Original Article

Hepatitis G virus in Saudi blood donors and chronic hepatitis B and C patients

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Abstract

Introduction: Screening blood donors for blood-borne pathogens is very critical for the recipient's safety. Similar to hepatitis B and C infections, hepatitis G infection is transmitted through contaminated blood and causes acute and chronic hepatitis. Previous reports have shown that the prevalence of hepatitis G virus (HGV) RNA among healthy Saudi donors was 1%-2%. However, the exposure rate of this virus has never been studied. We hypothesized that the prevalence of HGV infection may have changed overtime due to socio-economic and environmental factors. Since hepatitis B and C infections are endemic in Saudi Arabia, we investigated the exposure rate of HGV infection in healthy donors and chronically infected hepatitis B and C patients.

Methodology: A prospective study was done on healthy donors and patients with chronic HBV and HCV infections. Hepatitis B and C viral loads were measured by real-time polymerase chain reaction. HGV exposure rate was evaluated by detection of HGV antibodies.

Results: Analysis of samples from healthy donors (n = 210), chronic HBV+ patients (n = 169), and chronic HCV+ patients (n = 105) showed that nine samples (4.3%), seven samples (4.1%), and four samples (3.8%) were positive for HGV antibodies, respectively. The non-significant difference in the exposure rates of HGV between the study groups may indicate that HGV infection occurs independent of HBV or HCV infections.

Conclusions: We showed for the first time that the exposure rate of HGV infection among the Saudi population is 4.3%, and we recommend HGV screening for all blood donors.

Key words: blood donors; HGV; HBV; HCV; Saudi Arabia

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Introduction

Hepatitis is an endemic infection in many countries around the world including countries in the Middle East [1]. There are several viruses that target primarily the liver and cause hepatitis. These include viral hepatitis type A, B, C, D, E, and G [2]. Hepatitis A and E viruses are transmitted mainly by fecal-oral routes and usually cause acute enteric hepatitis. On the other hand, hepatitis B, C, D, and G viruses are transmitted parentally, and thus they can be transmitted sexually or through contaminated blood, and may cause acute and chronic hepatitis [3,4]. Hepatitis B and C viruses are among the most studied viruses, and they were the predominant types of viral hepatitis in Saudi Arabia between 2000 and 2005, with HBV comprising 49.3% and HCV comprising 40.7% of the identified cases [5]. On the other hand, very little information is available about hepatitis G virus (HGV) infection and pathogenesis. Also, its prevalence in healthy individuals and chronically infected hepatitis patients has not been comprehensively studied.

The hepatitis G virus, which is also known as GB virus C (GBV-C), was initially identified in 1995 and classified under the *Flaviviridae* family [6-9]. It is an enveloped virus with a 10kb positive-sense single-stranded RNA genome. Although HCV and HGV are structurally similar, it appears that HGV replicates more efficiently in white blood cells [4,9,10]. Studies have shown that HGV infection can occur as a single infection or in combination with other infections such as HCV or HIV [9,11,12]. It is unclear whether HGV has any role in the chronicity of hepatitis B or C infections. Therefore, HGV infection may contribute to the progression of chronic infections or to the development of drug resistance. Some reports have revealed a role of HGV infection in the pathogenesis of rare non-liver diseases such as aplastic anaemia [13,14]. The prevalence of HGV appears to vary between regions. It has been shown that the prevalence

of HGV viremia among healthy donors was high in Africa (17.2%) as compared with Asia (3.4%) or Europe (4.5%) [1]. Other studies have reported that the prevalence of HGV viremia in healthy Saudi donors was between 1%-2%, but this percentage was relatively higher in dialysis patients (5.5%) and cryptogenic hepatitis patients (25%) [15,16]. However, the exposure rate of HGV infection in the Saudi population has never been investigated. Several reports have shown that the prevalence of viral hepatitis infection has changed over time due to multiple factors, including economic and environmental factors [5,17]. For instance, reports from Saudi Arabia have shown that the prevalence of HAV and HCV infections was reduced from 53% and 4.7% to 18.1% and 0.65%, respectively, over a period of 20 years. Further, the prevalence of HBV carriers dropped from 6.7% to 0.3% in 18 years after the subunit HBV vaccine was introduced in 1989 [5,17]. Thus, we hypothesized that the prevalence of HGV infection in Saudi Arabia may have changed over the years.

Screening blood donors for blood-borne pathogens is very critical for the recipient's safety. Since HGV infection is a disease that can be transmitted via contaminated blood, our objective was to evaluate the exposure rate of the hepatitis G virus among healthy donors and chronically infected hepatitis B and C patients by enzyme-linked immunosorbent assay (ELISA).

Methodology

Study groups

A total of 484 participants were enrolled in this study. All serum samples were obtained from the virology and molecular biology laboratories from routine blood screening or follow-up patients at King Khalid University Hospital (KKUH) and were categorized into three groups. The first group included 210 serum samples that were collected randomly from healthy blood donors. These samples were negative for HBsAg, anti-HBc Ab, anti-HCV Ab, HIV Ag/Ab, and anti-HTLV I & II Ab. The second group included 169 serum samples that were collected from patients chronically infected with the hepatitis B virus. The third group included 105 serum samples that were collected from patients chronically infected with the hepatitis C virus. The patients' characteristics were summarized in Table 1. This study was approved by and performed according to the guidelines of KKUH and the College of Medicine Institutional Review Board (IRB) committee. The hospital provides primary and secondary care services for Saudi patients from the northern Riyadh area. It also provides tertiary care services to all Saudi citizens on referral bases.

Assessment of Hepatitis B and C viral loads and liver enzymes

The HBV and HCV viral loads were measured from serum samples using the COBAS TaqMan analyzer and COBAS AmpliPrep/COBAS TaqMan HBV Quantities test, version 2.0 (Roche Diagnostics, Germany), and COBAS AmpliPrep/COBAS TaqMan HCV Quantities test, version 2.0, respectively (Roche Diagnostics, Mannheim, Germany). Liver enzymes including alanine transaminse (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) were tested within one month of sample collection, and results were obtained from medical records. The levels of these enzymes were measured by Dimension RxL Max instrument (Siemens, New York, USA) and the normal range for ALT, AST, ALP were 20-65 U/L, 12-37 U/L, and 50-136 U/L, respectively.

Detection of anti-HGV antibodies by enzyme-linked immunosorbent assay (ELISA)

Serum samples from healthy donors and chronically infected hepatitis patients were assayed for HGV exposure by ELISA (Diagnostic Automation, CA, USA), following the manufacturer's instructions. The test is designed to detect HGV-specific antibodies in sera. Briefly, diluted samples or controls were loaded into a 96-well plate pre-coated with a recombinant HGV-specific antigen. The plate was then incubated for 30 minutes at 37°C to allow for the formation of the Ag-Ab complex. The plate was washed, horseradish-peroxidase conjugate was added, and the plate was incubated for 30 minutes at 37°C. After that, the washing step was carried out and a substrate solution (chromogen A and B) was added for detection. Finally, the reaction was stopped using H_2SO_4 and the colorimetric signal was measured by absorbance at 630 nm using a spectrophotometer.

Statistical analysis

Data were collected and entered into a Microsoft Office Excel file for statistical and descriptive analysis. The percentage and the mean values with standard division were applied to determine the significance and were used where applicable. The significance level was established at $p < 0.05$.

Results

In this study, among 210 serum samples from healthy male donors, nine samples (4.3%) were positive for HGV antibodies (Table 1). There was no significant difference between not exposed to HGV and exposed to HGV blood donors with respect to age. In chronic HBV-infected patients, seven samples (4.1%) out of 169 serum samples were HGV-antibodies positive; Five (71.4%) patients were males and two (28.6%) were females (Table 1,2). It also appeared that the rate of HBV infection among those not exposed to HGV was higher in males (n = 107; 63.3%) than in females (n = 62; 36.7%) (Table 1). HBV-infected patients exposed to HGV were significantly younger in age compared to those with HBV infection not exposed to HGV (33.8 ± 3.6 *vs.* 43 ± 12.5, p = 0.0001) (Table 1). Although the viral load for HBV in the patients exposed to HGV was relatively high in one male patient in comparison with six other patients in the same group (Table 2), the viral load of HBV among HBV-infected patients exposed to HGV was significantly lower compared to those with HBV infection not exposed to HGV ($0.68 \times 10^6 \pm 1.78 \times 10^6$ *vs*. $7.32 \times 10^6 \pm 31.63 \times 10^6$ IU/mL, p = 0.01) (Table 1). In the liver enzymes (ALT, AST, ALP), at least one was relatively high in three HBV-infected patients exposed to HGV, compared with four patients in the same group (Table 2). However, there was no significant difference in the levels of liver enzymes between HBV-infected patients exposed to HGV and non-exposed patients (Table 1). In chronic HCV-infected patients, four (3.8%) samples out of 105 serum samples were positive for HGV antibodies. Only one (25%) patient was male; three (75%) patients

Table 1. Demographic and laboratory characteristics of patient study groups

Populations	HGV- (non-exposed)	HGV+ (exposed)
Healthy donors	201/210	9/210 (4.3%)
Gender (M/F)	201/0	9/0
Age (mean ± SD)	27.2 ± 8.9 [18-54]	29 ± 10.1 [19-45]
HBV+	162/169	7/169 (4.1%)
Gender (M/F)	107/62	5/2
Age (mean ± SD)	43 ± 12.5***	33.75 ± 3.6***
Mean VL (IU/mL) ± SD	$7.32x10^6 \pm 31.63x10^6$*	$0.68x10^6 \pm 1.78x10^6$*
Mean ALT (U/L) ± SD	57.6 ± 58.4	128 ± 189.2
Mean AST (U/L) ± SD	34.7 ± 76.1	138.7 ± 306.7
Mean ALP (U/L) ± SD	110.6 ± 37.3	113 ± 45.3
HCV+	101/105	4/105 (3.8%)
Gender (M/F)	51/54	1/3
Age (mean ± SD)	49.9 ± 13.9	48 ± 17.1
Mean VL (IU/mL) ± SD	$1.65x10^6 \pm 3.11x10^6$**	$0.35x10^6 \pm 0.34x10^6$**
Mean ALT (U/L) ± SD	69.1 ± 42.3	85.7 ± 29.6
Mean AST (U/L) ± SD	42.3 ± 25.7	63.5 ± 26.7
Mean ALP (U/L) ± SD	129.6 ± 55.5	130.5 ± 24.6

* p = 0.01 (statistically significant)
** p = 0.001 (statistically significant)
*** p = 0.0001 (statistically significant)

Table 2. Clinical details of HBV- and HCV-infected patients exposed to HGV

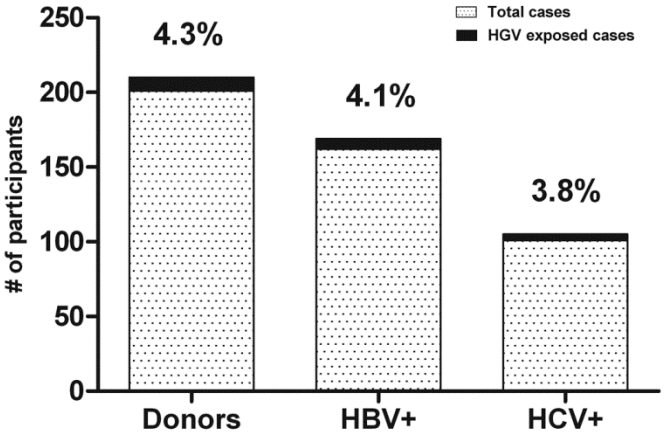
Infection	Patient #	Sex	Age	VL (IU/mL)	ALT (U/L)	AST (U/L)	ALP (U/L)
HBV	P11	M	31	53	131	36	106
	P38	F	36	548	30	13	122
	P72	F	35	930	30	11	69
	P80	M	27	13,794	32	18	100
	P99	M	34	222	48	26	92
	P110	M	38	4,738,316	549	834	209
	P120	M	34	113	76	33	93
HCV	P7	M	34	453,594	114	57	138
	P57	F	58	101,665	108	103	113
	P79	F	33	793,369	55	47	162
	P87	F	67	60,741	66	47	109

were females (Table 1, 2). Among HCV-infected patients not exposed to HGV, there was no significant difference in the HCV infection rate between males and females (Table 1). Also, there was no significant difference between HCV-infected patients exposed to HGV and non-exposed patients with respect to age (Table 1). The viral load of HCV was significantly lower in HCV patients exposed to HGV compared to those patients with HCV infection not exposed to HGV ($0.35 \times 10^6 \pm 0.34 \times 10^6$ *vs.* $1.65 \times 10^6 \pm 3.11 \times 10^6$ IU/mL, $p = 0.001$) (Table 1). In the liver enzymes (ALT, AST, ALP), at least one was relatively high in all HCV patients exposed to HGV (Table 2). However, there were no significant differences in the levels of liver enzymes between HCV-infected patients exposed to HGV and non-exposed patients (Table 1).

Discussion

We revealed in this study for the first time that the prevalence of HGV infection among healthy Saudi donors, chronic hepatitis B patients, and chronic hepatitis C patients was 4.3%, 4.1%, and 3.8%, respectively (Figure 1). The prevalence rate was determined based on the detection of HGV antibodies in the serum, which measures the real exposure rate to HGV. Since the detection of HGV RNA or viremia does not necessarily reflect the actual exposure rate to the virus, the occurrence of HGV infection is perhaps more frequent than studies of the prevalence of HGV RNA would suggest [11]. Also, HGV infection in high-risk populations determined by the presence of viral RNA may underestimate the true level of past and present infection [18]. To date, very little work investigating the role of HGV in liver disease in the Middle East, especially in Saudi Arabia, has been done. In addition, this report was the first study to investigate the prevalence of HGV infection in different Saudi populations using a diagnostic CE-approved HGV Ab ELISA kit. Previous studies have shown that the prevalence was between 1%-2% among Saudi donors, but that rate was based on the detection of HGV RNA by PCR, which showed current infection [15,16]. Thus, regardless of the test used, it appears that the prevalence rate of HGV infection in healthy Saudi donors is somewhat close to the rates that have been found in countries such as Iran, Japan, Taiwan, United States, Canada, France, and Norway, which range from 1%-7% [1,19-21]. However, other countries such as South Africa, Egypt, China, and Sweden have shown the prevalence of HGV to range between 12.2% and 22% [1,19,20]; the reasons for this geographic discrepancy are still not known.

Figure 1: Exposure rate of HGV among the study groups



As mentioned, the HGV exposure rates among Saudi chronic hepatitis B and C patients were 4.1% and 3.8%, respectively. These results are surprisingly similar to the exposure rate in healthy donors (4.3%). In agreement with this result, two reports in which detection of anti-HGV antibodies was used to assess prevalence revealed no significant difference in the prevalence of HGV between Colombian HBV and HCV seropositive donors (5.06% *vs.* 3.2%, respectively) [20]. Few studies have shown that no significant difference exists in the prevalence of HGV between healthy donors and HBV seropositive patients, but a significant increase was observed in HCV seropositive patients [22,23]. However, other reports have revealed that, in comparison with healthy donors, there was a significant increase in the prevalence of HGV not only in patients with chronic hepatitis B and C or liver diseases, but also in high-risk groups including intravenous drug users and thalassemia and hemodialysis patients [3,15,16,18,19,22]. Such variations in the results could be explained by the different methodologies used to assess the prevalence of HGV including measuring HGV RNA or anti-HGV antibodies. Two studies in which HGV RNA and anti-HGV antibodies were both used to evaluate the prevalence of HGV showed that no significant difference in the prevalence of HGV between the control group and chronic viral hepatitis with anti-HGV antibodies existed, but that there was a clear significant difference with HGV RNA [18,24]. The non-significant difference in the HGV exposure rates between the healthy donors and the chronic hepatitis B and C patients in the present study may indicate that HGV is capable of independent transmission, and neither HBV nor HCV infection is a predisposing factor for HGV infection or vice versa.

Interestingly, we observed that the chronicity of HBV infection was more prevalent in males than females. In addition, HGV infection was more prevalent in younger individuals among the HBV-infected patients. These results were not observed in chronic HCV-infected patients. Consistent with these findings, it has been reported in Saudi Arabia that the prevalence ratio between males and females in HBV infection was 1.8:1, whereas in HCV infection it was 1.1:1 [5,17]. Furthermore, other investigators in Taiwan have shown the frequency of HBV or HCV coinfection with HGV occurred more in males than in females [23,24]. However, HBV coinfection with HGV occurred more in older patients than in younger ones [23], which could be related to the variations in the population average age between different geographic regions.

In this study, the viral load levels of HBV and HCV were significantly reduced in hepatitis B and C patients exposed to HGV compared to those patients with hepatitis B or C infection and not exposed to HGV. Conversely, no significant difference in the levels of liver enzymes was found between HBV- or HCV-infected patients who were exposed to HGV and those patients not exposed to HGV. One report showed reduced levels of the viral load of HBV or HCV in HGV coinfected patients compared to those with single HBV or HCV infections, but these findings were not statistically significant. On the other hand, the ALT level was significantly reduced only in HBV and HGV coinfected patients compared to those with a single HBV infection [23]. Other studies, however, have revealed no correlation between HGV infection and liver enzymes [22,24]. These variations in the results could be due to the small sample size tested, and therefore, our results should be confirmed with a larger number of patients.

Conclusions

The prevalence of HGV infection among the Saudi population is still within the range of initial and worldwide reports (4.3%). Despite the fact that there was no sufficient data to support the clinical manifestations and the liver disease caused by HGV, blood-borne pathogen transmission and recipients' safety must be considered, especially for those who require blood transfusions and suffer from low immunity or chronic diseases. Thus, we recommend the initiation of HGV screening for all blood donors.

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