American Journal of Microbiological Research, 2017, Vol. 5, No. 1, 1-6
Available online at http://pubs.sciepub.com/ajmr/5/1/1

DOI:10.12691/ajmr-5-1-1

Prevalence of GBV-C and Its Impacts among Patients with Hepatitis B and C Viruses in Addis Ababa, Ethiopia

Mohamed Farouk[1,*], Abate Bane Shewaye[2], Desta Kassa[3], Mekuria Lakew[1]

[1]Department of Microbial Cellular & Molecular Biology, Addis Ababa University, Addis Ababa, Ethiopia
[2]Schools of Medicine, Addis Ababa University, Addis Ababa, Ethiopia
[3]Infectious and Non-infectious Diseases Research Directorate, Ethiopian Health & Nutrition Research Institute, Addis Ababa, Ethiopia
*Corresponding author: mohamed.f.mirghani@gmail.com

Abstract **Background:** Hepatocellular carcinoma (HCC) caused by hepatitis B virus (HBV) and hepatitis C virus (HCV) is currently one of the most common neoplasms worldwide. GB virus C/hepatitis G virus (HGV/GBV-C) is a virus in the *Flaviviridae* family isolated from patients with liver disease. It has the same mode of transmission with HBV and HCV and is common in high risk group. The impact of HGV/GBV-C in clinical outcome among HBV and HCV is controversy. Therefore, this study was conducted to determine the prevalence and the association of (HGV/GBV-C) in the clinical outcome among HCV and HBV patients. **Materials:** This case-control study was performed in Addis Ababa University, Ethiopia. The cases were 101 patients with viral hepatitis collected from Adera internal medical specialty center. The control group consisted of 50 healthy individuals collected from the Ethiopian Public Health and Research Institutes. The serological analysis and liver enzyme levels were determined for each of the participants. RNA was extracted, reversed transcribed, and amplified by Real Time polymerase chain reaction (PCR), using primers for 5- untranslated region (5-UTR) of the GBV-C. **Results:** Analysis of the 101 samples of the hepatitis patients showed that; 83(82.2%) were positive for HBV while only18 (17.8%) for HCV. The prevalence of (HGV/GBV-C) RNA was 11(13.2%) in HBV, 2 (11.1%) in HCV and rests of the control group were negative. There was no significant difference ($P > 0.05$) in the liver enzymes level among (HGV/GBV-C) negative and positive individuals. **Conclusion:** Our study showed that the co-infection rate of (HGV/GBV-C) RNA among hepatitis patients was significantly higher ($P < 0.05$) in HBV than in HCV patients, and the virus has no association in the course of the disease.

Keywords: *HGV/GBV-C, HBV, HCV, RNA, PCR*

Cite This Article: Mohamed Farouk, Abate Bane Shewaye, Desta Kassa, and Mekuria Lakew, "Prevalence of GBV-C and Its Impacts among Patients with Hepatitis B and C Viruses in Addis Ababa, Ethiopia." *American Journal of Microbiological Research*, vol. 5, no. 1 (2017): 1-6. doi: 10.12691/ajmr-5-1-1.

1. Introduction

For decades, hepatitis is considered a major significant public health problem and accounts for millions of deaths from chronic liver disease, cirrhosis, hepatocellular carcinoma (HCC) and even fulminant hepatitis.

Several hepatotropic viruses can target the liver and cause hepatitis, like viral hepatitis type A, B, C, D, E. Hepatitis B, C, and D, are transmitted parentally, sexually or through contaminated blood and can produce acute or chronic hepatitis [1,2,3,4], while hepatitis A and E viruses are transmitted by fecal-oral routes and cause self-limiting infections [5,6,7].

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the commonest causative agents of severe liver disease. They account for 60% of cirrhosis, 80% of HCC and cause around one million deaths every year, especially in developing countries [8]. HBV is a partially double-shelled circular DNA belonging to the family *Hepadnaviridae* [9] and HCV is an enveloped single stranded positive sense RNA virus of the genus *Hepacivirus* within the family *Flaviviridiae* [10,11]. Infection with HBV can be either acute or chronic, ranging from an inactive carrier state to progressive state which leads to significant liver disease [12,13,14]. On the other hand 80% of HCV infected individuals will become chronically carriers with high risk to develop sever liver disease ranging from mild minimal histological changes to extensive fibrosis and cirrhosis, HCC which may progress to liver cancer [15,16]. However, most HCC cases are due to HCV infection [17,18].

In the mid-90s, a novel viruses were identified by two independent groups; GB virus C (GBV-C) and hepatitis G virus (HGV) from patient with liver disease [19,20]. Genome analysis showed that the two viruses shared 96% of the amino acid sequence and 86% of the nucleotide sequences and this suggest that they are different isolates of the same virus, and the term GBV-C is the commonly used [19,20,21].

GBV-C is an enveloped RNA virus in the family *Flaviviridae* with HCV and proposed to be classified within the genus *Pegivirus* [11]. Although, GBV-C is the

most closet virus to HCV and they are sharing approximately 30% amino acid homology, but unlike HCV which replicate in the hepatocytes, GBV-C is a lymphotropic virus [22]. This virus is a positive single stranded RNA of approximately 9,400 nucleotides. The 5′ untranslated region (5′UTR) contains an internal ribosomal entry site and is followed by a single open reading frame that encodes for a polyprotein of ~3,000 amino acids. This polyprotein is cleaved by viral and cellular proteases resulting in several structural and non-structural viral proteins [23].The structural proteins of GBV-C are two putative envelope proteins (E1 and E2), while the non-structural proteins include NS2 (a protease), NS3 (an RNA helicase and trypsin-like serine protease), NS4, NS5A and NS5B (an RNA-dependent RNA polymerase) [11].

So far, there are seven different genotypes identified from different geographical locations: Genotype 1 predominant in West Africa; genotype 2 in Europe and USA; genotype 3 in Asia; genotype 4 in the Southeast Asian countries; genotype 5 in South Africa; genotype 6 in Indonesia and genotype 7 isolated in China recently. Within a given genotype, additional intra genotype diversities could occur based on their sequence diversity of either full genome length or a particular genomic range [24,25].

GBV-C is mainly transmitted through parenteral exposure to blood or blood products [20], sexual contact [26,27], and less frequently through mother-to-child transmission [28]. GBV-C infection is common worldwide, with rate of approximately 1% to 6% among healthy blood donors in the US and Europe, whereas it may reach up to 18% among healthy population in developed countries [29,30,31]. GBV-C infection is common among patients with HIV, HCV or HBV due to share mode of transmission approaches 50% [29,32].

Several studies from different countries have reported the positive role of GBV-C in improving the clinical infection and treatment outcomes of HIV patients [33,34,35]. On the other hand the impact of GBV-C in liver disease is controversy. Although the virus has been isolated from patients with fulminant hepatitis [36], but other studies failed to prove any association [37]. Co-infection of GBV-C with one or more hepatotropic viruses like HBV or HCV may have negative or positive effect on the disease evolution [38].

So the aim of this study is to determine the prevalence and the role of GBV-C infection among patients with hepatitis B and C infections and healthy control group in Addis Ababa, Ethiopia.

2. Material & Methods

2.1. Study Design and Settings

This case-control study was conducted in Addis Ababa University, Ethiopia, on frozen serum samples collected from adult patients with viral hepatitis seeking medical care and follow up in Adera Internal Medical Specialty Center. This center was purposely selected because it's an internal medicine clinic that provides healthcare to a great number of patients especially in liver disease, which makes the study sample the best possible representative of the target population.

2.2. Sample and Data Collection

The study population comprised a total of 101 patients with viral hepatitis collected from Adera Internal Medical Specialty Center and 70 healthy individuals collected from the Ethiopian Public Health and Research Institute. Following collections, the serum samples were divided into 2 microtubes and transported in an icebox to the Immunology and Molecular laboratories at the department of Microbial Cellular & Molecular biology, Addis Ababa University. Demographic data, including age, gender, medical history and health status were collected from the patients cards and archives.

2.3. Serological Analysis

HCV and HBV markers (HBsAg, anti-HBs, HBeAg and anti-HCV) were carried out by using a rapid and qualitative commercial fourth generation two-site sandwich immunoassay test device (Qualpro Diagnostics, 88/89, phase IIC, Verma Industrial Estate, Verna, Goa-403 722, India, 2004) according to the manufacturer's instructions. It uses a multi target region or epitopes that cross react with specific sites in the virus and the result interpretations were based on color formation.

2.4. Liver Function Test

The following parameters were measured: alanine aminotransferase GPT/ALT (normal <=40 IU/l), aspartate aminotransferase (GOT/AST, normal <=40 IU/l), alkaline phosphatase (normal <=270 IU/l), bilirubin total (normal <=1.2 mg/dl), and albumin (normal: 3.5-5.3 g/dL).

2.5. RNA Extraction

For the preparation of total RNA from human sera, we used the Ribo Virus kit (Sacace Biotechnologies, Italy) according to the manufacturer's instructions. In brief, 150 µl of serum was incubated with 600 ml of lysis buffer containing guanidine thiocyanate at 70°C for 5 min. After the addition of 600 ml of ethanol, the precipitated RNA was applied onto a silica-based spin column for purification and was finally eluted with 50 ml of Rnase-free H_2O.

2.6. RT-PCR & Amplification

One-step reverse transcription of the RNA and Real Time amplification of the cDNA was performed by using (V2-FRT, Sacace, Italy), which include all the components to generate RT-PCR amplified products from GBV-C/HGV RNA. Briefly, the total reaction volume was 25 ul, containing 10 ul of extracted RNA and 15 ul of the master mix that was prepared based on manufacturer's instructions. cDNA synthesis was performed at 50°C for 15 min, followed by inactivation of the reverse transcriptase enzyme at 95°C for 5 min. The two PCR rounds are the same. The amplification was done in 5 cycles for the first round (95°C for 5 s, 60°C for 20 s,

72°C for 15 s) and 40 cycles for the second rounds of the PCR (95°C for 5 s, 60°C for 30 s, 72°C for 40 s with a final extension at 72°C for 15 s). The fluorescence detection and analysis of the PCR product was performed during the final extension by using the FAM, JOE/HEX channels of the Mx3000P instrument. Negative control of amplification and positive control of amplification were maintained as provided by the kit. Internal control which serves as amplification control was used to identify possible reaction inhibition.

2.7. Ethical Consideration

Ethical clearance was obtained from the Ethical Review committee of the College of Natural Science, Addis Ababa University and Ministry of Science and Technology.

2.8. Statistical Analysis

Data were entered and analysis done by using SPSS v. 20. Student t- test was used to determine the differences of continuous variables. Chi-square tests were used to compare categorical variables. P value < 0.05 was considered statistically significant.

3. Results

Total of 151 subjects were tested, including 101 samples with liver disease and 50 samples of healthy individuals. Among liver disease patients eighty three (55%) were HBV positive, while the remaining 18 (12%) were HCV positive and two of them were co-infected with HBV and HIV respectively (Figure 1).

The socio-demographic characteristics of hepatitis patients as shown in (Figure 2), the majority 52 (62%) of HBV patients were male, and most of the participant 35 (42%) were age between 25 – 34 years, while among HCV the predominant patients were 11 (61%) females age between 45-60 years.

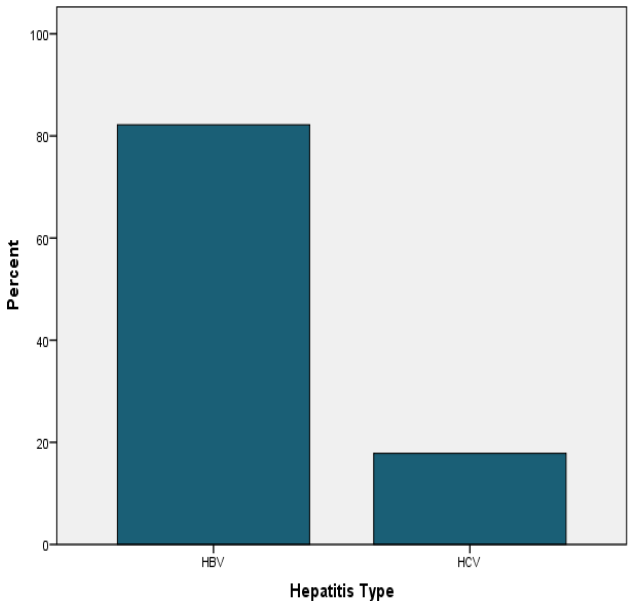

Figure 1. Type of Hepatitis Among Liver Disease Patients

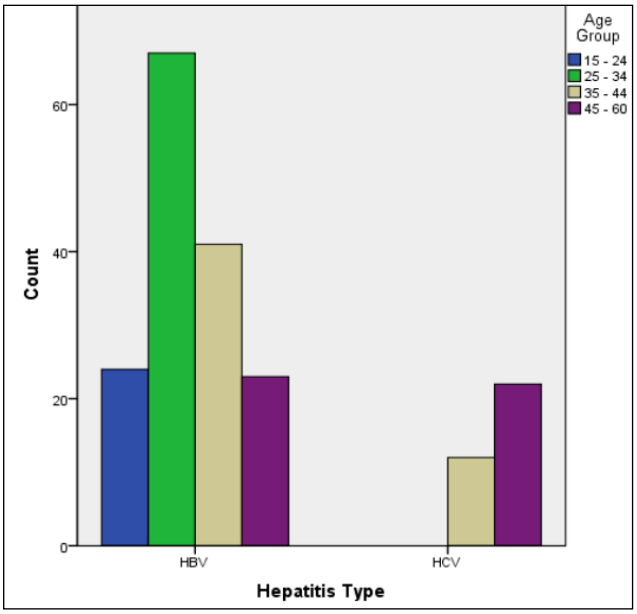

Figure 2. Distribution of sex and age among hepatitis patients

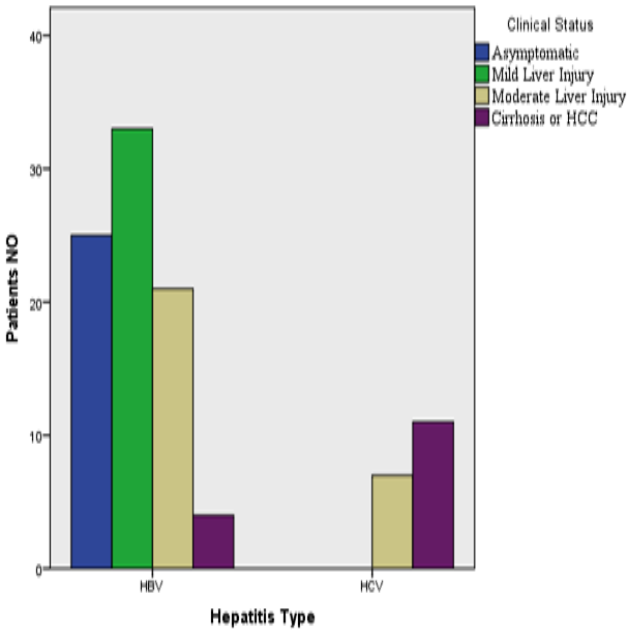

Figure 3. Clinical Statutes of the hepatitis patients

We categorized hepatitis patients into four groups based on the level of the liver enzymes and the abdominal Ultrasound scan as shown in (Figure 3). Most of HBV patients were with mild liver injury because their level of the liver enzymes was in the upper limit and with normal liver scan. In contrast, most of HCV patients were suffering from cirrhosis and or hepatocellular carcinoma.

The overall prevalence of GBV-C RNA were detected in 13 (12.9%) of the hepatitis patients, 2 (11.1%) HCV and 11 (13.25%) HBV respectively, while all the healthy subjects were negatives. The rate of GBV-C RNA was significantly higher among HBV patients.

Patients with HCV and GBV-C co-infection were in the age range 45-60 and there were no significant differences in sex and clinical status because all the HCV patients were in the end stage liver disease. Among the 83 patients with hepatitis B, there were no significant differences in sex, age and the clinical status between those with and

without GBV-C infection. GBV-C infection had no influence on the severity of chronic liver disease, because there were no significant differences in the clinical picture between GBV-C positive and negative patients.

Table 1. Socio-demographic and clinical characteristics of HBV & HCV patients with and without GBV-C infection

Characteristics	GBV-C RNA (+)		GBV-C (-)	
	HBV +	HCV +	HBV +	HCV +
No. (%)	11 (13.25%)	2 (11.1%)	72 (86.7%)	16 (88.9%)
Sex M/F	8/3	1/1	44/28	6/10
Age group				
15-24	2	0	11	0
25-34	3	0	32	0
35-44	5	0	18	6
45-60	1	2	11	10
Liver Status				
Asymptomatic	4	0	21	0
Mild Liver Injury	5	0	28	0
Moderate Liver Injury	2	0	19	7
Cirrhosis / HCC	0	2	4	9

1: Normal liver scan and the level of the liver enzyme in the upper limit
2: Fatty liver and high liver enzymes.

4. Discussion

GBV-C is a member of the *Flaviviridae* family and it is closely related to the Hepatitis C Virus (HCV). Although, this virus has been isolated from patients with fulminant hepatitis, but its impact in liver disease is still controversial.

In the present study, we investigated the prevalence and impact of GBV-C infection in 101 hepatitis patients and 50 healthy individuals. Analysis of the hepatitis patients samples showed that; 83(55%) were positive for HBV while only18 (12%) for HCV.

Most of the HBV patients were male's age between 25 – 34 years, while among HCV the predominant patients were female age between 45-60 years.

Interestingly, we observed that most of HCV patients were females and older than HBV patients. Furthermore, among the 18 hepatitis C patients 11 were suffering from cirrhosis and hepatocellular carcinoma while among HBV patients there were only 4 with hepatocellular carcinoma or cirrhosis.

Among hepatitis patients, from 83 HBsAg positive samples, 11 (13.25%) were positive for GBV-C and from 18 anti-HCV positive samples, 2 (11.1%) were positive for GBV-C, while all the healthy subjects were negatives. Our study showed that the co-infection rate of (HGV/GBV-C) RNA among hepatitis patients was significantly higher ($P < 0.05$) in HBV than in HCV patients. This is the first report showing the incidence of GBV-C in HBV and HCV patients in Ethiopia.

Our findings were consistent with other reports; Birgit *et al*. reported the incidence of co-infection with GBV-C in chronic hepatitis C to be 11% [39]. However, it has been reported from various regions that the prevalence of GBV-C among HCV patients range between 10-33.3% [40,41,42,43]. While among HBV patients in other countries such as Taiwan, Egypt, Turkey, United Arab Emirates and Saudi Arabia have shown the prevalence of GBV-C to range between 4.1-29% [42,49,50,51,52].

These differences in the prevalence of GBV-C infection may be due to sample size, the demographic and clinical features of patients. This controversy may be due to the number of patients and the laboratory systems in the detection of the virus RNA and the antibody which was one of our limitations. The detection of the viral RNA with reverse transcription polymerase chain reaction [44], indicated active infection, however the clearance of the virus is associated with the development of antibodies against envelope glycoprotein (E2) which are detected by immunoassays [45].

The prevalence of GBV-C in our study is higher among HBV than HCV; this might be duo to the impact of HCV treatment on GBV-C RNA clearance [46]. The presence of GBV-C infection among hepatitis patients was not associated with sex and age, which correlated with other reports [42,47]. GBV-C incidence was relatively higher in the age group of 35 – 44 years and in male, but the difference was not statistically significant ($p > 0.05$).

Some studies have shown that GBV-C infection is associated with significantly less compensated and decompensated cirrhosis, and with improvement in cirrhosis-free survival among patients with HBV or HCV [38,48]. Unfortunately, in agreement with previous reports GBV-C infection had no influence on the severity of chronic liver disease among HBV and HCV co-infection [37,47,53,54].

Our study was a preliminary survey to evaluate the incidence of GBV-C infection among hepatitis patients in Ethiopia. There were several limitations to our study including the small number of patients and measuring anti-GBV-C antibodies. Furthermore, there is no information regarding the predominant genotypes of GBV-C in Ethiopia which might have a different role on the disease progression as reported in various other geographical regions. Finally, wider studies with long follow ups are necessary to understand the role of GBV-C in liver disease.

Acknowledgments

The authors would like to thank the staff of Adera Internal Medical Specialty Center and the Ethiopian Public Health and Research Institutes for their help during this research.

References

[1] Hu DJ, Bower WA, Ward JW. Viral hepatitis. In: Morse S, Moreland AA, Holmes KK, eds. Atlas of sexually transmitted diseases and AIDS. London: *Elsevier*; 2010: 203-29.

[2] CDC. Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic disease. *MMWR* 1998; 47.

[3] Armstrong GL, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med* 2006; 144: 705-4.

[4] Peters MG. Special populations with hepatitis B virus infection. *Hepatology* 2009; 49(Suppl 5): S146-S155.

[5] Koopmans M., Duizer E. (2004): Foodborne viruses: an emerging problem. *International Journal of Food Microbiology*, 90, 23-41.

[6] Vasickova P., Dvorska L., Lorencova A., Pavlik I. (2005): Viruses as a cause of foodborne diseases: a review of the literature. *Veterinarni Medicina* 50, 89-104.

[7] Ashbolt N.J. (2004): Microbial contamination of drinking water and disease outcomes in developing regions. *Toxicology*, 198, 229-238.

[8] Global policy report on the prevention and control of viral hepatitis (2013).World Health organization; Geneva, Switzerland.

[9] Nassal M.HBV cccDNA: viral persistence reservoir and key obstacle for a cure of chronic hepatitis B. *Gut* 2015; 64: 1972-84.

[10] Penin F. structural biology of hepatitis C virus. *Clin Liver Dis* 2003; 7: 1-21.

[11] Stapleton JT, Foung S, Muerhoff AS, Bukh J, Simmonds P. The GB viruses: a review and proposed classification of GBV-A, GBV-C (HGV), and GBV-D in genus Pegivirus within the family Flaviviridae. *J Gen Virol* 2011; 92: 233-46.

[12] Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat*. 2004; 11(2): 97-107.

[13] Fattovich G. Natural history and prognosis of hepatitis B. *Semin Liver Dis* 2003; 23: 47-58.

[14] McMahon BJ. The natural history of chronic hepatitis B virus infection. *Semin Liver Dis* 2004; 24: 17-21.

[15] World Health Organization. Hepatitis C Fact Sheet N°164. Available from: Http: //www. who. int/ mediacentre /factsheets /fs164/en/. Last accessed: March 2016.

[16] Sultan MT, Rahman MM, Begum S. Epidemiology of hepatitis C virus (HCV) infection. *J Bangladesh Coll Phy Surg*. 2009; 27: 160-2.

[17] Lee MH, Yang HI, Yuan Y, Lltalien G, Chen CJ (2014). Epidemiology and natural history of hepatitis C virus infection. *World J. Gastroenterol*. 20(28): 9270-9280.

[18] Lavanchy D. Evolving epidemiology of hepatitis C virus. *Clin Microbiol Infect* 2011;17: 107-115

[19] Simons, J. N., T. P. Leary, G. J. Dawson, T. J. Pilot-Matias, A. S. Muerhoff, G. G. Schlauder, S. M. Desai, and I. K. Mushahwar. 1995a. Isolation of novelvirus-like sequences associated with human hepatitis. *Nat Med* 1: 564-569.

[20] Linnen, J., J. Wages, Jr., Z. Y. Zhang-Keck, K. E. Fry, K. Z. Krawczynski, H. Alter, E. Koonin, M. Gallagher, M. Alter, S. Hadziyannis, P. Karayiannis, K. Fung, Y. Nakatsuji, J. W. Shih, L. Young, M. Piatak, Jr., C. Hoover, J. Fernandez, S. Chen, J. C. Zou, T. Morris, K. C. Hyams, S. Ismay, J. D. Lifson, G. Hess, S. K. Foung, H. Thomas, D. Bradley, H. Margolis, and J. P. Kim. 1996. Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. *Science* 271: 505-508.

[21] Leary, T. P., A. S. Muerhoff, J. N. Simons, T. J. Pilot-Matias, J. C. Erker, M. L. Chalmers, G. G. Schlauder, G. J. Dawson, S. M. Desai, and I. K. Mushahwar. 1996. Sequence and genomic organization of GBV-C: a novel member of the flaviviridae associated with human non-A-E hepatitis. *J Med Virol* 48: 60-67.

[22] Cheung RC, Keeffe EB, and Greenberg HB, Hepatitis G virus: Is it a hepatitis virus? *West J Med* 167: 23-33. 1997.

[23] Pavesi A, Detection of signature sequences in overlapping genes and prediction of a novel overlapping gene in hepatitis G virus. *J Mol Evol* 50: 284-295. 2000

[24] Feng Y, Zhao W, Dai J, Li Z, Zhang X, Liu L, Bai J, Zhang H, Lu L, and Xia X, A novel genotype of GB virus C: its identification and predominance among injecting drug users in Yunnan, China, *PLoS One* 6: e21151. 2011.

[25] Sathar MA, York DF, Group 5: GBV-C/HGV isolates from South Africa, *J Med Virol* 65: 121-122. 2011.

[26] Scallan MF, Clutterbuck D, Jarvis LM, et al., Sexual transmission of GB virus C/hepatitis G virus, *J Med Virol*, 1998; 55: 203-8.

[27] Nerurkar VR, Chua PK, Hoffmann PR, et al., High prevalence of GB virus C/hepatitis G virus infection among homosexual men infected with human immunodeficiency virus type 1: evidence for sexual transmission, *J Med Virol,* 1998; 56: 123-7.

[28] Fischler B, Lara C, Chen M, et al., Genetic evidence for mother-to-infant transmission of hepatitis G virus, *J Infect Dis*, 1997; 176: 281-5.

[29] Dawson,G.J. et al. Prevalence studies of GB virus-C infection using reverse transcriptase-polymerase chain reaction. *J Med V irol* 50, 97-103 (1996).

[30] Liu,H.F., Muyembe-Tamfum,J.J., Dahan,K., Desmyter,J. & Goubau,P. High prevalence of GB virus C/hepatitis G virus in Kinshasa. *J Med Virol* 60, 159-165 (2000).

[31] Tucker,T.J., Louw,S.J., Robson,S.C., Isaacs,S. & Kirsch,R.E. High prevalence of GBV-C hepatitis G virus infection in a rural South African population. *J Med Virol* 53, 225-228 (1997).

[32] Bhattarai N, Stapleton JT, GB virus C: the good boy virus? *Trends Microbiol,* 20(3): 124-30. 2012.

[33] Toyoda H, Fukuda Y, Hayakawa T, Takamatsu J, and Saito H, Effect of GB virus C/hepatitis G virus co-infection on the course of HIV infection in hemophilia patients in Japan. *Journal Acquired Immune Deficiency Syndromes*.17: 209-13. 1998.

[34] Heringlake S, Ockenga J, Tillmann HL, Trautwein C, Meissner D, Stoll M, Hunt J, Jou C, Solomon N, Schmidt RE, and Manns MP, GB virus C/hepatitis G virus infection: a favorable prognostic factor in human immunodeficiency virus-infected patients? *Journal of Infectious Diseases*.177: 1723-6. 1998.

[35] Bjorkman P, Flamholc L, Molnegren V, Marshall A, Guner N, Widell A (2007). Enhanced and resumed GB virus C replication in HIV-1-infected individuals receiving HAART. *AIDS*. 21(12): 1641-1643.

[36] Yoshiba M, Okamoto H, Mishiro S. Detection of the GBV-C hepatitis virus genome in serum from patients with fulminant hepatitis of unkown etiology. *Lancet* 1995; 346: 1131-2.

[37] Alter J. A reassessment of the literature on the hepatitis G virus. *Transfusion* 1997; 37: 569-72.

[38] Chams V, Fournier-Wirth C, Chabanel A, et al. Is GB virus-C alias "hepatitis G" involved in human pathology. *Trans Clin Bioi* 2003; 10: 292 306.

[39] Birgit Kallinowski, Christine Buhrmann, Stefanie Seipp, Tobias Goeser, Wolfgang Stremmel, Gerd Otto, and Lorenz Theilmann (1998). Incidence, Prevalence, and Clinical Outcome of Hepatitis GB-C Virus Infection in Liver Transplant Patients. *Liver Transplantation and Surgery,* (4)1: 28-33.

[40] Yue Feng, Li Liu, Yue-Mei Feng, Wenhua Zhao, Zheng Li, A-Mei Zhang, Yuzhu Song, and Xueshan Xia (2014). GB Virus C infection in Patients With HIV/Hepatitis C Virus Coinfection: Improvement of the Liver Function in Chronic Hepatitis C. *Hepat Mon*. 14(3): e14169.

[41] Berg T, Naumann U, Fukumoto T, Lobeck W, Bechstein P, Neuhaus P, et al. GB virus C infection in patients with chronic hepatitis B and C before and after liver transplantation. *Transplantation* 1996;62: 711-714

[42] Alvarado-Mora MV, Botelho L, Nishiya A, Neto RA, Gomes-Gouvea MS, Gutierrez MF, Carrilho FJ, Pinho JR (2011) Frequency and genotypic distribution of GB virus C (GBV-C) among Colombian population with Hepatitis B (HBV) or Hepatitis C (HCV) infection. *Virol J* 8: 345.

[43] Tanaka E, Alter HJ, Nakatsuji Y, *et al*. Effect of hepatitis G virus infection on chronic hepatitis C. *Ann Intern Med*. 1996; 125(9): 740-3.

[44] Ruiz V, Espinola L, Mathet VL, Perandones CE and Oubina JR, Design, development and evaluation of a competitive RT-PCR for quantitation of GBV-C RNA, *J Virol Methods*, 2006; 136: 58-64.

[45] Thomas DL, Vlahov D, Alter HI, Hunt, Marshall R, Astemborski I and Nelson KE. (1998) Association of antibody to GB Virus C (Hepatitis G Virus) with viral clearance and protection from reinfection. *J. Infect. Dis*. 177, 539-542.

[46] Masaru Enomoto, Shuhei,Shiguchi, Katsuhiko Fukuda, Tetsuo Kuroki, Motoharu Tanaka, Shuzo Otani, Masayuki Ogami, and Takeyuki Monna (1998). Characteristics of Patients with Hepatitis C Virus With and Without GB Virus C/Hepatitis G Virus Co-infection and Efficacy of Interferon Alfa. *HEPATOLOGY*, (27)5.

[47] Tanaka Y, Mizokami M, Orito E, Ohba K, Nakano T, Kato T, Kondo Y, Ding X, Ueda R, Sonoda S, Tajima K, Miura T, Hayami M: GB virus C/hepatitis G virus infection among Colombian native Indians. *Am J Trop Med Hyg* 1998, 59: 462-467.

[48] Berzsenyi MD, Bowden DS, Kelly HA, Watson KM, Mijch AM, Ham¬mond RA, et al. Reduction in hepatitis C-related liver disease associated with GB virus C in human immunodeficiency virus coinfection. *Gastroenterology*. 2007; 133(6): 1821-30.

[49] El-Zayadi AR, Abe K, Selim O, Naito H, Hess G, Ahdy A: Prevalence of GBVC/ 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.

[50] Monica, V., Alvarado, M., Livia, B., Anna, N., Raymundo, A., Neto3, M. and Maria, F. (2011). Frequency and genotypic distribution of GB virus C (GBV-C) among Colombian population with Hepatitis B (HBV) or Hepatitis C (HCV) infection. *Virol J*. 8: 345.

[51] Abu Odeh RO, Al-Moslih MI, Al-Jokhdar MW, Ezzeddine SA. Detection and genotyping of GBV-C virus in the United Arab Emirates. *J Med Virol* 2005; 76: 534-40.

[52] Akcali S, Sanlidag T, Ozbakkaloglu B. Prevalence of GBV-C/ hepatitis G virus viremia among chronic hepatitis B, chronic hepatitis C and hemodialysis patients in Turkey. *Ann Saudi Med* 2006; 26: 68-9.

[53] Grassi M, Raffa S, Traditi F, Ferrazzi M, Cioschi S, Fontana M, et al. Detection and clinical evaluation of GBV-C/HGV in plasma from patients with chronic hepatitis of unknown etiology. *Clin Ter*. 2000 Jul-Aug; 151(4): 241-5.

[54] Ramezani A, Gachkar L, Eslamifar A, et al. Detection of hepatitis G virus envelope protein E2 antibody in blood donors. *Int J Infect Dis*. 2008; 12: 57-61.