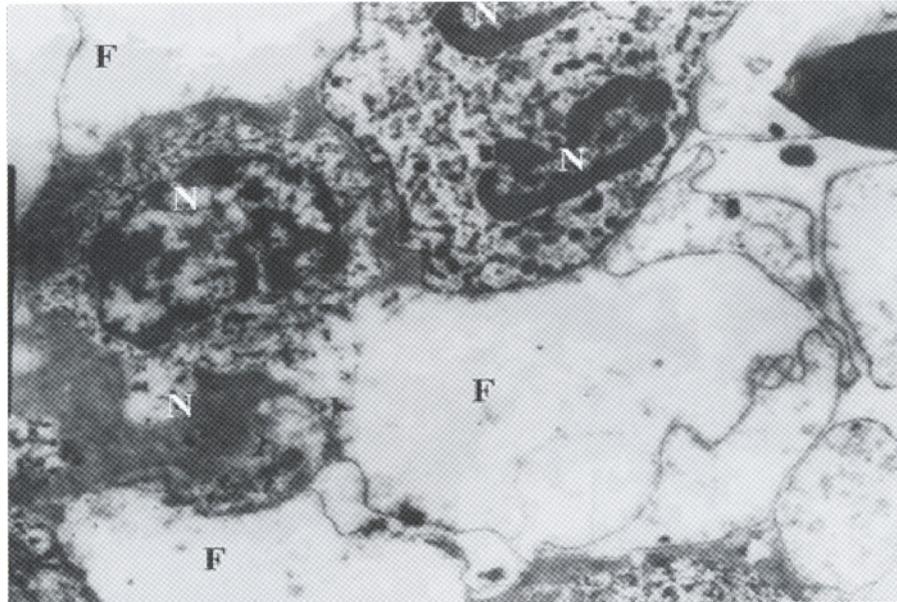
HGV infection is closely associated with HCV infection both in areas of endemicity and in areas of

no endemicity for HCV (Tanaka, 1998). This was confirmed in this study as among 23 HGV-positive cases, the association of HCV and HGV infections versus isolated HGV infection was 91.3% versus 8.7%. This probably reflects common exposure and transmission patterns rather than an interdependent relation. Moreover, this high association may be attributed to the reduced clearance of HGV viraemia among HCV-infected patients, as most immunocompetent individuals who become infected with HGV clear the virus, while fewer than 25% of HCV-infected patients spontaneously clear infection (Stapleton, 2004).

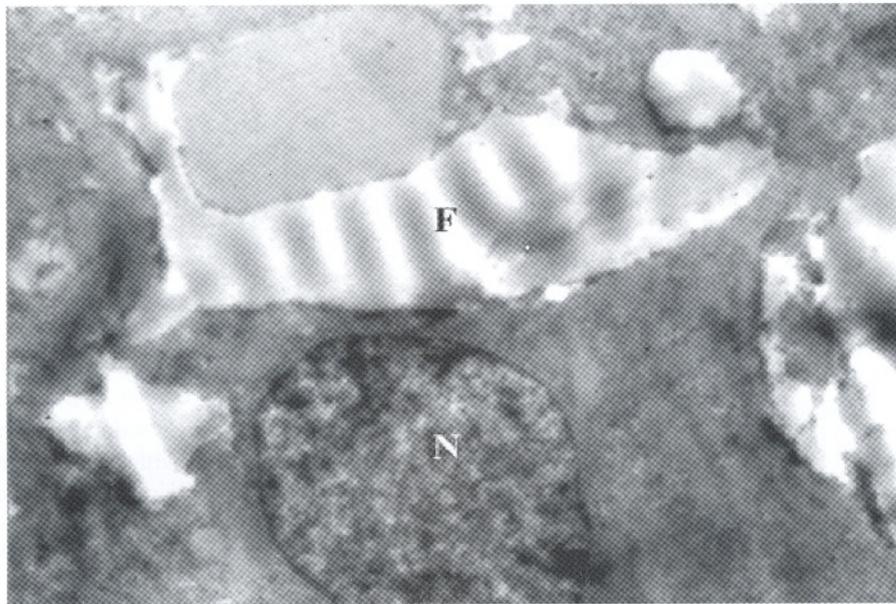
The rate of HGV viraemia in blood donors detected in this study (5%), was consistent with that reported in epidemiological studies of the general population and blood donors in Africa and South America (5% – 10%) (Desmet, 1994). In contrast it was lower than that reported among 82 apparently healthy, Egyptian blood donors (12%) (Hassoba, 1997). This difference could be due to

variabilities in the characteristics of population of blood donors as the age group, social standard and special habits. In addition, there is evidence that HGV can be transmitted intrafamilially, by non-

parenteral routes as saliva and semen, by accupuncture or sharp objects and it has an age-related prevalence (Semprini, 1998; Chen, 1999; Seifreid, 2004).



**Figure 2.** Electronmicrograph from a case of HCV coinfectd with HGV showing fat vacuoles (F) compressing 2 apoptotic binucleated (N) hepatocytes ( $\times 5\ 000$ )



**Figure 3.** Electronmicrograph from a case of HCV showing fat vacuoles replacing the cytoplasmic organelles of a hepatocyte ( $\times 7\ 000$ )

Egypt is a country known for its high seroprevalence of HCV (Hassan, 2001). The common exposure patterns of HCV and HGV may account for the overall high HGV viraemia among Egyptian

blood donors that exceeds that reported from Japan (0.8%), USA (1.7%), China (2%), Indonesia (2.7%) and Korea (1.8%) (Alter, 1997; Wang, 1998; Handajani, 2000; Li, 2001; Jeon, 2003).

Numerous studies performed on HGV led to the exclusion of its role as a significant aetiological agent of hepatitis. However coinfections with other viruses may contribute to changes in the progress and severity of liver disease patients (Chams, 2003). From the point of view of some authors, several facts must be considered before dismissing the possible pathogenic role for HGV in HCV chronic liver disease. First, the lack of detectable core protein, which may explain the absence of excess inflammation in HCV coinfecting patients. Second, the presence of a highly conserved E2 region and formation of an anti-HGV-E2 antibody that is indicative of an effective immune response in the host leading to clearance of viral RNA. Third, HGV isolates from widely separated geographic areas have been thought to be highly conserved, until the recent description of 5 major genotypes and 5 subclasses of genotype 1, which suggests the possibility of a relationship between specific genotypes and pathogenicity. Fourth, the virus occurs and appears to replicate *in vitro* in PBMCs and not in hepatocytes. It also inhibits replication of HIV in coinfecting cell cultures (Williams, 2004; Hattori, 2003; Liu, 2003; George, 2003).

In this study, analysis of HGV-positive versus negative patients in chronic HCV patients showed no significant difference between patient groups regarding age, sex, virologic markers or serotype distribution. Furthermore, no association was found between the presence of HGV viraemia and the severity of liver disease in terms of serum ALT levels or histopathologic examination in both severity of inflammation and degree of fibrosis. These findings agree with other workers on hepatitis C patients who also failed to detect a significant effect of coinfection with HGV on the indices of liver disease including biochemical, histologic and response to interferon therapy (Heiba, 1999; Wang, 1998; Slimane, 2000; Par, 2004).

However, our finding that HGV infection was significantly less prevalent in patients with severe disease (DC and HCC), than in those with mild liver disease (CH and CC) is noteworthy. It does not only deny the role of HGV in aggravating liver disease, but also raises the question of a possible beneficial role of HGV in chronic HCV patients through viral reciprocal inhibition. Because both HCV and HGV are capable of replicating in lymphocytes (Stapleton, 2003; Mazur, 2001), it is reasonable to speculate that viral interference might occur. In this study we found that the concentration of HCV RNA was lower in patients coinfecting with HCV and HGV than in those with HCV infection only. Although the difference was not sta-

tistically significant, it is suggestive of the possible reciprocal relationship between the two viruses. A reverse relation was found to exist between HCV RNA concentration and HGV infection in a study on chronic HCV patients coinfecting with HGV. HCV copy numbers in patients with HGV coinfection was significantly lower than that in patients without HGV (Yan, 2000). In contrast, such relation was not found in other studies (Chu, 2001). Further studies on viral load quantitation by more accurate methods, as real time PCR, are required to clarify this issue.

Also in favor of the possible beneficial role of HGV is the recent finding of the inhibitory effect of HGV on replication of HIV in the *in-vitro* models of coinfection. It has been demonstrated that this is achieved by the down-regulation of expression of major HIV coreceptors, by the increase in specific chemokines and by alteration in the Th cytokine production by PBMCs (Mazur, 2001). It was found that HGV may help maintain cytokine profiles associated with long-term non progression among HIV-positive patients and that HGV coinfection correlated with an intact Th1 cytokine profile among those patients (Nunnari, 2003). Since Th cytokines are involved in the pathogenesis of disease, so HGV may potentially influence other comorbid infections in a beneficial mode (Stapleton, 2004).

Results of histopathologic examination in this study revealed no difference in the inflammatory scores or fibrosis stage that could be attributed to HGV coinfection. These findings were consistent with other authors who showed that coinfection did not affect the liver lesion nor induced a more aggressive disease (Shang, 2000; Strauss, 2002; Petrik, 1998; Goldstein, 1997). Regarding the presence of lymphoid aggregation, steatosis and bile duct damage, the lesions mostly encountered in HCV infection, we did not detect a significant difference between the two groups, although other authors observed more severe bile duct damage in HGV coinfecting persons (Xu, 2001; Chu, 2001). EM examination also confirmed that there were no detectable specific ultrastructural morphological features in coinfecting patients. Also, no virus particles were detected in hepatocytes of the coinfecting patients. This is supportive with the suggestion that HGV may be a non hepatotropic virus. The replication site of HGV *in vivo* is still unknown. The virus appears to be primarily a lymphotropic virus rather than hepatotropic (Tucker, 2000). Evidence denoting that the negative strand of HGV could not be detected in the liver, suggest that the virus does not replicate in the liver (Laras, 1999).

In contrast, HGV replication was identified in the cytoplasm of hepatocytes of 10 donor livers. It was detected by in situ hybridization with HGV RNA probes and immunologic staining for HGV-E2 protein. However there was no evidence of liver disease in those HGV infected healthy liver donors despite viral replication in hepatocytes (Halasz, 2000).

Generally speaking, the EM studies on HGV infections are very few in the literature. In a study by Xu et al (2000), they observed that the ultrastructural changes in one case of acute single HGV infection were: shrinkage of liver cells, extension of rough endoplasmic reticulum, proliferation of collagen fibrils but they did not comment on the presence or absence of virus particles.

## 5 Conclusion

HGV infection is common in chronic HCV patients. It does not appear to aggravate the liver disease at the histopathologic and the ultrastructural levels, but the finding that it was less prevalent in clinically severe liver disease than in those with mild disease, plus the lower HCV RNA concentration in coinfecting patients raise the speculation of a possible beneficial role. But much more *in-vitro* and *in-vivo* studies are required to answer the question related to interaction of both viruses.

## Acknowledgments

This work was funded by Hepatitis Research Project number 74D of the Microbiology Department of TBRI.

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*Received October 25, 2005*