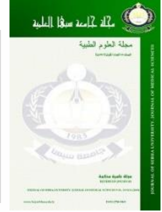
SEBHA UNIVERSITY JOURNAL OF JOURNAL OF MEDICAL SCIENCES VOL.17 NO. 1 2022
DOI: 10.51984/JOMS.V17I1.1919

مجلة جامعة سبها للعلوم الطبية
Journal of Medical Sciences

Journal homepage: www.sebhau.edu.ly/journal/index.php/joms



Genotyping of Hepatitis C Virus isolated from Libyan patients by line immune Probe Assay (LiPA)

Fatma I. E. Ali Najem[1], *Suliman A. Elgadi[2], Ibrahim A.Taher[1], Halema S. Ali[2], Amina .S. Abusedra[1]

[1]Benghazi Center of infectious diseases and immunity, Benghazi, Libya
[2]Medical laboratory department, Faculty of Engineering and Technology, Sebha university, Brack, Libya

Keywords:
Chronic HCV infection
Genotypes
Subtype
HIV-positive patients

A B S T R A C T

Chronic HCV infection contributed with the development of some diseases such as liver cirrhosis, cancer, and liver failure especially in HIV-positive patients during active antiretroviral therapy which may lead to death. This was the reason that genotyping has become a very important in routine diagnosis and management of chronic viral hepatitis infections. The purpose of this study was to see HCV genotypes among Libyan patients by LiPA technique, were a 75 blood samples were collected and processed at the laboratory of BIDIC (Benghazi Center of Infectious disease and Immunity) to perform the viral load and HCV genotyping. It was found that (60 %) of the collected samples belongs to HCV genotype 4, and (26.7 %) subtype 1a, while other subtypes 2a/2c, subtype 1b; Subtype 1a /1b; and genotype 2 all represented 5.3%; 5.3 %; 1.3 %; 1.3 % respectively. This concluded that HCVgenotypes in libya is distributed consuistently with Libyan neighboring countries. And the analysis of the 5′ UTR by LiPA with the INNO-LiPA HCV II kit can provide a quick, simple and reliable method for determination of the HCV genotype.

عزل التنميط الجيني لفيروس التهاب الكبد الوبائي (سي) من المرضى الليبيين عن طريق فحص مسبار المناعة الخطي (LiPA)

فاطمة علي نجم[1] و *سليمان القاضي[2] و إبراهيم طاهر[1] و حليمة صالح علي[2] و أمينة أبوسدرة[1]

[1]مركز بنغازي للأمراض المعدية والمناعة، بنغازي، ليبيا
[2]قسم المختبرات الطبية، كلية العلوم الهندسية والتقنية، جامعة سبها، براك، ليبيا

الكلمات المفتاحية:
عدوى التهاب الكبد المزمن
طرز وراثية
نوع فرعي
مرضى مصابين بفيروس نقص المناعة البشرية.

الملخص

ساهمت العدوى المزمنة بالتهاب الكبد الفيروسي في ظهور بعض الأمراض مثل تليف الكبد والسرطان وفشل الكبد خاصة في المرضى المصابين بفيروس نقص المناعة البشرية أثناء العلاج الفعال بمضادات الفيروسات القهقرية والذي قد يؤدي إلى الوفاة. كان هذا هو السبب في أن التنميط الجيني أصبح مهمًا جدًا في التشخيص الروتيني وإدارة عدوى التهاب الكبد الفيروسي المزمن. كان الغرض من هذه الدراسة هو التعرف على الأنماط الجينية لفيروس التهاب الكبد C بين المرضى الليبيين بتقنية LiPA ، حيث تم جمع 75 عينة دم ومعالجتها في مختبر BIDIC (مركز بنغازي للأمراض المعدية والمناعة) لأداء الحمل الفيروسي والتنميط الجيني لـ HCV. وجد أن (60٪) من العينات التي تم جمعها تنتمي إلى النمط الجيني 4 HCV، و (26.7٪) من النوع الفرعي a1، بينما الأنواع الفرعية الأخرى c/2a2، النوع الفرعي b1، النوع الفرعي a / 1b1، والنمط الجيني 2 يمثلون 5.3٪، 5.3٪، 1.3٪، 1.3٪ على التوالي. وخلص هذا إلى أن الأنماط الجينية لفيروس HCV في ليبيا تتوزع بالتوازي مع دول الجوار الليبي. ويمكن أن يوفر تحليل 5 ′ UTR بواسطة LiPA مع مجموعة INNO-LiPA HCV II طريقة سريعة وبسيطة وموثوقة لتحديد التركيب الوراثي لـ HCV.

Introduction

HCV genotyping is very important within the routine diagnosis and management of chronic viral hepatitis infections. The reference

*Corresponding author:
E-mail addresses: Sul.Elgadi@sebhau.edu.ly ,(F. I. E. Ali Najem) fatmanajam@yahoo.co.uk ,(I. A.Taher) tahermicro@gmail.com, (H. S. Ali) hsaleh198152@gmail.com

Article History : Received 8 January 2021 - Received in revised form 15 April 2022 - Accepted 25 May 2022

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, 17, 18]. These techniques are employed in molecular epidemiological studies, where exact subtyping is required. In clinical practice, HCV genotype will 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 within the 5' noncoding region (commercialized as INNO-LiPA HCV II, Innogenetics, Ghent, Belgium) [3-7]which was utilized in this study .

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

Materials and Methods
For a period of ten months, during 2009 and 2010, 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 male (57%). 43 (57. 3 %) have confection with HIV infection. All specimens were received and processed at the laboratory of BIDIC (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 (EIA) & confirmed by recombinant immunoblot assay (RIBA).The other stored at –80°C until genotyping assay was administrated, so as to optimize preservation of HCV RNA. HCV qualitative and quantitive assays were performed by semi-automated version Cobas® Amplicor® HCV v2.0 (Roche Molecular Systems),. Qualitative detection assays are supported the principle of target amplification using polymerase chain reaction (PCR). HCV RNA is extracted and reverse transcribed into a double stranded deoxyribonucleic acid (cDNA), which is subsequently processed into a cyclic enzymatic reaction resulting in the generation of an oversized 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 42000IU/ml to>850000 IU/ml. HCV genotyping was performed using the commercial kit, (INNO-LiPA HCV II, Innogenetics, Ghent, Belgium). The LiPA test is predicated on reverse hybridization with oligonucleotide probes representing type-specific sequence patterns within the HCV 5'NCR. Genotype identification relies on an interpretation chart that presents 20 individual patterns, each of which is particular for a genotype or subtype.

This was performed using 10µl 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 subsequent antigen where 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 applied for one hour at 50 ºC. it absolutely was followed by a stringent wash at 50 ºC for half-hour .After washing the strips with rinse solution for 2minutes , strepatividin labeled with alkaline phosphatase was added and certain to any biotinylated hybrid. This step was performed at temperature for half-hour. Incubation of half-hour with BCIP/NBT chromogen resulted in development of a purple positive band that occurred on condition that there was high homology at the nucleotide level between the probe and therefore the biotinylated PCR products . Genotypes were identified supported an interpretation chart that provided by the manufacture supported 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 %; 1.3 % respectively as shown in **Table I**.
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%); 1(2%) respectively. Similarly 20 (62.5%) out of 32female patients had HCV genotype 4; 9(28%) genotype 1a; while subtypes 1b; and 2a/2c represented 1(3.2%); 2(6.3%); respectively as shown in **Table II.**
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 show in **Table III**

Table I – HCV genotypes among Libyan patients

	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 II the pattern of genotypic distribution according to sex

	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 III the pattern of genotypic distribution among HIV patients and non HIV infected individuals

	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

DISCUSSION
The importance of HCV genotyping has considerably increased within the previous few years. It's been wont to study worldwide and native molecular epidemiology of HCV, and to trace sources of HCV infection in risk groups like drug users and blood products. Typing has also been accustomed study relationships between type/subtype and therefore the clinical status, pathogenesis and/or outcome of disease [3]. The foremost area of clinical application of HCV genotyping has been within the study of the importance of types/subtypes, in response to antiviral treatment of HCV infection with interferon and ribavirin, in addition because the identification of patients with mixed infections. It's also been a useful application in vaccine research and development [3, 8, 9].

Clinical laboratories will likely still face increasing HCV test volumes, in line with projected increases within the diagnosis of chronic HCV infection. As a result, clinical laboratories must still rapidly adopt new technologies capable of improving HCV test performance and efficiency. Additionally to sensitive detection and accurate quantification of HCV RNA, HCV genotype

determination also will likely still play a very important role in anti-HCV treatment algorithms [1, 2, 3, 8, 9].

In the present study HCV genotype 4 was the key predominant type comprising 60% of the tested sample, followed by genotype 1a. An identical picture has been identified within the geographic area, Egypt and a few African countries [8, 11, 12, 13, 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 were HCV genotype 1 was 26%. Furthermore an oversized sample number is required to see the accurate prevalence of HCV genotypes among Libyans patients.

When the patients were classified in keeping with age no significant differences in distribution of HCV genotypes were observed among our patients. These findings supported by reports of Stéphane et al; Stuyver L et al; and Osoba et al [10,11,12, 1]. Similarly no significant difference was also observed within 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].

CONCLOSION

In conclusion the distribution of HCVgenotypes in libya is consuistent with other neighboring countries. Additionally the analysis of the 5′ UTR by LiPA with the INNO-LiPA HCV II kit provides a quick and simple method for determination of the HCV genotype, and produce consistent results , but is comparatively expensive.

REFERENCES:

[1]- A.O. Osoba, M. Ibrahim , M; A. Abdelaal; A. Al-Mowallad ; B. Al Shareef, ; B. Al Haj Hussein. Hepatitis c virus genotyping by polymerase chain reaction and DNA enzyme immunoassay among saudi patients in the western province, Saudi Arabia .Annals of Saudi Medicine, Vol 20, Nos 5-6, 2000 394-397

[2]- Germer JJ, et al. Automated sample preparation for the Trugene HIV-1 genotyping kit using the MagNA pure LC instrument. Diagn Microbiol Infect Dis 2004, 49: 59-61.

[3]- Maertens G, Stuyver L. Genotypes and genetic variation of hepatitisC virus. In: Harrison TJ, Zuckerman AJ, editors. The molecular medicine of viral hepatitis. Chichester: John Wiley and Sons Ltd., 1997:182-233.

[4]- Pawlotsky JM, et al. What strategy should be used for diagnosis of hepatitis C virus infection in clinical laboratories? Hepatology 1998,27: 1700-2.9.

[5]- . Rodriguez-Perez F, Suarez-Perez E, Alvarez-Rohena M, Toro DH. Prevalence of chronic hepatitis C virus genotypes among patients between 21 to 65 years old in Puerto Rico. P R Health Sci J. 2004 Jun;23(2 Suppl):49-56

[6]- Sabine Yerly, Rafael Quadri, Francesco Negro, Klara Posafy Barbe, etal .Nosocomial Outbreak of Multiple Bloodborne Viral infections. JID 2001;184(1August):369-372

[7]- . Shobokshi OA, Serebour FE, Skakni L, Al Saffy YH, Ahdal MN. Hepatitis C genotypes and subtypes in Saudi Arabia. J Med Virol 1999;58:44-8..

[8]- Simmonds P. Viral heterogeneity of the hepatitis C virus. J Hepatol 1999, 31 (Suppl 1): 54-60.

[9]- Simmonds P, et al. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology 2005, 42: 962-73.

[10]-Stéphane Chevaliez, Jean-Michel Pawlotsky . Hepatitis C Virus Serologic and Virologic Tests and Clinical Diagnosis of HCV Related Liver Disease. *Int. J. Med. Sci.* 2006, 3:35-39

[11]- Stuyver L, et al. Second-generation line probe assay for hepatitis C virus genotyping. J Clin Microbiol 1996, 34: 2259-66.

[12]-Stuyver L, et al. Hepatitis C virus genotyping by means of 5'-UR/core line probe assays and molecular analysis of un type able samples. Virus Res 1995, 38: 137-57.

[13]- Zheng X, et al. Direct comparison of hepatitis C virus genotypes tested by INNO-LiPA HCV II and TRUGENE HCV genotyping methods. J Clin Virol 2003, 28: 214-6.

[14]-Abdel-Rahman M, Saad Y, El-Raziky M, Zayed N, El-Akel W, Said M, et al. Hepatitis C genotype 4 with normal transaminases: correlation with fibrosis and response to treatment, a cohort Egyptian study of 4277 patients. Clin Res Hepatol Gastroenterol 2013. Nov;37(5):479-484. 10.1016/j.clinre.2013.02.009

[15]- Daw MA, El-Bouzedi A, Dau AA. Geographic distribution of HCV genotypes in Libya and analysis of risk factors involved in their transmission. BMC Res Notes 2015. Aug;8:367. 10.1186/s13104-015-1310-x

[16]- Abdel-Naser Elzuoki, Islam Elzouki, Sabah Albarassi, Mohamed Gammo, and Abdalla Burwaiss. Hepatitis C Genotypes in Libya: Correlation with Patients' Characteristics, Level of Viremia, and Degree of Liver Fibrosis. Oman Med J. 2019 Sep; 32(5): 409–416.

[17]-Zakalashvili M, et al. Identification of hepatitis C virus 2k/1b intergenotypic recombinants in Georgia. Liver Int. 2018 Mar; 38(3):451-457.

[18]-Zakalashvili M, et al. Assessment of treatment options for patients with hepatitis C virus recombinant form 2k/1b. Hepatol Res. 2021 Feb;51(2):156-165.