Research Journal of Medical Sciences 10 (3): 91-96, 2016
ISSN: 1815-9346

The Status of Hepatitis G/C Virus among HIV Positive Patients and Effects of GBV-C on the Progress of HIV Infection

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Abstract: Individuals infected with the Human Immunodeficiency Virus (HIV) are often co-infected with other pathogens in particular hepatitis viruses. HCV and GBV-C are blood-borne viruses that are highly prevalent among HIV/AIDS patients. The aim of this study was to evaluate the presence of hepatitis G virus (GBV-C) among HIV positive patients and its effect on the progress of HIV disease. One hundred and fifty HIV infected patients (92 men and 58 women) were included in this study. Patients were categorized into four different high-risk groups: intravenous drug users (n = 48), hemophiliacs (n = 45), homosexuals (n = 32) and heterosexuals (n = 25). RNA was extracted from 100 μL of serum or plasma using RNX plus kit. Reverse Transcription Polymerase Chain Reaction (RT-PCR) and nested PCR were used to detect GBV-C-RNA and HCV-RNA. GBV-C-RNA and HCV-RNA were found in 26.66 and 30% of patients, respectively. The prevalence of GBV-C for intravenous drug users, hemophiliacs, homosexuals, heterosexuals those with history of kidney disease and patients with a history of liver disease was 31.25, 28.88, 21.88, 20, 28.58 and 25%, respectively. Data show that within 4 years, CD^{+4} cell counts increased from 398-502 cells/mm^3 while HIV-RNA load decreased to 2.1±0.7 log_{10} copies/mL and HGV-RNA load increased to 2.5±2.08 log_{10} copies/mL. The prevalence of GBV-C and HCV in HIV infection variy in different groups. Our study shows a significant relationship between co-infection with GBV-C and HIV since it slows the progression of HIV and liver diseases in 4 years after being infected.

Key words: Human Immunodeficiency Virus (HIV), GBV-C, HCV, cell counts, RT-PCR

INTRODUCTION

Human Immunodeficiency Virus (HIV) and hepatitis viruses are the most common chronic viral infections throughout the world. Patients infected with HIV are often co-infected with other pathogens, particularly hepatitis viruses. Among hepatitis viruses, HCV and HGV are more prevalent in certain populations such as Intravenous Drug Users (IDUs) (DL *et al*., 2005). As with HIV, these viruses can be transmitted efficiently through blood transfusion, sexual intercourse and parenteral routes. Available reports suggest that HGV and HCV are more prevalent in high-risk groups such as hemodialysis patients, hemophiliacs, intravenous drug abusers, homosexuals and heterosexuals (Leeratanapetch and Suseangrut, 2010; Karuru *et al*., 2005). These viruses are prevalent world wide in healthy populations such as blood donors and also in certain groups of patients such as HIV infected subjects. GB Virus C (GBV-C), formerly known as Hepatitis G Virus (HGV) can infect humans but does not cause disease. There have been reports that HIV patients co-infected with GBV-C can survive longer than those without GBV-C but they may face different outcomes (Stapleton *et al*., 2011; Jung *et al*., 2007). HGV and GBV-C are RNA viruses that were independently identified in 1995 (Simons *et al*., 1995). Although, GBV-C was initially thought to be associated with chronic hepatitis, extensive investigation failed to identify any association between this virus and any clinical symptoms (Alter, 1996; Reshetnya *et al*., 2008; Reshetnyak *et al*., 2008). However, the time interval between GBV-C infection and clearance of viremia (detection of GBV-C RNA in plasma) is not known. Approximately, 2% of healthy US blood donors are viremic with GBV-C and up to 13% of blood donors have antibodies to E2 protein, indicating possible prior infection (Muerhoff *et al*., 2003). Parenteral, sexua and vertical transmission of GBV-C have been documented. Because of the similarity in modes of transmission, individuals infected with HIV are often co-infected with GBV-C. The prevalence of GBV-C viremia

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in HIV patients ranges from 14-43% (George *et al*., 2006). The presence of HCV and GBVC/HGV in plasma can be detected by specific Reverse Transcriptase (RT) nested PCR. GBV-C appears to be lymphotropic and has been shown to replicate in vitro in peripheral blood mononuclear cells, CD^{4+}, CD^{8+} T lymphocytes and B lymphocytes (DL *et al*., 2005; Karuru *et al*., 2005). Epidemiological data indicate that HGV is transmitted predominantly through parenteral routes with a high seroprevalence among injection drug users although, sexual transmission has also been reported (Stapleton *et al*., 2011). However, though many people are infected with HGV worldwide, no clear association with a known disease state has been demonstrated (Mosam *et al*., 2007; Jung *et al*., 2007).

This study was performed in cellular and molecular gerash research center of shiraz university of medical sciences to determine the presence of GBV-C virus among HIV positive patients and evaluate its effects on the progress of HIV infection.

MATERIALS AND METHODS

Samples: One hundred and fifty plasma samples collected in 2007 and 2011 from HIV infected patients admitted to Shiraz Health Center were tested. HIV infection was diagnosed using two Enzyme-Linked Immunosorbent Assays (ELISAs) and confirmed using PCR. Patients were interviewed in detail about the duration and possible routes of transmission as well as risky behavior using standardized questionnaires. The sera obtained from the patients were aliquoted and stored at -70°C until being used.

HCV RNA detection: HCV RNA was extracted from 200 μL of serum using acid guanidine-phenol-chloroform method and stored at -70°C or directly used for synthesizing cDNA. RT-PCR products were further amplified using nested PCR. Nested PCR protocol was initiated with a first round at 95°C for 5 min followed by a second round of 35 cycles at 94°C for 40 sec, 64°C for 35 sec and 72°C for 40 sec and finalized with an extension at 72°C for 3 min.

HGV RNA detection: GBV-C RNA was reverse-transcribed. First, PCR amplification was performed according to the following conditions: denaturation at 95°C for 5 min followed by 25 cycles at 95°C for 50 sec, 55°C for 40 sec and 72°C for 50 sec. Second round cycling was carried out under the following conditions: denaturation at 95°C for 4 min followed by 35 cycles at 95°C for 40 sec, 64°C for 60 sec, 72°C for 40 sec and a final extension step at 72°C for 5 min. Primer pairs used were 5'-CGGCCAAAAGGTGGTGGATG3' (sense strand) and 5'-CACTGGTCCTTGTCAACTCG3' (antisense strand). In the second round they were 5'-GGTGATG ACAGGGTTGGTAG3' (sensestrand) an 5'GCCTATTGG TCAAGAGAGACAT' (anti-sense strand).

HGV and HCV serological analysis: Antibody against E2 glycoprotein of HGV (Anti-E2 Ab) was assayed in plasma samples using 3rd generation ELISA kit (DIAPRO, Italy and Dade Behring Murburg, Germany), according to manufacturer's instructions. Anti-HCV Antibody (HCVAb) was evaluated in studied plasma samples using ELISA according to manufacturer's instruction (DIAPRO, Italy).

HGV and HCV-RNA extraction and amplification: HGV and HCV-RNA genomes were extracted from plasma samples by RNX which is a kind of solution used in RNA extraction and the extraction procedure described previously. The quality of extraction technique was evaluated by spiking HGV and HCV-RNA in negative plasma samples. The existence of HGV and HCV-RNA genomes in samples was checked using an in-house HGV/HCV nested-RT-PCR protocol. Viral RNA was extracted from 140 mL plasma samples using QIAamp Viral RNA Mini kit (QIAGEN Inc., CA) according to manufacturer's instructions. The accuracy of the information related to all cases of this study was checked using hospital archives. This study was completed adhering to the ethical aspects form helsinki convention and was approved by Shiraz University of Medical Science Ethics Committee.

CD^{4+} T cell counts and Viral Load (VL) quantification: CD^{4+} cells were counted using lymphocyte staining with CD^{4} conjugated monoclonal antibodies (Tri test, BD Biosciences, San Diego, California, USA).

Statistical analysis: Parametric and non-parametric analyses, Chi-square and one way ANOVA (version 15, Chicago, IL, USA) tests were used for statistical analysis of serological and molecular results. A level of $p \leq 0.05$ was accepted as statistically significant.

RESULTS

HIV infected patients were categorized into four different high risk groups: intravenous drug users 32%, hemophiliacs 30%), homosexual (21.33%) heterosexuals (16.67%) and patients with a history of kidney diseases (23.33%) and liver diseases (40%). While using PCR, HGV-RNA was detected in 26.66% of patients with HIV, HCV-RNA was found in 30% of HIV patients. There was not any significant difference between the prevalence of GBV-C RNA in different age groups among GBV-C positive patients (Table 1-2).

Table 1: Prevalence of HGV and HCV in patients with HIV$^+$ (N = 150)

Characterization	Hemophiliacs	Homosexual	Heterosexuals	Intravenous drug users
HIV$^+$ (N = 150)				
Number (%)	45 (30)	32 (21.33)	25 (16.67)	48 (32)
Gender (M/F) (88/62)	27/18	18/14	15/10	28/20
Age	35.22±2.24	45.10±28.1	45.12±61.81	46.42±41.51
HCV 30 (45/150)	15/45 (33.33)	10/32 (31.25)	7/25 (28)	13/48 (27.08)
HGV 26.66 (40/150)	13/45 (28.88)	7/32 (21.88)	5/25 (20)	15/48 (31.25)
HGV/HCV 19.33 (29/150)	10/45 (22.22)	6/32 (18.75)	8/25 (32)	5/48 (10.41)
HIV only 24 (36/150)	7/45 (15.55)	9/32 (28.12)	5/25 (20)	15/48 (31.25)

N: Number of samples; NS: Not Significant; S: Significant; M: Male; F: Female

Table 2: Classified patients into four different high risk groups HIV$^+$ (N = 150)

Characterization	Hemophiliacs	Homosexual	Heterosexuals	Intravenous drug users
Number (%)	45 (30)	32 (21.33)	25 (16.67)	48 (32)
History of liver 40% (60/150)	10/45 (22.22)	14/32 (43.75)	10/25 (40)	26/48 (51.17)
History of kidney 23.33% (35/150)	6/45 (13.33)	8/32 (25)	9/25 (36)	12/48 (2520.83)
History of transfusion 36.67% (55/150)	29/45 (64.44)	10/32 (31.25)	6/25 (24)	10/48 (20.83)

Table 3: Classified patients according to history of diseases HIV$^+$ (N = 150)

Characterization	History of liver	History of kidney	History of transfusion
Number (%)	60 (40)	35 (23.33)	55 (36.67)
Gender (M/F) 92/58	42/18	16/19	34/21
Age	33.52±2.11	42.14±68.1	46.52±31.09
HCV 30 (45/150)	16/60 (26.66)	10/35 (28.58)	19/55 (34.55)
HGV 26.66 (40/150)	15/60 (25)	10/35 (28.58)	15/55 (27.27)
HGV/HCV 19.33 (29/150)	14/60 (23.34)	8/35 (22.86)	7/55 (12.72)
HIV only 24 (36/150)	15/60 (25)	12/35 (34.29)	9/55 (16.36)

Serological presentation of HGV and HCV: Anti-E2-HGV-Ab was found in 42 of 150 (28%) HIV positive patients. HIV positive patients were also HCVAb positive (n = 49, 30%). The difference between the presence of HGV and HCV antibodies among HIV positive patients was significant (p = 0.0001, OR = 0.814, 95% CI = 0.736-0.900).

Mono and polyviral infections with HGV and HCV: Serological data showed the prevalence of HCV-Ab markers (32.66%) and HGV-Ab (28%) among patients with HCV, HCV-HGV and HGV positive (data not shown). The co-infection of HGV and HCV was diagnosed through the evaluation of different markers of these two viruses in 29 (19.33%) patients with HIV (Table 1-2).

HGV and HCV and risk factors of HIV: Significant correlations were found between gender and HGV (p = 0.0002), HCV (p = 0.0001) and HIV (p = 0.0001). Also a significant relationship was observed between the prevalence of HGV and risk groups: intravenous drug users (p = 0.0001), hemophiliacs (p = 0.0003), homosexuals (p = 0.0001), heterosexuals (p = 0.0001), patients with a history of kidney diseases (p = 0.0002) and liver diseases (p = 0.0004). There was not a significant correlations between the diagnosis of HCV with the age of patients (p = 0.936). Conversely, significant correlations were observed between the prevalence of HCV and risk groups: intravenous drug users (p = 0.000), hemophiliacs (p = 0.000), homosexuals (p = 0.0001), heterosexuals (p = 0.0002) and patients with a history of kidney diseases (p = 0.0003) and liver diseases (p = 0.000) (Table 1-2).

CD^{4+} T cell counts and Viral Load (VL) quantification: Overall in our study, a trend towards a negative correlation between GBV-C load and HIV-1 load was found. Also a positive correlation between GBV-C load and the number of CD^{4+} cells within 4 year was observed. In the GBV-C RNA-positive group, the median CD^{4+} T lymphocyte count increased from 398-502 cells/mL and the median HIV-1 viral load was 2.01± 0.7 $\log_{10}$ copies/mL. However, in GBV-C RNA-negative group, the median CD^{4+} T lymphocyte count decreased from 428-351 cells/mL and the median HIV-1 viral load was 5.2±1.04 Log_{10} copies/mL (Table 3).

Many studies have shown that the coinfection of GBV-C and HIV is associated to higher CD^{4+} cell counts and lower HIV loads (Alter, 1996; Reshetnyak *et al*., 2008). In agreement with previous reports, a significant difference in CD^{4+} cell counts and HIV load with regard to the GBV-C infection status among patients with HIV/HCV coinfection was found (Mosam *et al*., 2007; Jung *et al*., 2007). Then, the study proceeded with analyzing the correlation between GBV-C viral load and HIV-1 viral load and CD^{4+} T cell counts. During 4 years, a persistent trend of negative correlation between the GBV-C load and HIV-1

load was documented (r = -0.626, p = 0. 000). At the same time, a trend of positive correlation between the GBV-Cload and CD^{4+} cell count (r = 0.317, p = 0.001) was observed inthe HIV infected patients group. There was a significant relationship between GBV-C viral load and CD^{4+} T cell count (r = 0.460 , p = 0.000).

DISCUSSION

Acquired Immune Deficiency Syndrome (AIDS) is the most fatal infectious disease and the fourth cause of death in the world (Karuru *et al*., 2005). It is estimated that one third of these deaths are related to liver diseases that are mainly caused by the co-infection of HIV and hepatitis B and C or G (Dodig and Tavill, 2001). Epidemiological data indicate that HGV is transmitted predominantly through parenteral routes with a high seroprevalence among Injection Drug Users (IDUs) in parallel with sexual transmission. In this study, the frequency of HGV infection was determined in four groups of HIV positive patients. All HIV-positive patients were evaluated for HCV and HGV markers that showed anti-HCV in 32.66% and anti-HGV in 28% of all HIV positive patients. HCV prevalence in the study group was similar to USA (Alter, 1996; Reshetnyak *et al*., 2008). HCV-HIV co-infection-positive patients was variably reported to be from 25-50% (Alter, 1996; Reshetnyak *et al*., 2008). Also the prevalence of HGV-HIV co-infection-positive patients was reported to be from 6-16% (Leeratanapetch and Suseangrut, 2010; Muerhoff *et al*., 2005; Dodig and Tavill, 2001; Bourlet *et al*., 1999). Ramia *et al*. (2004) found a high prevalence of HGV infection in both IDU and sexual groups with no statistically significant difference between them. In a study by Campo, HGV-RNA was positive in 12 out of 37 HIV infected patients (32.4%). Other investigators reported a relatively high prevalence of HGV-RNA among non-injection drug using homosexual and bisexual men and heterosexual individuals (McNair *et al*., 1992). Zehender *et al*. (1997) showed a considerable spread of HGV among Italian HIV-positive IDUs and indicated that HGV infection does not enhance liver impairment. In our study, patients with HIV and HCV co-infection (30%) were more likely to be HGV-RNA positive (26.66%). HGV-RNA was found more frequently in patients with injection drug use (31.25%) than in those with sexual risk of exposure (21.88, 20%). Our findings are in agreement with those by Campo *et al*. (1999) studies but are in contrast to the results by Frey *et al*. (2002). Also, the frequency of HGV viremia is detected in 3-7 (21.88%) homosexuals and 5-25 (20%) heterosexuals which is similar to the results by Scallan *et al*. (1998) in Scotland and Lefrere in France while it is relatively higher than the results of Hattori (12.5%). In our study, the frequency of hepatitis G virus infection after blood transfusion in liver patients is 25% which is higher than the rate reported by Kobayashi (13.2%) (Bowden *et al*., 1996). The prevalence of HCV and HGV among hemophiliacs are 33.33 and 28.88%, respectively. Both rates are 28.58% in patients with a history of kidney diseases. These findings are similar to those by Yu *et al*. (2000), Fabrizi *et al*. (2002) and Nerurkar *et al*. (1998)) while they are relatively higher than those of Ramon in Brazil and Cesaire (14-35%). A significant relationship between the increase in the quick progress of liver diseases in HIV infected patients co-infected with HCV (p = 0.001) was found while there was a slow progress of liver diseases in HIV patients co-infected with HGV (p = 0.001). In this study, CD^4 count is higher in GBV-C positive group compared to the GBV-C negative group in the HIV positive individuals. Therefore, a significant difference was observed. Also there is a significant difference in the HIV viral loads between GBV-C positive and negative groups. Data show that GBV-C viremia affects HIV and slows down the progression of liver disease in HIV infected patients. Williams *et al*. (2004) reported that a negative correlation between GBV-C load and HIV-1 load as well as a positive correlation between GBV-C load and CD^{4+} T cell count had been previously demonstrated among chronically HIV infected patients. Also, Maria Teresa showed that a trend towards a negative correlation between GBV-C load and the HIV-1 load but no correlation between GBV-C load and the number of CD^{4+} cells. Giret *et al*. (2011) showed that CD^{4+} T cell counts tended to be higher in patients infected with GBV-C load. In this study, the statistical analysis shows that an increase in HGV-RNA load has a negative effect on the load of HIV-RNA. On the other hand, it can lead to a slower progression of HIV-RNA replication while an increase in CD^{4+} T cell count can be observed. Then, the study carried on with analyzing the correlation between GBV-C viral load and HIV-1 viral load or CD^{4+} T cell counts (Table 4).

Table 4: The valuation of CD^4/HIV-RNA/HGV-RNA in patients with HIV

First year	HIV^+/HGV^+	HIV^+/HGV^-
CD^4 count cells/mL	398	428
HIV-RNA laod log_{10} copies/mL	3.8±1.02	4.6±9.02
HGV-RNA laod log_{10} copies/mL	1.09±1.05	0
After 4 years	HIV^+/HGV^+	HIV^+/HGV^-
CD^4 count cells/mL	502±117	351±241
HIV-RNA laod log_{10} copies/mL	2.1±0.7	5.2±1.04
HGV-RNA laod log_{10} copies/mL	2.5±2.08	0

CONCLUSION

The presence of GBV-C RNA was frequent in populations at risk of blood-borne or sexually transmitted viruses. Most of the GBV-C RNA-positive individuals had no biochemical evidence of liver damage within 4 years. However, further studies are required to investigate the mechanism of its transmission and clinical significance of an acute and persistent HGV infection among high-risk groups. GBV-C recently received considerable attention, largely owing to its potential role in decelerating HIV-1 disease progression by interfering with HIV replication. GBV-C is parentally transmitted, similar to the serum hepatitis viruses and HIV-1. Most of the efforts have been devoted to understanding the beneficial role of GBV-C in HIV. Results suggest that HGV probably changes the pathology of HIV in liver diseases as there is a significant relationship between the increase in the frequency of HGV and the slow progression of liver diseases among HIV infected patients. In the absence of HGV, co-infection of Hepatitis C Virus (HCV) in patients may have played a role in HIV progression.

ACKNOWLEDGEMENT

This study was financially supported by Shiraz Transplant Research Center and Gerash University of Medical Science, Shiraz, Iran.

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