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RESEARCH ARTICLE

Genotyping of Hepatitis C Virus isolated from Libyan Patients by Line Immune Probe Assay

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ABSTRACT

Introduction: Hepatitis C virus (HCV) genotyping is important in the routine diagnosis and management of chronic hepatitis C infections. The reference method used for HCV genotype determination is direct sequencing of the NS5B or E1 regions of HCV genome by means of "in-house" techniques followed by sequence alignment with prototype sequences and phylogenetic analysis. **Material and Method:** Blood samples were collected from 75 HCV- infected Libyan patients (age range 8–70 years mean 24 years), out of them 32 of them females (43%) and 43 males (57%). Forty-three (57.3%) have confection with human immunodeficiency virus (HIV) infection. **Result:** Out of the 75 samples examined in this study, 45 (60%) were found to belonging HCV genotype 4 followed by 20 (26.7%) subtype 1a. Other subtypes 2a/2c, subtype 1b; subtype 1a/1b; and genotype 2 all represented 5.3%; 5.3%; 1.3%; and 1.3%, respectively. **Conclusion:** In conclusion, the distribution of HCV genotypes in Libya is consistent with other neighboring countries.

Keywords: Genotyping, Hepatitis C virus (HCV), immune

INTRODUCTION

Hepatitis C virus (HCV) genotyping is important in the routine diagnosis and management of chronic hepatitis C infections. The reference method used for HCV genotype determination is direct sequencing of the NS5B or E1 regions of HCV genome by means of "in-house" techniques followed by sequence alignment with prototype sequences and phylogenetic analysis.[1,2] These techniques are used in molecular epidemiological studies, where exact subtyping is needed. In clinical practice, HCV genotype can be determined by various commercial kits, using direct sequence analysis of the 5' noncoding region (Trugene® 5'NC HCV Genotyping Kit, Bayer HealthCare, Diagnostics Division, Tarrytown, New York) or reverse hybridization analysis using genotype specific probes located in the 5' noncoding region (commercialized as INNO-line immune probe assay (LiPA) HCV II, Innogenetics, Ghent, Belgium)[3-7] which was used in this study.

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Objective of the Study

The purpose of this study was to determine HCV genotypes among Libyan patients by LiPA technique.

MATERIALS AND METHODS

Blood samples were collected from 75 HCV-infected Libyan patients (age range 8–70 years mean 24 years), out of them 32 of them females (43%) and 43 males (57%). Forty-three (57.3%) have confection with human immunodeficiency virus (HIV) infection. All specimens were received and processed at the laboratory of Benghazi Center

of Infectious Disease and Immunity to perform the viral load and HCV genotyping. The separated sera were aliquoted in two groups one examined for HCVAb by enzyme immunoassay and confirmed by recombinant immunoblot assay. The other stored at –80°C until genotyping assay was carried out, to optimize preservation of HCV RNA. HCV qualitative and quantitative assays were performed by semi-automated version Cobas® Amplicor® HCV v2.0 (Roche Molecular Systems). Qualitative detection assays are based on the principle of target amplification using polymerase chain reaction (PCR). HCV RNA is extracted and reverse transcribed into a double-stranded complementary DNA, which is subsequently processed into a cyclic enzymatic reaction leading to the generation of a large number of detectable copies. Detection of amplified products is achieved by hybridizing the produced amplicons onto specific probes after the reaction in PCR. All of the cases had detectable viral load ranging from 42,000 IU/ml to >850,000 IU/ml. HCV genotyping was performed using the commercial kit (INNO-LiPA HCV II, Innogenetics, Ghent, Belgium). The LiPA test is based on reverse hybridization with oligonucleotide probes representing type-specific sequence patterns in the HCV 5'NCR. Genotype identification is based on an interpretation chart that presents 20 individual patterns, each of which is specific for a genotype or subtype.

This was performed using 10 µl of the amplified product s of Cobas Amplicor. Biotin labeled amplified products hybridized to specific probes which are tailed with a poly (T) tail by terminal deoxynucleotidyl transferase and attached to nitrocellulose membranes. The following antigens were screened for 1a; 1b; 1; 2a/2c; 2b; 2k; 3a; 3b; 3c; 3; 4a; 4b; 4c/4d; 4e; 4f; 4h; 4; 5a; 6a; and 10a. The hybridization step was carried out for 1 h at 50°C. It was followed by a stringent wash at 50°C for 30 min. After washing the strips with rinse solution for 2 min, streptavidin labeled with alkaline phosphatase was added and bound to any biotinylated hybrid. This step was performed at room temperature for 30 min. Incubation of 30 min with 5-Bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium chromogen resulted in development of a purple positive band that occurred only if there was high homology at the nucleotide level between the probe and the biotinylated PCR products. Genotypes were identified based on an interpretation chart that provided by the manufacture based on the precipitation bands on the nitrocellulose paper.

RESULTS

Out of the 75 samples examined in this study, 45 (60%) were found to belonging HCV genotype 4 followed by 20 (26.7%) subtype 1a. Other subtypes 2a/2c, subtype 1b; subtype 1a/1b; and genotype 2 all represented 5.3%; 5.3%; 1.3%; and 1.3%, respectively, as shown in Table 1.

Of 43 male patients, 25 (58%) infected with HCV genotype 4; 11 (26%) genotype 1a. However, subtypes 1b; 2a/2c; 1a/1b; and 2 represented 3 (7%); 2 (5%); 1 (2%); and 1 (2%), respectively. Similarly, 20 (62.5%) out of 32 female patients had HCV genotype 4; 9 (28%) genotype 1a; while subtypes 1b and 2a/2c represented 1 (3.2%) and 2 (6.3%), respectively, as shown in Table 2.

Of the 43 HCV-infected patient with HIV infection, 60% had genotype 4; 38% had genotype 1a and 2% had genotype 2; while 59.3% of the non-HIV-infected patients had genotype 4; 12.5% had genotype 1a; 12.5% had genotype 1b; 12.5% had genotype 2a/2c; and 3.2% had genotype 1a/1b, as shown in Table 3.

DISCUSSION

The importance of HCV genotyping has considerably increased in the past few years. It has been used to study worldwide and local molecular epidemiology of HCV, and to trace sources of HCV infection in risk groups such as drug users and blood products. Typing has also been used to study relationships between type/subtype and the clinical status, pathogenesis, and/or outcome of disease.[3] The major area of clinical application of HCV genotyping

Table 1: HCV genotypes among Libyan patients

Category	HCV genotypes						
	Total	4	1a	1b	2a/2c	1a/1b	2
Patients no.	75	45	20	4	4	1	1
%	100	60	26.7	5.3	5.3	1.3	1.3

Table 2: The pattern of genotypic distribution according to sex

Gender	HCV genotypes						
Sex	Total	4	1a	1b	2a/2c	1a/1b	2
Males (%)	43	25 (58)	11 (26)	3 (7)	2 (5)	1 (2)	1 (2)
Females (%)	32	20 (62.5)	9 (28)	1 (3.2)	2 (6.3)	0	0

Table 3: The pattern of genotypic distribution among HIV patients and non-HIV-infected individuals

Category	HCV genotypes						
	Total	**4**	**1a**	**1b**	**2a/2c**	**1a/1b**	**2**
HIV infected (%)	43	26 (60)	16 (38)	0	0	0	1 (2)
Non-HIV infected (%)	32	19 (59.3)	4 (12.5)	4 (12.5)	4 (12.5)	1 (3.2)	0

HIV: Human immunodeficiency virus

has been in the study of the significance of types/subtypes, in response to antiviral treatment of HCV infection with interferon and ribavirin, as well as the identification of patients with mixed infections. It has also been a useful application in vaccine research and development.[3,8,9]

Clinical laboratories will likely continue to face increasing HCV test volumes, according to projected increases in the diagnosis of chronic HCV infection. As a result, clinical laboratories must continue to rapidly adopt new technologies capable of improving HCV test performance and efficiency. In addition to sensitive detection and accurate quantification of HCV RNA, HCV genotype determination will also likely continue to play an important role in anti-HCV treatment algorithms.[1-3,8,9]

In the present study, HCV genotype 4 was the major predominant type comprising 60% of the tested sample followed by genotype 1a. A similar picture has been identified in the Middle East, Egypt, and some African countries.[8,10-14] Our finding supported the published literature from Libya where genotype 4 was detected more frequently in patients from East Libya (Benghazi) compared to West Libya (Tripoli) (75.9% vs. 41.6%, $P = 0.245$).[15,16] For genotype 1 was more frequent in patients from West Libya compared to East Libya (34.1% vs. 16.8%, $P = 0.657$), this support our finding was HCV genotype 1 was 26%. Furthermore, a large sample number are required to determine the accurate prevalence of HCV genotypes among Libyan patients.

When the patients were classified according to age, no significant differences in distribution of HCV genotypes were observed among our patients. These findings supported by reports of Rodriguez *et al*.; Shobokshi *et al*.; and Osoba *et al*.[10-12] Similarly, no significant difference was also observed in the distribution of genotype 4 between patients with HIV infection and patients with non-HIV infection. However, genotype 1a was found in 38% of HIV-infected patients compared with 12.5% of non-infected individuals. These findings are supported by the report of Yerly *et al*.[13]

CONCLUSION

In conclusion, the distribution of HCV genotypes in Libya is consistent with other neighboring countries. In addition, the analysis of the 5' UTR by LiPA with the INNO-LiPA HCV II kit provides a fast and easy method for the determination of the HCV genotype, and produces consistent results, but is relatively expensive.

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