Hepatitis C virus infection and genotypes in blood donors

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Abstract: Genotyping of the hepatitis C virus (HCV) is important for designing therapeutic strategies and regional specific diagnostic assays. The aim of this study was to identify the HCV genotypes in HCV infected blood donors. This is the first report on HCV genotypes in blood donors in Iran. In this cross-sectional study, 103 blood donors 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. From 103 blood donors, only96 cases had genotypes which could be typed. The highest frequency genotype 1a, with 53 (51.5%) of subjects. Genotype 3a and 1b were the other frequent genotypes with 39 (37.9 %) and 4 (3.9%) subjects, respec-tively. These results indicate that the dominant HCV genotypes among blood donors were 1a, 3a and 1b respectively. It was also noticed that more of the blood donors infected with genotypes 1a and 3a had history of intravenous drug abuse and tattooing.

Key Words: ❺HCV ❺genotypes ❺blood donors ❺Iran

Introduction

Hepatitis C virus is the main causative agent of chronic post-transfusion hepatitis and poses an elevated risk for development of liver cirrhosis and hepatocellular carcinoma. HCV infects approximately 3% of the world population, an estimated 200 million people (1).

Epidemiological studies in different regions of the world show wide variance in HCV prevalence patterns, though it is clearly evident that the incidence of HCV is higher among less developed nations. The prevalence of hepatitis C is lowest in Northern European countries, including Great Britain, Germany and France. The prevalence of HCV antibodies in blood donors averages less than 1% for these regions. In contrast, higher rates have been reported in Southeast Asian countries, including India (1.5%), Malaysia (2.3%), and the Philippines (2.3%). Alarming rates were reported for many African nations, reaching as high as 14.5% in Egypt (2).

In Iran, the Prevalence of HCV infection is about 0.12% in blood donors (3).

It seems that the prevalence of HCV infection is less than 1 percent in general population, but the infection is emerging mostly because of problems such as intravenous drug abuse and needle sharing among drug addicts. HCV infection is the most prevalent cause of chronic hepatitis and cirrhosis in hemophiliac and thalassemic patients and patients with renal failure in Iran (4-6).

HCV is spread primarily by contact with infected blood and blood products. Blood transfusion and the use of shared, unsterilized, or poorly sterilized needles, syringes and inject-ion equipment have been the main routes of the spread of HCV.

With the introduction of routine blood screening for HCV antibody and improvements in the tests for detecting HCV in mid-1992, transfusion-related hepatitis C has virtually disappeared. At present, injecting drug abuse and Hemodialysis are the most common risk factor for contracting the infection.

HCV is a small (40 to 60 nanometers in diameter), enveloped, single strand-ed RNA virus of the family Flavi-viridae and genus hepacivirus. There are at least six major genotypes and more than 50 subtypes of HCV (7). 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 (8-9).The genotypes of HCV show a distinct geographical distribution. Genotypes 1a, 1b, and 2a are the predominant genotypes in the United States and Western Europe. Genotype 4 is the predominant genotype of the Middle East. Types 5 and 6 are largely confined to South Africa and South East Asia, respectively (10)

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[DOI: 10.21859/isv.1.4.17]

Moreover, as it is known, the current screening assays are based on epitopes derived from only genotypes 1a or 1b; causing variation in seroreactivity among different HCV genotypes. Therefor, development of serological screening assays would require antibodyies to all the genotypes and variants of hepatitis C.
The aim of this study was to identify the HCV genotypes in HCV infected blood donors .This is the first report on HCV genotypes in blood donors in Iran.

Materials and methods

This cross sectional study was performed on 103 blood donors with HCV infection from blood transfusion centers in the different provinces, between the period December 2006 and January 2008. The number of 34(or 35) samples from blood transfusion centers Tehran, Kerman and Kermanshah were collected. The frozen samples were placed on dry ice were carried to our laboratory and stored at -80˚C freezer. A questioner was applied in order to assess employment status and to evaluate potential risk factors for infection. The sources of infection were mainly included: intravenous drug abusers (IVDU), blood transfusion, tattooing, and surgery. The study population included 101 (98.1%) men and 2 (1.9 %) women, in the age range of 17–60 years. None of the blood donors had been on anti-viral therapy for HCV at the time of sampling for this study. All of the 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 BLOT 3.0.
All blood donors were identified as HCV infected by a qualitative PCR earlier standardized in our laboratory.
Viral RNA was extracted from 100 µl of serum using the Roche High Pure Viral RNA kit (Roche Diagnostics Corporation) according to the manufacturer's instructions .The viral RNA was eluted in 50 µl of nuclease free water.
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 (11). This method allows for the determination of genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a and 6a in two separate reaction tubes (11).
For the reverse transcription -PCR, 8 µl of the extracted nucleic acid, 1.5 mM MgCl2 and 1X PCR buffer containing 10 mM Tris-HCl50 mM KCl(pH 8.3) 10mM DTT, 10 nmol of each dNTP and 25 pmol of outer primers (11) in a total volume of 10 µl were used. The reaction mixture was incubated at 95 ºC 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 42ºC, the reaction was heated for 5 min at 95ºC. Briefly, 2 µl of the cDNA was amplified in a 50 µl reaction volume containing 1.5 mM $MgCl_2$, 10 mMTris–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

Table 1. Comparison of age-groups with genotypes of HCV.

HCVgenotypes / age-groups	1a No.	1a (%)	3a No.	3a (%)	1b No.	1b (%)	Unknown No.	Unknown (%)	Total No.	Total (%)
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)

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min and 72 °C for 1 min. For Second round of PCR, two mixes were made. One mix contained primers for the specific detection of HCV genotypes 1b, 2a, 2b and 3b. The second mix contained primers for the selective amplification of genotypes 1a, 3a, 4, 5a, and 6a. 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. The products were viewed on an UV transilluminator. Samples were assigned genotypes based on the band size of the final amplified product, as recommended (11).

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. The genotype profile most frequently detected was genotype 1a seen in 53 (51.5%) blood donors followed by infection with genotype 3a seen in 39(37.9%)(Table 1).Genotype 1b was seen in four blood donors (3.9%). The HCV strains in 7 blood donors (6.8 %) could not be typed using this genotyping technique. Infected blood donors were detected more frequently in age groups 31-40 years old, as compared with other age groups. Genotypes 1b, 3a and 1a were detected 3(6.25%), 17 (35.42%) and 25 (52.1%) in blood donors in age groups 31-40 years old respectively. 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). In the present study, 52(50.5%), 12(11.7%) and 43(41.7%) blood donors had history of minor surgery, transfusion, intravenous drug users (IVDU), and tattooing respectively. Since HCV infected blood donors had multiple routes of acquiring the infection, no statistically significant association between the route of HCV transmission and genotype (P>0. 1) was found.

Discussion

Infection by hepatitis C virus (HCV) is the leading cause of chronic liver disease worldwide. Hepatitis C shows significant genetic variation in worldwide populations, evidence of its frequent rates of mutation and rapid evolution. There are six basic genotypes of HCV which vary in prevalence across different region of the world. Each of these major genotypes may differ significantly in their biological effects in terms of replication, mutation rates, type and severity of liver damage, and detection and treatment option.

The results of this study indicate the preponderance of HCV genotypes 1a and 3a in Iranian blood donors. Similar studies in Iranian patients have reported the predominance of HCV genotypes 1a, 3a and 1b. In the first report in Iranian patients, the prevalence of specific genotypes in 15 samples was studied in Tehran. Seven patients were with genotype 1a, three patients with genotype 1b and four patients with genotype 3a. One patient was with genotype 4(12). 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 (13-14).

Genotypes 1b, 3a and 4 are prevalent in our neighboring countries. Genotype 1b in Russia (15) and Turkey (16), Genotype 3a in Pakistan (17) and also Genotype 4 in Iraq are more prevalent (18).

In comparison with Middle East countries, although genotype 4 is found in most of the Arab countries (Yemen, Kuwait, Lebanon, Iraq, and Saudi Arabia) in the Middle East (19), there is a contrast with data reported from non-Arab Middle East countries in this region including our country. In the present study, genotype 4 was not seen in infected blood donors with HCV. Previous study has shown that genotype 4 is rare (12). Genotype 4 is prevalent in most of the Arab countries in the Middle East. In the future, it is possible that Iranian patients with the history of travel to these countries (Iraq, Saudi Arabia, and Lebanon) cause changing in the distribution of HCV genotypes in Iran.

Some studies suggest that different types of HCV may be associated with different transmission routes. Subtype 3a appears to be prevalent among injection drug abusers and it is believed that they were introduced into North American and the United Kingdom with the widespread use of heroin in the 1960s. Genotypes 3a and 1a are more prevalent in IVDU in Europe and USA (20-22).

Fifty-two (50.5%) and 6 (5.8%) were blood donors were the first- time and repeat donors. Forty-five (43.7%) blood donors had previous experience of blood donation (multiple-time donors). There was no significant association between HCV genotypes and the number of blood donations (P=0.20) There was no significant association between the place of HCV infected blood donors and genotypes.

Genotypes 1a, 3a and 1b are the most common genotypes respectively in Iran. In the present study, 43(41.7%) blood donors had history of intravenous

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drug users (IVDU) and tattooing. Despite that 29(28.2%) and 14(13.6%) of blood donors had history of intravenous drug abuse (IVDU) and tattooing, but there was no significant association between HCV genotypes and mode of transmission (P=0.9). Genotypes 1a and 3a most often infect injecting drug abusers and appear to be increasing in prevalence (23-24).Genotype 1b was seen in four blood donors (3.9%). Genotypes 1b and 2a are most strongly associated with infected blood products and the relative prevalence of these genotypes has decreased in the recent years due to improved blood screening (25-26). Mocmish et al investigated on infected volunteer blood donors from nine countries (Scotland, Finland, The Netherlands, Hungary, Australia, Egypt, Japan, Hong Kong, and Taiwan).They showed the presence of genotypes 1, 2, and 3 for almost all infections in donors from Scotland, Finland, Netherlands, and Australia (27). They reported that genotypes 2 and 3 were not found in the eastern European country (Hungary), where all but one of the donors was infected with type 1(27). Donors from Japan and Taiwan were infected only with type 1 or 2, while types 1, 2, and 6 were found in those from Hong Kong. HCV infection among Egyptians was almost always by type 4 (27). The genotype distribution of hepatitis C virus (HCV) in blood donors from southeast France showed that the most frequent genotypes were 1b (30.2%), 1a (27.7%), and 3a (22.4%)(28).In another study in Brazil country, the proportions of blood donors with HCV types 1, 2, and 3 were 70.0%, 2.9%, and 25.7%, respectively(29). The study on HCV genotypes among blood donors in Korea showed that HCV genotype 1b was most dominant (80%), followed by genotype 2a (13.3%), and 2b (6.7%) (30). Therefore, distribution of genotypes HCV in infected blood donors in Iran is similar to epidemiological data from West Europe.

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