

The Different Distribution of Hepatitis C Virus Genotypes in Eastern Black Sea Region of Turkey

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Abstract

Hepatitis C virus (HCV) has infected about 170-200 million people worldwide and HCV genotypes have been related with a particular geographic distribution. The information of genotypes in chronic hepatitis C is critical for the decision of the pharmaceutical regimen, and for the therapeutic outcome. The aim of the study was determining the dispersion of hepatitis C Virus genotype in our area. The study was conducted on 42 patients who referred from various clinics infected with HCV. Serum HCV RNA levels were quantified by using the COBAS AMPLICOR HCV Monitor 2.0. An 851 bp long fragment spanning codons 63 to 347 of the RNA-dependent RNA polymerase in the NS5b part of the HCV genome was amplified. PCR amplifications were conducted in a BioRad DNA Engine using Quantitect SYBR Green PCR mix. Purified PCR products expressively sequenced with the ABI PRISM 310 Genetic Analyzer appliance utilizing DYEnamic ET Terminator Cycle Sequencing Kit. The most prevalent genotype was type 1b (90.4%) in our study. In the other types, type 3 and 4 were detected.

We believe that distribution of HCV genotype in this region should be followed strictly due to difference from reports, which reported other parts of Turkey.

Keywords: Genotype; Hepatitis C virus; PCR; RNA; Sequencing

Introduction

Hepatitis C virus (HCV) has infected about 170-200 million people worldwide, and only half of patients treated with the actual accepted therapies achieve a sustained viral reply [1,2]. In industrial domains, the HCV accounts for 20% of acute and 70% of chronic cases of hepatitis [3]. The majority of persons with recently acquired HCV infection are asymptomatic. Only 20% of patients develop complaints, such as fatigue and abdominal suffering. Once affected, 15-25% of HCV-infected individuals are able to discharge the virus without treatment. The remainders go on to develop chronic hepatitis C [4]. 50% of patients with progressive hepatitis can be healed with antiviral treatment if the infection is diagnosed in time and treatment is achievable [5]. Hepatitis C virus (HCV) is an enveloped, about 9.6 kb, positive-sense, single-stranded RNA virus and is classified in the family *Flaviviridae* [6]. All of its proteins are encoded in a single open reading frame that encodes a polyprotein of about 3010 amino acids [7]. HCV emerges abundant genetic heterogeneity, as a result of aggregation of mutations during replication. The genetic heterogeneity is not evenly distributed across the genome, the most highly conserved regions of the genome are parts of the 5' non-coding region (NCR) and the terminal 3' NCR, followed by the core region. Accumulation of nucleotide substitution in the HCV genome results in alteration and evolution into distinct genotypes, subtypes [1]. At least six major HCV genotypes and hundreds of subtypes have been determined based on nucleotide distinctness within core worldwide so far [7-9]. The knowledge of genotypes in chronic hepatitis C is crucial for the decision of the therapeutic regimen and for the therapeutic outcome; hence genotypes 2 and 3 are curable in more than 80%, whereas genotypes 1 and 4 are curable in only 40-50% of circumstance [5]. The HCV genotypes have been related with a precise geographic dispersion. Some seem to have spread worldwide (genotypes 1a, 1b, 2a, 2c, 3a), while others have been detected in more restricted areas only (genotypes 4, 5a, and 6a) [10].

HCV genotype 1b was reported as the most common in Turkey [11,12]. Our hospital is located in the Eastern Black Sea region and

sometimes accepts patients from neighboring countries, particularly in Georgia, and there is not a report published in this area. Therefore, the aim of the study was determined the genotype distribution of HCV in our region.

Materials and Methods

This study was conducted on 42 patients (19 females, 23 males, mean age 49.3 ± 16.30 years) who referred from various clinics infected with HCV. The Table 1 shows the demographic features of patients. All patients had chronic active hepatitis, as approved by ultrasonographic investigation, abnormal aminotransferase levels were seropositive

Patients, n	42
Gender, M/F (%)	23/19
Age, mean year (range)	49.38 ± 16.30
AST, median U/L (range)	89.8 ± 26.8
ALT, median U/L (range)	102.0 ± 92.7
HBV viral load, median (range) (copies/mL)	139355.2 ± 1.38
Co infection status	
HCV+HBV	nd
HCV+HIV	nd

M/F: Male/female
nd: Not detected

Table 1: The demographic features of patients.

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for anti-HCV antibodies, and were also positive by polymerase chain reaction (PCR) testing for HCV RNA. All statistical analyses were performed using SPSS software version 18.0.

Detection of HCV RNA

Blood samples were centrifuged immediately, sera/plasma were separated, aliquoted and then kept at -70°C until testing. HCV RNA was isolated from serum/plasma samples by a biorobot workstation using magnetic-particle technology (NucliSENS-easyMAG, BioMérieux, Boxtel, Holland). Samples were assayed for HCV RNA by reverse transcription (RT)-PCR with primers from the NS5B part of the viral genome. Serum HCV RNA levels were accounted by using the COBAS AMPLICOR HCV Monitor 2.0 (Roche Diagnostics GmbH, Mannheim, Germany). For each sample and control, the Cobas Amplicor Analyzer (Roche Diagnostics GmbH, Mannheim, Germany) automatically determined the HCV RNA titer.

HCV genotyping

HCV RNA was extracted from patient serum using the Ionpure Viral Nucleic Acid Isolation Kit (Iontek Inc., Istanbul, Turkey). A 851 bp long fragment spanning codons 63 to 347 of the RNA-dependent RNA polymerase in the NS5b domain of the HCV genome was amplified using Primer-F: CAYTACCRGGACGTGCTCAAGGAG and Primer-R: TACCTRGTCATAGCCTCCGTGAA. PCR amplifications were conducted in a BioRad DNA Engine® (Bio-Rad, Mexico city) using Quantitect SYBR Green PCR mix (QIAGEN GmbH, Hilden, Germany). HCV RNA was reverse transcribed and amplified in a single-step RT-PCR reaction starting with 40 min at 50°C and an initial denaturation for 15 min at 95°C, followed by 45 cycles at 95°C for 40 s, 54°C for 60 s and 72°C for 90 s. After the last cycle, samples were incubated for an additional 5 min at 72°C. Three microlitres of each reaction mixture were analyzed by electrophoresis through a 1% agarose gel. All PCR products were purified using the High Pure PCR Products Purification Kit® (Roche Diagnostics GmbH, Mannheim, Germany), and straightly sequenced with the ABI PRISM 310 Genetic Analyzer® equipment using DYEnamic ET Terminator Cycle Sequencing Kit® (Amersham Pharmacia Biotech Inc, Piscataway, USA). The forward primer was used as the sequencing primer (primer-F: CAYTACCRGGACGTGCTCAAGGAG).

Results

The outcomes of this study established that the most prevalent genotype among these patients is genotype 1b (38 patients, (90.47%)). In the other genotypes, type 3 and 4 were detected (type 3 in 3 patients (7.14%) and type 4 in 1 patient (2.38%), 2 of patients were from neighboring countries).

Discussion

HCV has remarkable genetic heterogeneity, and as a result of comparative analysis of complete or partial genomic sequences of HCV from different geographic areas identified, at least six major genotypes based on a sequence divergence of 30% among isolates [13,14]. Some regions of HCV genome, such as E1/E2 are highly variable and the open reading frame length of each genotype is characteristically different [15,16]. The reference procedure for HCV genotype determination is direct sequencing of the NS5B or E1 areas of HCV genome by means of “in-house” techniques, followed by sequence alignment with prototype sequences and phylogenetic analysis. These methods must be used in molecular epidemiology studies, where exact subtyping is needed [17]. Restriction fragment length polymorphism could be insufficient

for subtyping of HCV genotypes [18]. In clinical performance, HCV genotype can be determined by distinct commercial kits, using direct sequence analysis of the 5'NCR or reverse hybridization analysis using genotype specific probes located in the 5'NCR. Usually, mistyping is rare with these techniques [17].

Recently, hepatitis C treatment has been increasingly used in some areas and/or risk community; it would be expected to have an impact on the most recent distribution of HCV genotypes [19]. While genotype 3a is very sensitive to interferon medication compared to the other genotypes, genotype 1b is more resistant to interferon in HCV genotypes [20,21]. In Europe and in North and South America, HCV subtypes 1a and 1b have been reported as the most prevalent genotype, likewise subtype 1b is responsible in most of HCV infection in Japan [9,13]. In other subtypes, HCV-2a and 2b subtypes are mostly common in North America, Europe and Japan, and subtype 2c is found commonly in northern Italy [9]. HCV subtype 3a is the most common genotype circulating in India, Nepal and Pakistan [20]. In the other types of HCV, genotype 4 is common in the Middle East, and in Africa, HCV genotype 5 and HCV genotype 6 are establish only in South Africa and Hong Kong, respectively [9,10].

In southern part of Turkey, the prevalence of HCV genotypes were detected as 82.2% for genotype 1b, 14.5% for genotype 1a and 3.3% for 2a [18]. In another study conducted in our country, the prevalence of genotype 3a was detected as 4% [22]. The prevalence of HCV genotypes were detected as 97.4% for genotype 1b, 2.6% for 1a in western Black-Sea region of Turkey [23]. These findings are different from ours especially for genotype 3, prevalence of genotype 3 is higher than reported studies in our study [18,22]. In general, 2-10% of anti-HCV-positive patients have markers of HBV infection, but co-infection with HBV and HIV has not been determined in our study [24].

While the genotype 1b was found as the most common genotype among chronic hepatitis C patients, higher prevalence of genotype 3 in our study may be due to regional difference. As mentioned above, regional differences exist this difference may be related to acceptance of patients from neighboring countries. We believe that distribution of HCV genotype in this region should be followed strictly due to difference from reports which reported from Turkey.

Conflict of Interest

The authors declare no conflict of interest.

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