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Molecular epidemiology of hepatitis C virus genotypes in Bushehr province, Iran

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Abstract. – *Background and Objectives:* **Molecular epidemiology of hepatitis C virus (HCV) is very important for the treatment of hepatitis C infection. The aim of this study was to determine the distribution of HCV genotypes in Bushehr province (South West of Iran).**

Materials and Methods: **A total of 100 patients who were detected as positive for HCV antibody (by using ELISA method and RIBA test) referred to Arya Virology Laboratory between 2007-2009 in order to molecular diagnosis and furthermore virus genotyping. After detection of HCV, RNA genotyping of virus was done by using genotype specific primers.**

Results: **Genotype 1a was found in 49% of the patients and genotype 3a was found in 40% of the patients and 1b in 5% of patients, while the genotype of the virus could not be identified in 5% of the patients. Finally, in 1% of patients coinfection due to 1a-3a genotypes was identified.**

Conclusion: **The dominant genotype of HCV in Bushehr province, Iran, was determined as 1a.**

Key Words:

Hepatitis C virus, Genotyping, RT-PCR, Bushehr, Iran.

Introduction

Hepatis C virus (HCV) is an enveloped, single-stranded RNA virus, a member of the Flaviviridae family and genus hepacivirus[1,2]. HCV is a causative agent for chronic, acute and fulminant hepatitis[3,4]. About 75 percent of patients with acute hepatitis C ultimately develop chronic infection[1]. Only a minority of cases of acute HCV recover completely, with spontaneous virus eradication. In most cases the acute infection progresses to chronicity. Chronic HCV infection is defined as an infection that persists for more than 6 months, with or without clinical manifestations of hepatic or extrahepatic disease. Chronic type of this infection can cause cirrhosis, liver failure, and liver cancer. HCV infection is a global health problem and it is estimated that 200 million people of the world population are infected[5]. The global spread of chronic HCV infection coincided with the widespread use of transfused blood and blood products and with the expansion of intravenous drug use but decreased prior to the wide implementation of anti-HCV screening[6]. There are at least six major genotypes designated by Arabic numerals and more than 50 subtypes of HCV identified by lower case letters. The different genotypes have different geographic distributions[1,4]. Genotype determination of HCV is one of the most important factors in order to prediction of the viral persistency, pathogenicity and resistancy to antivirals[7]. The success and the treatment period of interferon and ribavirin seems to be related to the genotype of virus[8]. Furthermore, HCV genotyping is a useful tool to determine its molecular epidemiology, as they are indicative of transmission route of infection[9,10]. There is no published data about the distribution of HCV genotypes from Bushehr province (South West of Iran). Prevalence of HCV genotypes in Bushehr is an issue that is not sufficiently investigated and there is a need, therefore, to study this in detail.

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Materials and Methods

This research was approved by the Ethical Committee of Bushehr University of Medical Sciences. The sera were collected from 100 patients referred to the Arya Virology Laboratory from 2007 to 2009. The mean age of patients was 27.8 y.o.

SeroIgocical Assay

The sera were collected from 100 patients. All sera were initially tested for anti-HCV antibody by Enzyme Linked Immuno Sorbent Assay (ELISA) test (Ortho; HCV 3.0 ELISA Test System; Ortho-Clinical Diagnostics, Raritan, NJ, USA). For positive sera by ELISA, the confirmatory Recombinant Immunoblot Assay (RIBA) test (Deciscan HCV plus; Sanofi Diagnostics Pasteur, SA Marnes la Coquuette, France) was carried out[3].

The sera which were detected as positive for HCV antibody aliquoted and stored immediately at –70°C for further studies.

Extraction of Hepatitis C Virus RNA and cDNA Synthesis

In this study, RNA extraction kit (QIAGEN) was used in roder to HCV RNA extraction from the sera of patients. Extracted HCV-RNA was reverse transcribed into cDNA by using random primers (Sensicript RT kit; QIAGEN, Poison Information Centre Mainz, Germany)[11].

Hepatitis C Virus-RNA Detection

Synthesized cDNA was amplified by using nested-PCR which directed at the 5' untranslated region. Two sets of primers were synthesized (ROCHE; Roche Diagnostics GmbH, Roche Applied Science, Mannheim, Germany) which the first set 5`-AGC GTC TAG CCA TGG CGT -3`, called forward external primer, and 5`- GCA CGG TCT ACG AGA CCT-3`, named reverse external primer. The second set 5`-GTG GTC TGC GGA ACC GG-3`, called forward inner primer, and 5`- GGG CAC TCG CAA GCA CCC-3`, named reverse inner primer of 5'UTR region of HCV genome. The RT-PCR was performed as described previously[9].

Genotyping

Viral RNA which was extracted from the sera by using RNA extraction kit(QIAGEN) was immediately reverse transcribed to the cDNA as mentioned above. HCV genotyping was carried out by using primer based genotyping method as described previously[12].

Results

Out of 100 patients, 96 (96%) were males and 4 (4%) were females. All patients were positive for the presence of HCV RNA in their sera. The results of HCV genotyping by using primer based method showed that 49 samples (49%) were 1a, 40 (40%) were identified as 3a, 5 (5%) were detected as 1b, while the genotype of five samples(5%) didn't reveal in our study. Also, one patient (1%) was infected by two genotypes (1a and 3a) simultaneously.

Discussion

Epidemiological studies in different regions of the world show the differences in distribution of HCV genotypes. The geographical distribution of HCV genotypes is important for the epidemiological studies in terms of distribution and possible risk groups[13]. Also, HCV genotype determination is an important need for clinicians in order to decide about the duration of antiviral treatment[14]. In this context, the goal of our study was to identify the prevalence of HCV genotypes in Bushehr, Iran. Based on results, 49 patients (49%) were infected by 1a genotype, 40 patients (40%) showed the infection with genotype 3a, 5 patients (5%) were infected by 1b and 1 patient (1%) was coinfected by two genotypes simultaneously (1an and 3a). Meanwhile the genotype of 5 patients (5%) didn't identify. Our results are nearly similar to those studies which have already been conducted by different investigators in Tehran, Iran[15,16]. Our investigation confirmed that the dominant genotype in Bushehr province is 1a which is in accordance to other reports from the different area of Iran[9,15,16]. Regarding the scientific reports, the prevalence of chronic HCV infection in patients with liver diseases (chronic hepatitis, cirrhosis and hepatocellular carcinoma) is very high in southern Europe and Japan (60-90%), intermediate in other part of Europe, the United States, Australia and Africa (30-60%) and lower in China and other countries of the Far East (10-30%)[17,18]. The prevalence of chronic HCV carriers varies greatly far depending on geographical location and the characteristics of the population analysed. Low rates are found in the general adult population in North America and Western Europe, the Middle East and South Ameri-

ca. The number of infected individuals is particularly high in Egypt, in the elderly population in some Mediterranean areas, including southern Italy[19]. HCV prevalence in general population is less than one percent in Iran[20]. Higher rates have been reported in South East Asian countries, including India (1.5%), Malaysia (2.3%), and the Philippines (2.3%)[5]. HCV genotype 1a is prevalent in blood donors from Mexico[21]. HCV genotype 1b is dominant in the northeastern Bosnia and Herzegovina and Indonesia and Turkey[13,22,23]. HCV genotype 3a is the most predominant genotype in Pakistan and Thailan[24-26]. Also, in one study which was conducted in Pakistan, showed that there is a strong association between chronic HCV infection and hepatocellular carcinoma[24]. HCV genotype determination is important when treatment is being considered, since some genotypes respond more favorably to the medications. Also HCV genotyping could determine the length of therapy, e.g., treatment for genotypes 2 and 3 requires only 24 weeks while genotypes 1 and 4 require 48 weeks[7]. Also, the results of this study have some similarities with other studies in Pakistan and India, which showed that genotype 3 is very prevalent and genotype 2 is very rare[10,27]. Although high prevalence of 49% HCV 1a genotype and low prevalence of 5% 1b HCV genotype were observed among patients in Bushehr province, Iran.

References

1) Chronic hepatitis C: Current disease management. Available from: http://digestive.niddk.nih.gov/ddiseases/pubs/chronichepc/

2) Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Prevalence of hepatitis C virus (HCV) infection and HCV genotypes of hemodialysis patients in Salvador, Northeastern Brazil. Science 1989; 244: 359-362.

3) Silva LK, Silva MB, Rodart IF, Lopes GB, Costa FQ, Melo ME, Gusmão E, Reis MG. Prevalence of hepatitis C virus (HCV) infection and HCV genotypes of hemodialysis patients in Salvador, Northeastern Brazil. Braz J Med Biol Res 2006; 39: 595-602.

4) Alavian SM, Gholami B, Masarrat S. Hepatitis C risk factor in Iranian volunteer blood donors: A case-control study. J Gastrol Hepatol 2002; 17: 1092-1097.

5) Koop CE. Hepatitis C: An epidemic for any one, worldwide prevalence. DHMC 2008. Available from: http://www.epidemic.org/theFacts/theEpidemic/worldprevalence/.

6) Magiorkinis G, Magiorkinis E, Paraskevis D, Ho SY, Shapiro B, Pybus OG, Allain JP, Hatzakis A. The global spread og hepatitis C virus 1a and 1b: a phylodynamic and phylogeographic analysis. PLOS Med 2009; 6: e1000198.

7) Lee CM, Hung CH, Lu SN, Changchien CS. Hepatitis C virus genotypes: Clinical relevance and therapeutic implications. Chang Gung Med J 2008; 31: 16-25.

8) Park JC, Kim JM, Kwon OJ, Lee KR, Chai YG, Oh HB. Development and clinical evaluation of a microarray for hepatitis C virus genotyping. J Virol Methods 2010; 163: 269-275.

9) Farshadpour F, Makvandi M, Samarbafzadeh AR, Jalalifar MA. Determination of hepatitis C virus genotypes among blood donors in Ahvaz, Iran. Indian J Med Microbiol 2010; 28: 54-56.

10) Narahari S, Juwle A, Basak S, Saranath D. Prevalence and geographic distribution of hepatitis C virus genotypes in Indian patient cohort. Infect Genet Evol 2009; 9: 643-645.

11) Sambrook J, Russell D. Molecular cloning: A laboratory manual. 3 rd ed. CSHL Press; 2001.

12) Ohno O, Mizokami M, Wu RR, Saleh MG, Ohba K, Orito E, Mukaide M, Williams R, Lau JY. New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. J Clin Microbiol 1997; 35: 201-207.

13) Sanlidag T, Akcali S, Ozbakkaloglu B, Ertekin D, Akduman E. Distribution of hepatitis C virus genotypes in Manisa region, Turkey. Mikrobiyol Bul 2009; 43: 613-618.

14) Chevaliez S, Bouvier-Alias M, Brillet R, Pawlotsky JM. Hepatitis C virus (HSV) genotype 1 subtype identification in new HCV drug development and future clinical practice. PLOS one 2009; 4: e8209.

15) Samimi-Rad K, Nategh R, Malekzadeh R, Norder H, Magnius L. Molecular epidemiology of hepatitis C virus in Iran as reflected by phylogenetic analysis of the NS5B region. J Med Virol 2004; 74: 246-252.

16) Keyvani H, Alizadeh AH, Alavian SM, Ranjbar M, Hatami S. Distribution frequency of hepatitis C virus genotypes in 2231 patients in Iran. Hepatol Res 2007; 37: 101-103.

17) Pawlotsky JM. Use and interpretation of virological tests for hepatitis. Hepatology 2002; 36 (5 Suppl 1): S65-S73.

18) Pawlotsky JM. Molecular diagnosis of viral hepatitis. Gastroenterology 2002; 122: 1554-1568.

19) Alberti A, Benvegnù L. Hepatitis C. In: Textbook of hepatology: from basic science to clinical practice. Third Edition Ed. Juan Rodés, Jean-Pierre Benhamou, Andres T. Blei, Jürg Reichen, Mario Rizzetto. Blackwell Publishing, Malden, Massachusetts, USA, 2007. Vol. 1, pp 881-892.

20) Alavian SM. Hepatitis C, Chronic renal failure, control is possible. Hepatitis Monthly 2006; 6: 51-52.

21) Sosa-Jurado F, Santos-Lopez G, Guzman-Flores B, Ruiz-Conde JI, Melendez-Mena D, Vargas-Maldonado MT, Martinez-Laguna Y, Contreras-Mioni L, Vallejo-Ruiz V, Reyes-Leyva J. Hepatitis C virus infection in blood donors from the state of Puebla, Mexico. Virol J 2010; 7: 18.

22) Ahmetagic S, N Salkic N, Cickusic E, Zerem E, Mott-Divkovic S, Tihic N, Smriko-Nuhanovic A. Hepatitis C virus genotypes in chronic hepatitis C patients and in first time blood donors in northeastern Bosnia and Herzegovina. Bosn J Basic Med Sci 2009; 9: 278-282.

23) Utama A, Budiarto BR, Monasari D, Octavia TI, Chandra IS, Gani RA, Hasan I, Sanityoso A, Miskad UA, Yusuf I, Lesmana LA, Sulaiman A, Tai S. Hepatitis C virus genotype in blood donors and associated liver disease in Indonesia. Intervirology 2008; 51: 410-416.

24) Idrees M, Rafique S, Rehman I, Akbar H, Yousaf MZ, Butt S, Awan Z, Manzoor S, Akram M, Aftab M, Khubaib B, Riazuddin S. Hepatitis C virus genotype 3a infection and hepatocellular carcinoma: Pakistan experience. World J Gastroenterol 2009; 15: 5080-5085.

25) Waheed Y, Shafi T, Safi SZ, Qadri I. Hepatitis C virus in Pakistan: a systematic review of prevalence, genotypes and risk factors. World J Gastroenterol 2009; 15: 5647-5653.

26) Jutavijittum P, Jiviriyawat Y, Yousukh A, Pantip C, Maneekarn N, Toriyama K. Genotypic distribution of hepatitis C virus in voluntary blood donors of northern Thailand. Southeast Asian J Trop Med Public Health 2009; 40: 471-479.

27) Chowdhury A, Santra A, Chaudhuri S, Dhali GK, Chaudhuri S, Maity SG, Naik TN, Bhattacharya SK, Mazumder DN. Hepatitis C virus infection in the general population: A community-based study in West Bengal, India. Hepatology 2003; 37: 802-809.

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