Identification of HCV genotypes in HCV infected blood donors

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Abstract HCV infection is a leading cause of chronic liver disease, including cirrhosis of the liver. There are at least six major genotypes and more than 50 subtypes of HCV. The prevalence and distribution of HCV genotypes depend on geographical location. The aim of this study was to identify and compare the HCV genotypes in HCV infected blood donors and patients. In this cross-sectional study, 167 serum samples from 103 blood donors and 64 patients with hepatitis C were investigated for HCV genotypes. HCV genotyping was carried out using type-specific primers from the core region of the viral genome. The highest frequency was for genotype 1a, with 53 and 34 (51.5% versus 53.1%) of subjects in blood donors and patients respectively. Genotype 3a and 1b were the other frequent genotypes with 4 and 16 (3.9% versus 25%) and 39 and 10 (37.9% versus 15.6%) subjects, respectively. There was not any statistical significant association between the place of infection of the patients and genotype. The results of this study indicate that the distribution of genotypes in the two populations was similar. The dominant HCV genotypes between blood donors and patients were 1a, 3a and 1b respectively.

Keywords HCV · Genotypes · Blood donors · Patients

Introduction

The hepatitis C virus belongs to the Flaviviridae family and hepacivirus genus of viruses, and is spread primarily through direct contact with the blood or bodily fluids of infected individuals. HCV is a linear RNA virus, with a positive-sense single stranded genome of approximately 9600 nucleotides [1]. HCV infection is a leading cause of chronic liver disease, including cirrhosis of the liver.

The estimated global prevalence of HCV infection is 2.2%, corresponding to about 130 000 000 HCV-positive persons worldwide [1]. Region-specific estimates range from less than 1.0% in Northern Europe to more than 2.9% in Northern Africa. The lowest prevalence (0.01–0.1%) has been reported from countries in the United Kingdom and Scandinavia; the highest prevalence (15–20%) has been reported from Egypt [1]. An estimated 27% of cirrhosis and 25% of hepatocellular carcinoma (HCC) worldwide occur in HCV-infected people [1].

HCV currently infects an estimated 1.8% of the US population [2]. It is estimated that only 20% of infected individuals will recover from this viral infection, while the rest become chronically infected [3]. While the majority of chronically infected individuals never exhibit symptoms, approximately 10–30% of these patients will eventually develop cirrhosis or hepatocellular carcinoma, both of which are associated with significant morbidity and mortality [4]. HCV infection is currently estimated to cause 40–60% of chronic liver disease and as a consequence is recognized as the leading cause for liver transplantation in the United States [5].

In Iran, the Prevalence of HCV infection is about 0.12% in blood donor [6].

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It seems that the prevalence of HCV infection is less than 1 percent in general population in Iran, but the infection is emerging mostly because of problems such as intravenous drug use and needle sharing among drug addicts.

There are at least six major genotypes and more than 50 subtypes of HCV. These differ in nucleotide sequence by more than 30% over the complete virus genome. A number of subtypes, which differ in nucleotide sequence by more than 20% have also been described [7].

Some HCV genotypes are distributed worldwide, while others are more geographically confined. Genotypes 1a, 1b, 2a, 2b, 2c, and 3a account for more than 90% of the HCV infection in North and South America, Europe, Russia, China, Japan, Australia, and New Zealand [8]. Genotype 3a is more common among younger populations [8]. Other subtypes of genotype 3 are highly prevalent in Nepal, Bangladesh, India, and Pakistan [8]. Most infections in Egypt are genotype 4a, and this and other subtypes of genotype 4 are found in Central Africa [8]. Genotype 5a accounts for about 50% of infections in South Africa. Genotypes 4 and 5 are found only sporadically outside Africa. Genotype 6 isolates are primarily found in Southeast Asia [8]. It should be noted that the genotype distribution can vary significantly among different population groups in the same geographical area.

HCV genotype may be an important factor influencing the severity of liver disease. Infection with genotype 1b has been associated with more advanced liver disease and the development of both liver cirrhosis as well as hepatocellular carcinoma [9].

In this study, the distribution of HCV genotypes in blood donors and patients were identified. Then, the distribution of HCV genotypes in both groups were compared with each others.

Materials and methods

This cross sectional study was performed on blood donors and patients with HCV infection. The study population included 103 blood donors in the age range of 17–65 years who donated blood in Tehran Transfusion Center during the period December 2006 and *January* 2008 and also 64 patients in the age range of 10–65 years who attended in outpatient department of Iranian Medical Council, Hepatitis unit during the same period.

None of the blood donors and patients had been on antiviral therapy for HCV at the time of sampling for the study. All patients were also tested for HBsAg (DADE Behring, Germany) and HCV antibody by third generation enzyme immunoassay (Hepanostika HCV Ultra Biomerieux, France). Positive results of HCV antibody were confirmed

by the Recombinant Immuno Blot Assays (RIBA) HCV 20. All blood donors and patients were identified as HCV infected by a qualitative PCR earlier standardized in our laboratory.

All HCV infected blood donors were recalled and interviewed using a structured questionnaire. The sources of infection mainly included: intravenous drug abusers (IVDU), blood transfusion, tattooing, and surgery.

The HCV infected patients included patients presenting with chronic liver disease. The elicited risk factors for patients with HCV included intravenous drug abusers (IVDU), blood transfusion, renal dialysis, hemophilia, thalassemia and occupational exposure.

Viral RNA isolation

Viral RNA was extracted from 100 µl of serum using the Roche High Pure Viral RNA kit (Roche Diagnostics Corporation) as per the manufacturer's instructions. The viral RNA was eluted in 50 µl of nuclease free water.

Genotyping of HCV

In HCV RNA positive samples, genotypes were determined by performing PCR using primers specific for the core region of the HCV genome, using two separate reaction tubes containing different primer mixes, as described previously. This method allows for the determination of genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a and 6a in two separate reaction tubes [10].

For the reverse transcription -PCR, 1 µg of the extracted nucleic acid, 1.5 mM MgCl₂ and 1X PCR buffer containing 10 mM Tris-HCl 50 mM KCl (pH 8.3) 10mM DTT, 10 n mol of each dNTP and 25 pmol of outer primers in a total volume of 10 µl were used for reverse transcription. The reaction mixture was incubated at 95 °C for 5 min before the addition of 20U ribonuclease inhibitor (Roche Molecular Biochemicals) and 20 U of reverse transcriptase from avian myeloblastis virus (Roche Molecular Biochemicals). After 60 min at 42°C, the reaction was heated for 5 min at 95°C. Briefly, 2 µl of the cDNA was amplified in a 50 µl reaction volume containing 1.5 mM MgCl₂, 10 mM Tris-HCl, 50 mM KCl, and 2.5 pmol each of sense and antisense outer primers. The first round of amplification was performed under the following conditions: twenty cycles of amplification at 94°C for 1 min, 45°C for 1 min and 72°C for 1 min followed by an additional 20 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 1 min. One microlitre of first round product was taken as input for the second round PCR. The products of the second round PCR were electrophoresed on a 2% agarose gel. Samples were assigned genotypes based on the band size of the final amplified product, as recommended [10].

The χ^2 -test was used to analyze significance of difference between proportions.

Results

Samples were classified as belonging to genotype 1a, genotype 3a, and genotype 1b or as untypeable using the type specific primer based PCR. From 103 blood donors and the 64 patients, only 96 and 60 cases had typeable genotypes. The highest frequency was for genotype 1a, 53 and 34 (51.5% versus 53.1%) of subjects in blood donors and patients respectively. Genotype 3a was seen in 39 blood donors (37.9%) and 16 patients (25%). Genotype 1b was seen in 4 blood donors (3.9%) and 10 patients (15.6%). The HCV strains in 7 blood donors (6.8%) and 4 patients (6.3%) could not be typed using this genotyping technique. Genotype 1a was detected more frequently in age group 21–30 years old, as compared with other age groups in patients (Table 2). This difference was statistically significant ($P=0.00$).

Genotypes of HCV were detected more frequently in age groups 31–40 years old, as compared with other age groups in blood donors. Genotypes 1b, 3a and 1a were detected 3 (6.25%), 17 (35.42%) and 25 (52.1%) blood donors in age groups 31–40 years old respectively (Table 1). This difference was statistically significant ($P<0.01$). There was not any statistical significant association between the place of infection of the patients and genotype ($P>0.05$). Due to the patients had multiple routes of contamination, there was not any statistical significant association between the route of HCV transmission and genotype ($P>0.1$).

Discussion

HCV genotypes and subtypes are distributed differently in different parts of the world, certain genotypes predominate in certain areas. Genotypes 1–3 are widely distributed throughout the world. Subtype 1a is prevalent in North and South America, Europe, and Australia. Subtype 1b is common in North America and Europe, and is also found in parts of Asia. Genotype 2 is present in most developed countries, but is less common than genotype 1.

In the present study, 34 (53.1%) and 16 (25%) patients had genotype 1a and 3a respectively. Genotype 1a was seen in 53 (51.5%) blood donors and genotypes 3a and 1b were seen in 39 (37.9%) and 4 (3.9%) blood donors respectively. The results of this study indicate the predominance of HCV genotypes 1 and 3 in Iranian blood donors and patients. Similar studies in Iranian patients have reported the predominance of HCV genotypes 1a, 3a and 1b. In the first study in Iranian patients, the prevalence of specific genotypes in 15 samples was studied in Tehran. Seven patients were with genotype 1a, 3 patients with genotype 1b and four patients with genotype 3a. One patient was with genotype 4 [11]. The results of other studies on circulating HCV genotypes in the Iranian patients showed that genotypes 1a, 3a and 1b are predominant respectively and that type 4 is rare [12,13].

In comparison with Iran's neighboring countries, subtype 1b in Russia and Turkey [14, 15] and genotype 4 in Iraq and Kuwait [16] and also genotype 3a in Pakistan are more prevalent [17].

Table 1 Comparison of age-groups with genotypes of HCV in blood donors

HCV genotypes age-groups	1a No. (%)	3a No. (%)	1b No. (%)	Unknown No. (%)	Total No. (%)
17–20	0 (0)	2 (100)	0 (0)	0 (0)	2 (1.9)
21–30	14 (48.3)	12 (41.4)	0 (0)	3 (10.3)	29 (28.2)
31–40	25 (52.1)	17 (35.4)	3 (6.3)	3 (6.4)	48 (46.6)
41–50	9 (60)	4 (26.7)	1 (6.7)	1 (6.7)	15 (14.6)
51–60	5 (55.6)	4 (44.4)	0 (0)	0 (0)	9 (8.7)
Total	53 (51.5)	39 (37.9)	4 (3.9)	7 (6.8)	103 (100)

Table 2 Comparison of age-groups with genotypes of HCV in patients

HCV genotypes age-groups	1a No. (%)	3a No. (%)	1b No. (%)	Unknown No. (%)	Total No. (%)
11–20	3 (42.9)	2 (28.6)	2 (28.6)	0 (0)	7 (10.9)
21–30	18 (64.3)	7 (25)	2 (7.1)	1 (3.6)	28 (43.8)
31–40	7 (50)	4 (28.6)	2 (14.3)	1 (7.1)	14 (21.9)
41–50	4 (57.1)	2 (28.6)	1 (14.3)	0 (0)	7 (10.9)
51–60	1 (14.3)	1 (14.3)	3 (42.9)	2 (28.6)	7 (10.9)
>60	1 (100)	0 (0)	0 (0)	0 (0)	1 (1.6)
Total	34 (53.1)	16 (25)	10 (15.6)	4 (6.3)	64 (100)

HCV is spread primarily by contact with infected blood and blood products. With the introduction in 1991 of routine blood screening for HCV antibody and improvements in the test in mid-1992, transfusion-related hepatitis C has virtually disappeared. The distributions were similar for Subtypes 1a (53.1% versus 51.5%), 3a (25% versus 37.9%) in patients and donors respectively. Subtypes 1a and 3a most often infect injecting drug users and appear to be increasing in prevalence [18, 19]. At present, injection drug use is the most common risk factor for contracting the infection. Genotypes 1a and 3a are related to parenteral transmission, associated with intravenous drug abuse.

Intravenous drug abuse is the major risk factor for HCV infection [20]. There are several studies in Iran showing that injecting drug use is one of the main factors associated with the prevalence of HCV infection [20–23]. Intravenous drug abusers not only have the highest prevalence of HCV infection but also constitute a potential reservoir of HCV in the community [20]. In a study that was done in 2004 among 202 IVDU, 105 (52.0%) were found to be positive for HCV antibodies using oral samples [21]. In the present study, 43(41.7%) blood donors had history of intravenous drug users (IVDU) and tattooing. Genotype 1a was detected in age group 21–30 years old, as compared with other age groups in patients. The result of this study is similar with previous studies. A recently published article in Iranian patients showed that 60% patients with intravenous drug abuser had genotype 1a, also the majority of HCV infection in young people was detected as 1a [24].

Subtypes 1b and 2a are most strongly associated with infected blood products and the relative prevalence of these subtypes has decreased in recent years due to improved blood screening [25–26]. At the present study, Subtype 1b was significantly more prevalent in patients than donors (15.6% versus 3.9%, $p < 0.05$). HCV infection is the most prevalent cause of chronic hepatitis and cirrhosis in hemophiliac and thalassemic patients and patients with renal failure in Iran [27, 28].

The most likely explanations for this pattern 1b are concerned by blood products receiving hemophiliac and thalassemic patients. Recently the thalassaemia data bank has been reported that the prevalence of HCV infection in these patients is 16%. It has also reported that over 70% of thalassaemia patients in Iran are older than 10 years [29]. In Iran, the prevalence of anti-HCV in hemophiliac is from 15.65% to 76.7% in patients from various geographical regions [30]. The majority of positive cases among hemophiliacs reflect treatment with unscreened or not efficiently inactivated blood products in the past.

In the present study, genotypes 2 and 4 were not seen in HCV infected blood donors and patients. Genotype 4 is

prevalent in most of the Arab countries in the Middle East. It is also possible that Iranian patients with the history of travel to these countries such as Iraq, Saudi Arabia, and Lebanon and also mass migration from Iraq to Iran are the cause of changing the distribution of HCV genotypes in Iran in future.

In conclusion, genotypes 1 and 3 were most frequently found in this geographical region. It was also seen that more of the blood donors and patients infected with genotypes 1a and 3a had history of intravenous drug users (IVDU) and tattooing. Subtype 1b was significantly more prevalent in patients than blood donors that it is due to population samples of patients were hemophiliac and thalassemic patients.

References

1. Alte MJ (2007) Epidemiology of hepatitis C virus infection. *World J Gastroenterol* 7; 13(17):2436–2441
2. Alte MJr, Kruszon-Moran D, Nainan OV, McQuillan GM, Gao F, Moyer LA, et al. (1999) The epidemiology of hepatitis C virus infection in the United States, 1988 through 1994. *N Engl J Med* 341:556–562
3. Cohen J (1999) The scientific challenge of hepatitis C. *Science* 285:26–30
4. Di Bisceglie AM, Order SE, Klein JL, Waggoner JG, Sjogren MH and Kuo G (1991) The role of chronic viral hepatitis in hepatocellular carcinoma in the United States. *Am J Gastroenterol* 86:335–338
5. Arens M (2001) Clinically relevant sequence-based genotyping of HBV, HCV, CMV and HIV. *J Clin Virol* 22: 11–29
6. Alavian SM, Gholami B and Masarrat S (2002) Hepatitis C risk factors in Iranian volunteer blood donors, a case control study. *J Gastroenterol Hepatol* 17:1092–1097
7. Simmonds P, Holmes EC, Cha TA, Chan SW, McOmish F, Irvine B, Beall E, Yap PL, Kolberg J and Urdea MS (1993) Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. *J Gen Virol* 74:2391–2399
8. Maertens G and Stuyver L (1997) Genotypes and Genetic Variation of Hepatitis C Virus. *The Molecular Medicine of Viral Hepatitis*. Chichester, England, John Wiley & Sons
9. Forns X and Bukh J (1999) *The Molecular Biology of Hepatitis C Virus: Genotypes and Quasispecies*. *Clinics in Liver Disease* 3(4):693–716
10. Ohno T, Mizokami MR, Wu R, Saleh MG, Ohba KI, Orito E, Mukaide M, Williams R and Lau JYN (1997) New hepatitis C virus genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a and 6a. *J Clin Microbiol* 35:201–207
11. Zali MR, Mayumi M, Raoufi M and Nowroozi A (2000) Hepatitis C virus genotypes in the Islamic Republic of Iran: a preliminary study. *East Mediterr Health J* 6:372–377
12. Keyvani H, Alizadeh AHM, Alavian SM, Ranjbar M and Hatami S (2007) Distribution of frequency of hepatitis C virus genotypes in 2231 patients in Iran. *Hepatology Research* 37(2):101–103

13. Samimi-Rad K, Nategh R, Malekzadeh R, Norder H and Magnius L (2004) Molecular epidemiology of hepatitis C virus in Iran as reflected by phylogenetic analysis of the NS5B region. *J Med Virol* 74:246–252
14. Viazov S, Kuzin S, Paladi N, Tchernovetsky M, Isaeva E, Mazhul L, Vasychova F, Widell A and Roggendorf M (1997) Hepatitis C virus genotypes in different regions of the former Soviet Union (Russia, Belarus, Moldova, and Uzbekistan). *J Med Virol* 53:36–40
15. Abacioglu YH, Davidson F, Tuncer S, Yap PL, Ustacelebi S, Yulug N and Simmonds P (1995) The distribution of hepatitis C virus genotypes in Turkish patients. *J Viral Hepatol* 2: 297–301
16. Mellor J, Holmes EC, Jarvis LM, Yap PL and Simmonds P (1995) Investigation of the pattern of hepatitis C virus sequence diversity in different geographical regions: implications for virus classification. *J Gen Virol* 76:2493–2507
17. Shah HA, Jafri W, Malik I, Prescott L and Simmonds P (1997) Hepatitis C virus (HCV) genotypes and chronic liver disease in Pakistan. *Gastroenterol Hepatol* 12:758–761
18. Dal Molin G, Ansaldi F, Biagni C, D'Agaro P, Comar M, Croce I, Tiribelli C and Campello C (2002) Changing molecular epidemiology of hepatitis C virus infection in northeast Italy. *J Med Virol* 68(3):352–356
19. Kalinina Q, Norder H, Vetrov T, Zhdanov Z, Barzunova M, Plotnikova V, Mukomolov S and Magnius LO (2001) Shift in predominating subtype of HCV from 1b to 3a in St. Petersburg mediated by increase in injecting drug use. *J Med Virol* 65:517–524
20. Alavian SM, Adibi P and Zali MR (2005) Hepatitis C virus in Iran: epidemiology of an emerging infection. *Arch Iranian Med* 8(2):84–90
21. Zamani S, Ichikawa S, Nassirimanesh B, Ichikawa K, Vazirian M, Gouya MM, Ravari SM, Ono-Kihara M, Afshar P and Kihara M (2007) Prevalence and correlates of hepatitis C virus infection among injecting drug users in Tehran. *International Journal of Drug Policy* 18:359–363
22. Zali MR, Aghazadeh R, and Nowroozi A and Amir-Rasouly H (2001) Anti-HCV antibody among Iranian IV drug users: is it a serious problem? *Arch Iranian Med* 4:115–119
23. Alavian SM (2005) Are the real HCV infection features in Iranian patients the same as what is expected? *Hepatitis Monthly* 5(1):3–5
24. Hajiani E, Hashemi J, Masjedizadeh R, Shayesteh A A, Idani E and Rajabi T (2006) Seroepidemiology of hepatitis C and its risk factors in Khuzestan Province, south-west of Iran: A case-control study. *World Journal of Gastroenterology* 14:4884–4887
25. Hejazi MS, Ghotaslou R, Farshdoosty Hagh M and Mohammadzadeh Sadigh Y (2007) Genotyping of Hepatitis C Virus in Northwest of Iran. *Biotechnology* 6(3):302–308
26. Elghouzzi M H, Bouchardeau F, Pillonet J, Boiret E, Tirtaine C, Barlet V, Montcharmont P, Maisonneuve P, Puy-Montbrun M C, Lyon-Caen D and Courouce A M (2000) Hepatitis C Virus: routes of infection and genotypes in a cohort of anti-HCV positive French blood donors. *Vox Sang.* 79:138–144
27. Alavian SM, Ardesliri A and Hajarizadeh B (2001) Seroprevalence of anti-HCV Ab among Iranian hemophilia patients. *Transfusion Today* 49:4–5
28. Alavian SM, Kafaee J, Yektaparast B, Hajarizadeh B and Doroudi T9 (2002) The efficacy of blood donor screening in reducing the incidence of hepatitis C virus infection among thalassemic patients in Iran. *Transfusion Today* 53:3–4
29. Alavian SM, Einollahi B, Hajarizadeh B, Bakhtiari S, Nafar M and Ahrabi S (2003) Prevalence of hepatitis C virus infection and related risk factors among Iranian hemodialysis patients. *Nephrology* 8:256–260
30. Abolghssemi H, Amid A and Zeinali S, et al. (2007) Thalassemia in Iran. *Journal of Pediatric Hematology/Oncology* 29:233–238