Gene Reports 4 (2016) 190–193

Contents lists available at ScienceDirect

Gene Reports

journal homepage: www.elsevier.com/locate/genrep

Genotype distribution of hepatitis C virus among Lebanese patients: Results from a major tertiary care center

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ARTICLE INFO

Article history:
Received 14 March 2016
Received in revised form 16 May 2016
Accepted 5 June 2016
Available online 16 June 2016

Keywords:
Hepatitis C
Virus
Genotype
Lebanon

ABSTRACT

Aims: The determination of hepatitis C virus (HCV) genotype is a crucial element in the therapeutic management of HCV infected patients. Studies showed that different treatment responses were obtained for different genotypes. The aim of this research study is to assess the distribution of hepatitis C virus genotypes in chronic HCV patients from a major tertiary care center in Lebanon.

Materials and methods: For all HCV patients referred for viral load testing at the American University of Beirut Medical Center, we reviewed the HCV genotyping results for 203 patients positive for HCV RNA using the *Linear Array Genotyping* kit.

Results: HCV genotypes were distributed as follows: *Genotype 1* (39.6 42.86%), *Genotype 3* (27.6 26.11%), *Genotype 4* (26.0 24.63%), *Genotype 2* (3.6 3.45%), and *Genotype 5* (3.1 2.96%). *Genotype 6* was not detected in any case from our patient population.

Conclusion: Unlike previous studies in the country, our study interestingly showed a shift in viral genotype from *Genotype 4* as the most predominant few years back to a current *Genotype 1* predominance. Future epidemiological investigations, especially in light of these interesting results which differ from other populations in the Middle East region and show HCV viral profile changes, are warranted.

1. Introduction

Since its discovery in 1989, hepatitis C virus (HCV) has been considered a global public health problem that affects about 3% of the world's population (about 185 million individuals worldwide) (Shepard et al., 2005; Mohd Hanafiah et al., 2013) and 3.4 to 4.4 million individuals in the United States (NIH consensus, 2002; Armstrong et al., 2006; Chak et al., 2011). In most European countries, the prevalence of HCV in the general population ranges from 0.5% to 2% (about 5 to 10 million infected individuals) (WHO, 2003). Studies in the Middle East showed that *Genotype 4* predominates in the Arab countries (with the exception of Jordan), while *Genotype 1* predominates in the non-Arab countries (Poustchi et al., 2010). For example, a recent study showed that the most predominant HCV genotype in East Turkey is *Genotype 1b* (İba Yilmaz et al., 2015). In addition, a study from Iran showed a high prevalence for the HCV *Genotype 1* (Liakina et al., 2015). However, another study in Libya reported a scattered HCV genotype prevalence that varied by geographical location, demographic, and risk factors (Daw et al., 2015).

HCV can cause chronic hepatitis and is recognized to be a leading cause of end-stage liver disease with high progression to cirrhosis and hepatocellular carcinoma (Poynard et al., 2000; Kim, 2002; WHO, 2003). Approximately 80% of acutely infected HCV patients progress to chronic infection, 20% of whom develop cirrhosis within 25 years, with 25% of patients with a cirrhosis developing hepatocellular carcinoma (Freeman et al., 2001; Reddy et al., 2011). HCV is the primary cause of liver transplantation in the United States (Davis et al., 2010). Sequence heterogeneity in the viral genome of the hepatitis C virus was described. Okamoto et al. (1992) classified HCV into four different genotypes while Simmonds et al. (1993) classified it into six major types and a series of subtypes. The knowledge of the genotypes in chronic hepatitis C is a crucial element to be identified since treatment response varies among the different genotypes and subsequently therapeutic management options change according to the genotype, thus different treatments, treatment duration, and therapy dose are required in patient care (Hnatyszyn, 2005). The purpose of this study is to describe the distribution pattern of HCV genotypes in chronic hepatitis patients in a major tertiary care center in Lebanon.

Abbreviations: HCV, hepatitis C virus; **RNA**, Ribonucleic Acid; **RT-PCR**, Reverse-transcriptase Polymerase Chain Reaction; **DNA**, Deoxyribonucleic Acid; **IFN**, interferon; **RBV**, ribavirin; **PEG**, pegylated; **DAA**, direct-acting antiviral; **SVR**, sustained viral response.

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http://dx.doi.org/10.1016/j.genrep.2016.06.002
2452-0144/

2. Materials and methods

2.1. Samples

This Institutional Review Board-approved study was performed at the American University of Beirut Medical Center (AUBMC), which is a major tertiary care center in Lebanon accommodating patients from the different districts and communities of the country. It is a retrospective analysis of HCV genotype testing performed in the Molecular Diagnostics Laboratory at AUBMC where we reviewed the HCV genotypes of 203 samples positive for HCV RNA in order to determine the distribution of genotypes among all patients. These patients have been referred to our center from all districts and ethnic communities of the country; however, no clinical data is available regarding the mode of transmission as it is not within the scope of this research.

2.2. HCV quantification and HCV genotyping

Samples were tested for the presence of HCV RNA and its quantification by polymerase chain reaction in real time (RT-PCR) using Cobas Ampliprep/Taqman 48 platform (Roche Diagnostics GmbH, Germany). Then, HCV genotyping was performed using the *Linear Array HCV Genotyping Test* (Roche Diagnostics GmbH, Germany) and the *Linear Array Detection Kit* (Roche Diagnostics GmbH, Germany). The test utilizes reverse transcription of target RNA to generate complementary DNA (cDNA), amplification of target cDNA by the Polymerase Chain Reaction (PCR), and nucleic acid hybridization for the genotyping of HCV RNA in human serum or EDTA plasma.

The *Linear Array HCV Genotyping Test* is based on five major processes: Specimen preparation; reverse transcription of the target RNA to generate complementary DNA (cDNA); PCR amplification of target cDNA using HCV specific complementary primers; hybridization of the amplified products to oligonucleotide probes specific to the target(s); and detection of the probe-bound amplified products by colorimetric determination.

2.3. Interpretation of results

The *Linear Array HCV Genotyping Strip* is read visually by comparing the pattern of blue bands to a reference table of genotype patterns (as provided by the manufacturer).

2.4. Statistical analysis

The HCV genotyping results of 203 patients were collected and the prevalence of the different genotypes was determined by direct counting methods.

3. Results

Using the *Linear Array Genotyping* test, the distribution of different HCV genotypes was determined. It was found that HCV *Genotypes 1*, *3*, and *4* are the most prevalent (39.6 42.86%, 27.6% 26.11, and 24.63%, respectively) and *Genotypes 2* and *5* are less frequent (3.45% and 2.96%, respectively), however, *Genotype 6* was not found (Table 1).

Table 1
Distribution of HCV genotypes among the 192 hepatitis C patients.

Genotype	# of patients	Frequency (%)
1	87	42.86
2	7	3.45
3	53	26.11
4	50	24.63
5	6	2.96
6	0	0.00
Total	203	100

The demographic data, mainly pertaining to age and gender, is shown in Table 2.

4. Discussion

Hepatitis C virus genotypes differ in three major properties including their prevalence, pathogenicity, and response to treatment. According to the international literature, the prevalence of certain HCV genotypes is frequently associated with certain geographical areas. For example, HCV *Genotype 1* is prevalent in North America and Japan, *Genotype 3* is most common in the Indian subcontinent, *Genotype 4* is most common in Africa and the Middle East, while *Genotype 5* can be found in South Africa and *Genotype 6* in Southeast Asia (Sy and Jamal, 2006). The countries located in the region closest to Lebanon (Arab and non-Arab countries) showed a predominant prevalence of HCV *Genotype 1* and *4*. In Turkey and Iran, HCV *Genotype 1b* and *1* were the most dominant, respectively; while many Arab countries (Egypt, Saudi Arabia…) recorded a high prevalence of HCV *Genotype 4* (İba Yilmaz et al., 2015; Liakina et al., 2015; Poustchi et al., 2010).

Previous studies about the prevalence of HCV genotype in Lebanon showed that *Genotype 4* was found to be the predominant genotype among 142 HCV-infected Lebanese patients followed by Genotype 1, these patients included 27 patients with β-thalassemia major or intermedia, 30 patients on haemodialysis, 32 multi-transfused patients and 15 IDUs (Sharara et al., 2007). The same distribution pattern was seen in a study among 395 thalassemics (Ramia et al., 2002). Another study conducted among 106 intravenous drug users (IDUs) in Lebanon found that *Genotype* 3 was the predominant HCV genotype followed by *Genotypes 1* and *4*, a situation similar to that among IDUs in Western Europe (Mahfoud et al., 2010). As shown in Table 3, the genotypic distribution is variable among the different studies so far conducted in Lebanon. Although the study by Mahfoud et al., is showing a predominance of Genotype 3, this only refers to the IDUs subgroup representing only 28 positive HCV-RNA in IDU cases. On the other hand, the study by Sharara et al., shows a wider variation of the predominant genotype among the different categories of patients included. Another hospital-based study showed a significantly different HCV genotype prevalence among dialysis and non-dialysis patients (a total of 77 infected cases). In the dialysis group (27 hemodialysis patients), *Genotype* 2 was predominant (80%, $p < 0.001$) while *Genotype* 1 (subtype 1b) was frequently detected in nondialysis cases (34.4%) whereas *Genotype 1* was found in only 5% of dialysis cases (Irani-Hakime et al., 2003). Our study showed that HCV *Genotypes 1*, *3*, and *4* are the most prevalent (42.86%, 26.11%, and 24.63%). This was surprising and in contrast with earlier findings where *Genotype 4* was the predominant genotype (47.5%) in Lebanese patients (Ramia et al., 2002; Sharara et al., 2007). This might be due to the fact that this study was done among different groups of HCV-positive patients referred from all Lebanon, representing, thus more of a population-based sample.

The pathogenicity of HCV infection also varies according to the different genotypes. For example, while HCV *Genotype 3* infection is

Table 2
Demographic data of HCV genotypes among the 203 hepatitis C patients.

		# of patients	Frequency (%)
Gender	Female	47	23.2
	Male	156	76.8
Age group	0–10	2	1.0
	11–20	12	5.9
	21–30	57	28.1
	31–40	29	14.3
	41–50	37	18.2
	51–60	36	17.7
	61–65	6	3.0
	>65	17	8.4
	Missing	7	3.4
	Total	203	100.0