



Distribution of Genotypes of B and C Hepatitis Viruses in Guilan Province

**Behrang Ashourizadeh*(M.D), Hanieh Bashizadeh-Fakhar (Ph.D student),
Seyede-Sarah Fallahchai (Ph.D student), Reza Goleijani-Moghaddam (Ph.D student)**

Young Researchers Club, Islamic Azad University, Tonekabon Branch, Tonekabon, Iran

ARTICLE INFO

Article history:

Received 5 July 2012

Accepted 17 September 2012

Available online 1 November 2012

Keywords:

Frequency of distribution genotype,

HBV,

HCV,

Guilan

ABSTRACT

Today, chronic viral hepatitis is considered as a public health problem in the world. Totally, 5% of the world population, especially in Asia and Africa suffer from this problem. The prevalence of acute hepatitis in America is 0.1-0.2 percent, 50% of those with hepatitis C (HCV) and 5-10% of them with hepatitis B (HCV) develop chronic hepatitis. Determining HBV and HCV genotypes is important to clarify the disease and pathogenicity of the virus since genotypes are different in terms of pathogenicity and response to treatment. In this project, the number of 90 patients with hepatitis B and C referred to a private medical diagnostic laboratory in Rasht was included in this study. After obtaining blood samples from patients, genotyping and determining the amount of hepatitis B and C virus were performed. It was found that out of 90 patients, 56 ones (62.2%) had HBV and 34 (37.8%) HCV. In all 56 patients with genotype HBV and in 34 patients with genotype HCV observed genotypes including 1a, 1b, and 3a. The most frequent genotype was 1a which was seen in 18 patients (20%) followed by 3a in 14 patients (15.6%). Only 2 patients (2.2%) had genotype 1b. Knowing the genotype and the viral load of HBV and HCV in each region is important in disease development and treatment. Determining HBV and HCV genotypes is important to clarify the disease progression and pathogenicity of the virus.

1. Introduction

Today, chronic viral hepatitis is considered as a public health problem in the world. Totally, 5% of the world population, especially in endemic areas such as Asia and Africa suffer from this problem. The prevalence of acute hepatitis in America is 1-2 per 1000 people, out of which 50% of those with hepatitis C and 5-10% of them with hepatitis B that develop chronic hepatitis (Chan et al., 1999). Determining hepatitis B virus (HBV) and hepatitis C virus (HCV) genotypes is important to clarify the disease progression and pathogenicity of the virus

since genotypes are different in terms of pathogenicity and response to treatment.

According to the World Health Organization (WHO) in 2001 and the Center for Disease Control and Prevention in 2005, the prevalence of chronic hepatitis B is between 2% to 7% in Iran (Combo et al., 2005). Overall, HCV is the common cause of viral hepatitis in the world, serum prevalence of which is based on anti-C antibody is estimated to be about 1%. However, there are significant differences in the prevalence of this infection (CDC, 2005). Studies suggest that patients with HBV and HCV infections eventually lead to active, developed

*Corresponding author: Dr. Behrang Ashourizadeh

Tel: 00989121992042

E-mail: Behrang_ashoorizadeh@yahoo.com

and chronic hepatitis, liver cirrhosis and hepatocellular carcinoma cells (HCC) (Zyzik et al., 1986; Lioeje et al., 2006).

HBV is a member of Hepadnaviruses family, which is now, based on a comparison of genomic sequences, classified into eight genotypes, including A to H (Kidd-Ljunggren et al., 2002). HCV has 6 major (Kidd- Ljunggren et al., 2002; Chan et al., 1999) and 11 minor genotypes (6a, 5a, 4a, 3b, 3a, 2b, 2a, 1c, 1b, 1a) (Lau et al., 1996). Determining HBV and HCV genotypes is important to clarify the disease progression and pathogenicity of the virus since genotypes are different in terms of pathogenicity and response to treatment. Therefore, knowing the genotypes of HBV and HCV viruses in each region is important in disease progression and treatment, growing chronic infection, and high risk for cirrhosis and liver cancer. This is the first study is has been performed in Guilan province.

2. Materials and Methods

In this study, 90 patients infected with hepatitis B and C referring to a private medical diagnostic laboratory in Rasht, Iran, from 2001-2002 were evaluated. DNA was extracted from the peripheral blood and the HCV and HBV genotypes were analyzed.

2.1. Extraction

In order to extract RNA and DNA, high pure viral nucleic acid kit (Roche, Germany) and DNP kit (Cinagene, Iran) were used, respectively. DNA extraction of hepatitis B virus and RNA extraction of hepatitis C virus was separately performed based on protocol of kits from blood serum and plasma of patients, respectively. Quantification of DNA and RNA extraction was done by $1.6 < OD < 1.9$. Synthesis of cDNA was performed by a kit from Fermentas, Inc and Oligo (dT) primer in final volume of 20 ml and by revers transcriptase enzyme according to kit instructions.

2.2. HCV genotyping

HCV-Genotyping AG kit manufactured by Biodiversity Company (Italy) was used by

multiplex nested PCR method based on specific primers (Table 1).

2.3. HBV genotyping

HBV genotyping was performed by nested PCR method and using 3' PrsS2-F:5' GGG ACA CCA TAT TCT TGG-3' and TAG GGT TTA AAT GTA TAC CCA-3' S1-R: 5'- by A pair of external primers. This PCR product was performed by primers YS1-F: 5'-GCG GGG TTT TTC TTG TTG A-3', YS2-R: 5'-GGG ACT CAA GAT GTT GTA CAG-3. By this nested PCR method, a 585 bp piece of nucleotides 203 to 787 of S gene of HBV virus was replicated. Then, PCR products were purified by purification kit PCR product (Roche Diagnostics GmbH, Mannheim, Germany) and sent for sequencing. Sequence analysis using the software and reference genes was CEQ8000. Sequence analysis was performed using CLUSTAL W software, then the obtained sequence was arranged by reference from different genotypes.

2.4. Statistical Method

Results with demographic data were recorded on data sheets for each patient. Collected data were analyzed using SPSS 16.0 software after coding. The relationship between serum status of patients and each of Qualitative variables was evaluated by chi-square test in terms of viral molecular markers. Confidence coefficient was 95% in all calculations and $P < 0.05$ was considered significant.

3. Results

Out of 90 patients, 62 (68.9%) were men and 28 (31.1%) women. The mean age of participants was 55.51 ± 8.3 years. Of the 90 patients studied, 56 patients (62.2%) with HBV and 34 patients (37.8%) were infected with HCV. In all 56 patients with HBV, genotype D and in 34 patients with HCV, 1a, 1b and 3a was observed. The most frequent genotype was 1a (n=18, 20%), followed by 3a (n=14, 15.6%) and the least frequent genotype was 1b (n=2 2.2%) (Table 2).

Table 1. Oligonucleotide primers used for genotype determination

Step one			
5'-CGCGCGAGTAGCAAGACTTC-3'	139-158	Core	Sense HCV O1
5'-CGCGCGACTACGAAGACTTC-3'	139-158	Core	Sense HCV O2
5'-ATGTACCGGATGAGGTCGGC-3'	391-410	Core	Anti-sense HCV O
Step two			
5'-AGGAAGACTTCGGAGCGGTC-3'	148-167	Core	Sense HCV In1
5'-AGGAAGACTTCGGAACGATC-3'	148-167	Core	SenseHCV In2
5'-TGCCTTGGGCATAGGCTGAC-3'	185-204	Core	anti-sense HCV O1a
5'-GAGCCATCCTGCCCACCGGA-3'	272-291	Core	anti-sense HCV O1b
5'-CCAAGAGGGACGGGGAACGT-3'	302-321		anti-sense HCV O2a
5'-ACCCTCGTTTCCGTACAGAG-3'	251-270		anti-sense HCV O2b
5'-GCTGAGCCCAGGACCGGCT-3'	216-235		anti-sense HCV O3a

Table 2. Genotype distribution of hepatitis viruses B, C

Total	Number	Percent	Genotype
D	62.2	56	62.2
1a	20.0	18	82.2
1b	2.2	2	84.4
3a	15.6	14	100.0
Total	100.0	90	

4. Discussion

The present research is the first study to determine the genotypes of hepatitis viruses B, C among patients with HCV and HBV chronic infection in Guilan province. More than 5% of the world's population are suffering from chronic infection of HBV and HCV and is considered as one of the biggest health problems in the world as well as in our country (Farzadegan et al., 1979). The association of infection with HBV and HCV viruses and hepatocellular carcinoma (HCC) has been reported (Ganem et al., 2004).

According to the World Health Organization (WHO) in 2001 and center for disease control and prevention in 2005, Iran is among the prevalence of chronic hepatitis B is between 2% to 7% in Iran (CDC, 2005). It has been shown that that 3% of the general population of Iran had been suffering from chronic hepatitis B (Merat et al., 2003). In two other studies by Zali and colleagues conducted in 1990 and 1999 at a national level, the overall prevalence

of chronic hepatitis B infection was reported about 1.7 % (Zali et al., 2005). Overall, HCV is another common cause of viral hepatitis in the world, and the prevalence of this infection based on anti-C antibody is estimated about 1%. However, there are significant differences in prevalence of this infection (CDC, 2005).

HBV is classified into 8 genotypes (Kidd-Ljunggren et al., 2002). Hepatitis B virus genotypes are of special geographical distribution. Genotype D seems to be plentiful in the Mediterranean Sea region and the Middle East (Sharifi et al., 2008). The determination of HBV genotype is important to clarify the disease progression and pathogenicity of the virus since genotypes are different in terms of pathogenicity and response to treatment. Various methods including sequencing are used to determine the genotype of hepatitis B virus (Robinson et al., 2000).

Studies conducted in Iran to determine the genotype of hepatitis B, were more on patients and S and C genes of hepatitis B virus. Goodarzi and

colleagues in a study in 2007 examined the genotypes of HBV and reported that all isolated variations belong to genotype D (Goodarzi et al., 2007). In other studies in Tehran on 55 blood donors using PCR-RFLP method, it was identified that all variations had genotype D (Milani et al., 2009). Identified genotype in this study (genotype D) is consistent with the results of previous studies in Iran. In neighboring countries such as Turkey, Pakistan, Afghanistan and Russia, D is reported as the most common genotype. In India, Saudi Arabia and Egypt, D is the most common genotype. Therefore, the most common genotype reported in the eastern Mediterranean region is genotype D (Amini et al., 2006; Alam et al., 2007).

HCV has 6 major and 11 minor genotypes (Lau et al., 1996). The standard method for determining the HCV genotype is the determination of direct sequencing of conserved and amplified areas of virus genome by PCR method. However, determining the sequence of viral genome seems not practical due to technical problems and being expensive as well as lack of conventional methods and availability in all laboratories (Lopez-Labrador et al., 1997). Generally three methods of determining HCV genotypes that are available including: 1- Type-specific amplification with specific primers of genotype, 2- PCR by conserved primer followed by performing RFLP with restriction enzymes, and 3- hybridization of amplified DNA fragments of the 5-NCR area with marked specific probes of genotypes 1, 2 and 3 (Takada et al., 1993). Currently, determining HCV genotype by specific primers of virus genotype for core area which was first introduced by Okamoto and colleagues (Okamoto et al., 1993) is one of the most common methods of genotyping determination.

In this study, distribution of 1a, 1b and 3a genotypes of HCV in 34 patients were analyzed. The most frequent genotype was 1a in 18 patients (20%), then 3a in 14 patients (15.6%) and the least frequency dedicated to genotype 1b in 2 patients (2.2%). In a study by Asarzadegan et al. in Khuzestan province in 2008, the frequency 1a, 3a and 1b of HCV genotype were found 46.4%, 35.4% and 16.4%, respectively (Asara Zadegan et al., 2007). These results were consistent with our results. Jokar and colleagues in 2010 studied the HCV genotype on hemodialysis patients in Guilan

province and the most common genotypes were reported 1a (59.38%) and 3a (40.62%) (Joukar et al., 2009). In a study by Somi et al. similar results were obtained in East Azerbaijan in Iran (Somi et al., 2008). In another research by Keivani and colleagues on patients with chronic hepatitis C in Tehran, genotypes 1a and 3a were the most common (Keyvani et al., 2007). Confirming the above results in Tehran, Samimi Rad and colleagues also put genotypes 1b and 4 in next places (Samimi-Rad et al., 2004). In our western neighbor country (Turkey) genotype 1b was the predominant genotype (Adami et al., 2004). While in India and Pakistan, 3a was the dominant one which may play a role in high prevalence of genotype 3a in Iran (Somi et al., 2008).

Several studies about the relationship between response to treatment of hepatitis b and c have been performed with genotype and number of viruses (Bozdayi et al., 2002). An eminent clinical relevance exists between HCV genotype and the response to treatment. Patients with genotype 1a, showed a weaker medical response compared to 2a and 3a. Also, patients with genotype 1b are at increased risk of developing liver cirrhosis and hepatocellular carcinoma. A relationship between genotype 3a and hepatic stenosis has also been found (Somi et al., 2008). In a study in Japan in 2004 it has been reported that the response rate to interferon in patients with genotype 1b and high viral count was higher (Arase et al., 2004).

In another study in Japan, appropriate medical response in patients with high viral count and genotype 2a has been reported (Nakamura et al., 2002). In a study conducted in Norway on 71 HCV patients, it was noted that patients with genotypes 2b and 3a with low viral count without liver cirrhosis showed a better initial response to interferon treatment (Bjoro et al., 2002).

Knowing the genotype and the viral load of HBV and HCV in each region is important in disease development and treatment. Determining HBV and HCV genotypes is important to clarify the disease progression and pathogenicity of the virus.

References

- Adami, V., Falasca, E., Dorotea, L., Malangone, W., Astori, G., Marini, L., 2004. Qualitative multiplex RT-PCR for simultaneous detection of hepatitis C virus and human immunodeficiency virus in plasma samples. *Clin Microbiol Infect.* 10(12), 1075-80.

- Alam, M.M., Zaidi, S.Z., Malik, S.A., Shaukat, S., Naeem, A., Sharif, S., 2007. Molecular epidemiology of Hepatitis B virus genotypes in Pakistan. *BMC Infect Dis.* 7,115.
- Amini Babil-Olyae, S., Sarrami-Forooshanim, R., Adeli, A., Sabahi, F., Abachi, M., Azizi, M., 2005. Complete genomic sequence and phylogenetic relatedness of hepatitis B virus isolates from Iran. *J Med Virol.* 76 (3), 31-26.
- Amini-Babil-Olyae, S., Alavian, S.M., Adeli, A., Sarrami-Forooshani, R., Sabahi, F., Sabouri, E., et al. 2006. Complete genomic sequence and phylogenetic relatedness of hepatitis B virus genotyping, core promoter, and precore/core mutations among Afghan patients infected with hepatitis B: A preliminary report. *J Med Virol.* 78(3), 358-64 .
- Arase, Y., Suzuki, F., Tsubota, A., Suzuki, Y., Saitoh, S., Kobayashi, M., et al. 2004. Sustained negativity for HCV RNA over 24 or more months by long-term interferon therapy correlates with eradication of HCV in patients with hepatitis C virus genotype 1b and high viral load. *Intervirol.* 47,19-25.
- Asare zedegan, M., Shakeri, G.H., Norozi, R., Amini, A., 2007. Survey of HCV genotypes in patient with HCV infection at K Hozestan .*Medical Sciences Jour.* 7:25-30.
- Bjoro, K., Bell, H., Myrvang, B., Skaug, K., Raknerud, N., Sandvei, P., Storseth, S., Ritland, S., Lund-Tonnesen, S., Bucher, A., Hellum, K.B., 2002. Effect of interferon-alpha induction therapy on genotype 2b/3a and low viral load hepatitis C virus infection. A randomized multicentre study. *Scand J Gastroenterol.* 37, 344-9.
- Bozdayi G, Rota S, Verdi H, Derici U, Sindel S, Bali M, et al. The presence of hepatitis C virus (HCV) infection in hemodialysis patients and determination of HCV genotype distribution. *Mikrobiyol Bul.* 2002;36(3-4):291-300.
- CDC. 2005. A Comprehensive Immunization Strategy to Eliminate Transmission of Hepatitis B Virus Infection in the United States; Recommendations of ACIP Part 1: Immunization of Infants, Children, and Adolescents. *MMWR.* 54, RR-16.
- Chan, H.L., Ghany, M.G., Lok, A.S.F., 1999. Hepatitis B. In: Schiff ER, Sorell MF, Maddreg WC editors *Diseases of liver.* Ch. 29, 8th ed. Philadelphia: Lippincott Williams & Wilkins. 758-791.
- Combo, D. 2005. A Comprehensive Immunization Strategy to Eliminate Transmission of Hepatitis B Virus Infection in the United States; Recommendations of ACIP Part 1: Immunization of Infants, Children, and Adolescents. *MMWR.* 54, RR-16.
- Farzadegan, H., Harbour, C., Ala, F., 1979. The prevalence of hepatitis B surface antigen and its antibody in blood donors and high risk groups in Iran. *Vox Sang.* 37(3), 182-6.
- Ganem, D., Prince, A.M., 2004. Hepatitis B virus infection, natural history and clinical consequences. *N Engl J Med.* 1, 350.
- Goodarzi, Z., Malekzadeh, R., Montazeri, G., Alavian, S.M., Qurbanalizadgan, M., Daram, M., et al. 2007. Phylogenetic Analysis of HBV Based on PreS Region in Iranian Hepatocellular Carcinoma Patients. *Hepatitis Monthly.* 7(4), 201-5.
- Iloeje, U.H., Yang, H.I., Su, J., Jen, C.I., You, S.I., Chen, C.J., 2006. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology.* 130, 678-86.
- Joukar, F., Khalesi, A., Besharati, S., Mansour ghanee, F., 2009. Survey of HCV genotypes in hemodialysis patient with HCV infection at Guilan. *Medical Jour.* 28, 277-284.
- Keyvani, H., Alizadeh, A.H.M., Alavian, S.M., Ranjbar, M., Hatami, S., 2007. Distribution frequency of hepatitis C virus genotypes in 2231 patients in Iran. *Hepatology Research.* 37,101-3.
- Kidd-Ljunggren, K., Miyakawa, Y., Kidd, A.H., 2002. Genetic variability in hepatitis B viruses. *J Gen Virol.* 83 (Pt6), 1267-80.
- Lau, J.Y., Davis, G.L., Prescott, L.E., Maertens, G., Lindsay, K.L., Qian, K., et al. 1996. Distribution of hepatitis C virus genotypes determined by line probe assay in patients with chronic hepatitis C seen at tertiary referral centers in the United States. Hepatitis Interventional Therapy Group. *Ann Intern Med.* 124, 868-76.
- Lopez-Labrador, F.X., Ampurdanes, S., Forns, X., Castellas, A., Saiz, J.C., Costa, J., et al. 1997. Hepatitis C virus (HCV) genotypes in Spanish patients with HCV infection: relationship between HCV genotypes 1b, cirrhosis and hepatocellular carcinoma. *J Hepatol.* 27(6), 959-65.
- Merat, S., Malekzadeh, R., Rezvani, H., Khatibian, M., 2000. Hepatitis B in Iran. *Arch Iran Med.* 3, 192-201.45.
- Milani, S., Sharifi, Z., Hosseini, M., Mahmoodian, Shooshtari, M., 2009. Determination of HBV genotypes among HBs Ag positive blood donors in Tehran, Iran using PCR-RFLP. *Iranian J Publ Health.* 38(1), 41-7.
- Nakamura, H., Ogawa, H., Kuroda, T., Yamamoto, M., Enomoto, H., Kishima, Y., et al. 2002. Interferon treatment for patients with chronic hepatitis C infected with high viral load of genotype 2 virus. *Hepatogastroenterology.* 49, 1373-6.
- Okamoto, H., Tokita, H., Sakamoto, M., et al. 1993. Characterization of the genomic sequence of type V (or 3a) hepatitis C virus isolates and PCR primers for specific detection. *J Gen Virol.* 74(Pt 11), 2385-90.
- Robinson, S.W., 2000. Hepatitis B virus and hepatitis D virus. In: Mandell GL, Bennet JE, Dolin R. *Principles and Practice of Infectious Diseases.* 5th ed. New York: Churchill Livingstone. 1406-39.
- Samimi-Rad, K., Nategh, R., Malekzadeh, R., Norder, H., Magnius, L., 2004. Molecular epidemiology of hepatitis C virus in Iran as reflected by phylogenetic analysis of the NS 5B region: *J med virol.* 74(2), 246-52.
- Selcuk, H., Kanbay, M., Kormaz, M., Gur, G., Accay, A., Arslan, H., et al. 2006. Distribution of HCV genotypes in patients with end-stage renal disease according to type of dialysis treatment. *Dig Dis Sci.* 51(8), 1420-5.
- Sharifi, Z., Gahrebaghian, A., Norozi, M., 2008. Survey HBV genotyping by sequencing in blood donor at Kerman ,Esfahan, Yazd .*Blood Jour.* 6(4), 248-256.
- Somi, M.H., Keivani, H., Ardalan, M.R., Farhang, S., Pouri, A.A., 2008. Hepatitis C virus genotypes in patients with end-stage renal disease in East Azerbaijan, Iran. *Sudia journal of kidney disease and transplantation.* 19(3), 461-5.
- Takada, N., Takase, S., Takada, A., Date, T., 1993. Differences in the hepatitis C Virus genotypes in different countries. *J Hepatol.* 17(3), 277-83.
- Watanabe, K., Yoshioka, K., Yano, M., Ishigami, M., Ukai, K., Ito, H., Miyata, F., Mizutani, T., Goto, H., 2005. Mutations

- in the nonstructural region 5B of hepatitis C virus genotype 1b: their relation to viral load, response to interferon, and the nonstructural region 5A. *J Med Virol.* 75, 504-12.
- Zali, M.R., Mohammad, K., Noorbala, M., Nayer, B.N., Shahraz, S., Mohraz, M., 2005. HBsAg-Positivity Rate in Iran Following Hepatitis B Mass Vaccination. *East Mediterr Health Journal.* 11, 62-67.
- Zyzik, E., Gerlich, W.H., UY, A., Kochel, H., Thomssen, R., 1986. Assay of hepatitis B virus genome titers in sera of infected subject. *Eur J Clin Microbiol.* 5, 330-5.

Archive of SID