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 hepato cellular 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].
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-HCl50 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 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 MgCl2, 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 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].