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NS5B SEQUENCING TO ASSESS THE MOST POPULAR HCV GENOTYPES IN MOROCCAN PATIENTS

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ABSTRACT

Hepatitis C virus (HCV) infection is the major cause of chronic hepatitis that can lead to fibrosis and/or hepatocellular cancer, with approximately 71 million chronically infected people worldwide. HCV exhibits significant genetic variability, with eight genotypes and more than 70 subtypes identified so far. The objective of this study was to determine the most popular HCV genotypes and subtypes among Moroccan patients by partial sequencing of NS5B, in order to guide treatment initiation and select the most appropriate treatment regimen. Genotypes 1, 2, 3, and 4 were observed in 100 patients newly diagnosed with HCV infection by two methods (Abbott test, and NS5Bgenotyping using the Sanger method). The concordance between the two methods was also evaluated. The viral load and genotype determination were performed using an automated Abbott real-time HCV Genotype II assay. The "NS5B" region was sequenced by the Sanger method using a validated in-house

protocol. Genotype 1 was the most prevalent (53%) genotype, followed by genotype 2 (39%), genotype 3 (6%) and genotype 4 (1%). Different HCV subtypes were also detected; 1b, 1a, 1d, 3a, 2, 2q, 2a, 2i 4d. 1b (30%) and 2i (29%) were the predominant subtypes. The concordance between the Abbott automated results and the NS5B sequencing for genotypes 1and 2 was 100% (45/45) and 96,77% (31/30) respectively.

Keywords: HCV; NS5B; Sanger sequencing; genotype / subtypes

1. INTRODUCTION

Hepatitis C virus is a non-cytopathic single-stranded RNA pathogen belonging to the Hepacivirus genus and the Flaviviridae family. The most significant pathological complications of this virus occur in 80% of infected individuals who develop chronic hepatitis, which can lead to steatosis, fibrosis, cirrhosis, and hepatocellular carcinoma **[1]**. According to the World Health Organization (WHO), an estimated 71 million people were chronically infected with HCV in 2015, and hepatitis-related deaths were largely due to chronic liver disease (720 000 deaths due to cirrhosis) and primary liver cancer (470 000 deaths due to hepatocellular carcinoma).

HCV infection is a major global health problem, with significant differences between and within countries (CDC 2019, centers for disease control and prevention). The highest prevalence is reported in two main regions; the Eastern Mediterranean Region and the European Region (WHO 2017). Morocco is classified as a low endemic country, with the HCV prevalence estimated at 1.58% in the general population **[2]**.

The regional prevalence is associated with HCV's variability, in terms of genetic diversity **[3]**. The HCV has a high molecular diversity in structural and non-structural coding regions. The HCV is currently classified into 8 genotypes (GT) and 86 subtypes, with possibly even more genotypes and subtypes to be identified **[4]**. HCV subtypes causing both phases of liver infections, acute and chronic (cirrhosis and hepatocellular carcinoma)are identified by letters (a, b, c, etc.) **[5]**. These genotypes have distinct geographical distributions.

Genotypes 1, 2, 3, 4, 5, 6, and 7 contain 67 confirmed subtypes and 20 provisional subtypes, with a nucleotide difference estimated to be less than 15% from one subtype to another **[6]**. The novel HCV genotype(GT8), is genetically distinct from previously identified HCV genotypes(GT1–7), with >30% nucleotide sequence divergence from the established HCV subtypes **[7]**. This genetic variability is implicated in the mechanisms of viral persistence and response to treatment **[8]**. Clinical and therapeutic management is based on the prior determination of the

HCV genotype. This process also enables the prediction of the antiviral response and the duration of the treatment **[9]**. The use of ribavirin and pegylated alpha interferon (PEG-IFNα) as a treatment against HCV has proved to be more effective against genotypes 2 and 3 compared to genotypes 1 and 4 **[8-10]**. The HCV genotype 1b also has a poor liver damage prognosis compared to other genotypes **[13]**.

Despite improving the treatment of HCV by combining PEG-IFNα and Ribavirin, several side effects and a lack of sustained efficacy for certain genotypes have been reported. Therefore, considerable progress has been made to develop numerous molecules with pan-genotypic effects.

Various technologies have been developed for the identification of HCV genotypes. The standard method is sequence analysis, which is based on the direct sequencing of a region of the genome, followed by PCR amplified **[14]**.

The obtained sequences were then compared with the NCBI sequence database in order to identify the genotypes **[15]**.

In Morocco, HCV genotyping was initiated by the introduction of hybridization tests and molecular tests on automated systems. These methods provided limited information related to circulating HCV subtypes.

The present study highlights the additive value of NS5B base sequencing for the genotyping of HCV in Moroccan patients and evaluates the concordance between the Abbott test and NS5B genotyping by the Sanger sequencing method.

2. MATERIALS AND METHODS

2.1. *Sample collection*

From March 2016 to September 2017, a total of 146 patients with hepatitis C serology reactive were received at the Department of Virology of the National Institute of Hygiene (NIH), for viral confirmation of active HCV infections, and assessing the baseline of HCV viral load and genotyping.

Blood (5 mL) was drawn from each patient by venipuncture into ethylene diamine tetra-acetic acid (EDTA) anti-coagulated tubes. The blood was mixed right away, and centrifuged. The plasma was collected, aliquoted in Eppendorf tubes, and stored at minus 20°C, until analysis.

2.2. *HCV viral load quantification*

HCV viral load quantification was performed using the Abbott RealTime HCV assay (Abbott, USA). HCV RNA was extracted from 500 µL plasma in the automated extractor (m2000sp), according to the manufacturer's instructions. Target RNA was concentrated to make them accessible and amplified on the m2000rt device used for automated real-time PCR amplification and quantification,

according to the manufacturer's instructions. The amplification cycle at which the HCV probe fluorescent signal detected by the Abbott m2000rt is proportional to the log of the HCV RNA concentration present in the original sample. HCV RNA concentration level is expressed in IU/ml.

The upper limit of quantitation (ULQ) for the Abbott RealTime HCV assay is 100,000,000 IU/mL (8.00 log IU/mL) and the lower limit of quantitation (LLQ) is equivalent to LoD (12 IU/mL or 1.08 log IU/mL).

2.3. *HCV genotyping on m2000sp-m2000rt (Abbott, USA)*

Genotyping of confirmed samples was performed on the M2000sp and M2000rtapparatuses, using the Abbott RealTime HCV genotype II (GT II) assay, targeting the 5′untranslated (UTR) and nonstructural (NS)5 B regions of the HCV genome. The HCV genotyping assay approved for in vitro diagnostic use (Abbott, USA) was performed according to manufacturer instructions. HCVGT II consists of reverse transcription-PCR performed in 3 individual reaction wells. These assay reactions utilize minor groove binder (MGB)-TaqMan probes targeting the HCV 5′ UTR for qualitative detection and differentiation of HCV GTs 1, 2, 3, 4, and 5 and ST-specific probes targeting the NS5B region for ST 1a and 1b differentiation.

2.4. *Sequencing core and NS5B fragments*

2.4.1 RNA extraction

HCV-RNA was extracted from 140 µL plasma samples using the Qiamp Viral Mini Kit 250 (Qiagen, France) according to the suppliers' instructions. Elution of the viral RNA was carried out on a final volume (60 µL) of the elution buffer. The eluates were stored at -20 ° C until analysis **[16]**.

2.4.2 Amplification

The amplification of the NS5B region was performed using a reverse transcription-polymerase chain reaction (RT-PCR), One-step RT-PCR Kit (Qiagen, France).

The final concentration of the primers NS5B-F(8256-8278) and NS5B-R (8622-8644) in the reaction volume was 0.6 µM (Mallory *et al*. 2014; Cai *et al*. 2013) **[19]**.

A reaction volume (50 µL) contained2µL One-Step RT-PCR Enzyme Mix enzyme, 10µL QIAGEN OneStep RT-PCR Buffer, 2µL dNTP Mix, 10µL extracted RNA, 3µL for each primer and 20µLRNase-free water to complete the final volume.

Reverse transcription and conventional PCR were performed usingthe GeneAmp PCR 9700 thermocycler, according to the following thermal cycling profile: 45 cycles (50°C) for 30 minutes, Reverse transcription (95°C) for 15 minutes, Activation of DNA Polymerase, reverse

transcription inactivation, and denaturation of cDNA (94°C) for 30 seconds, denaturation (51°C) for 45 seconds, elongation (72°C) for 1 minute, extension (72°C) for 10 minutes and final extension (4°C).

2.4.3 Sequencing reaction

All PCR products of the NS5B regions were purified using the ExoSAP-IT™ product cleanup reagent to hydrolyze excess primers and unincorporated nucleotides.

The BigDye ™ Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher) was used for the sequence reaction. The same NS5B-F and NS5B-R primers (3.2µM) were used, the final volume of the mixture (20 µL) contained the enzyme, buffer, primers (forward or Reverse), deionized water **(Table 2)** and 2 µL of the amplicon purified by ExoSAP-IT. The tubes put in the thermocycler and the following thermal cycling profile was utilized for the Polymerase Chain Reaction (PCR):

25 Cycles; 96°C for 1 minute (incubation), 96°C for 10 seconds (denaturation), 50°C for 5 seconds (elongation), 60°C for 4 minutes (extension).

2.4.4 Sequence analysis

The sequence was entered in the NCBI database (https://www.ncbi.nlm.nih.gov/). A phylogenetic tree was built using the same software (MegaX) from the reference genomes (genome 1: NC_004102.1/ genome 2: NC_009823.1/ genome 3: NC_009824.1/ genome 4: NC_009825).

The phylogenetic tree was constructed for the data using the maximum likelihood method. In this method, the tree is built using a document containing all obtained sequences aligned with the reference genomes.

2.4.5 Statistical analysis

The statistical data analysis was carried out using the Epi Info 7software.

ETHICS APPROVAL:

The study was approved by the director of the national institute of hygiene -Rabat (NIH), by Dahir N1–15-110 dated August 4, 2015, promulgating the law N28–13 relating to the protection of persons participating in biomedical research. The law provides special provisions for non-interventional or observational research as stipulated in its articles 2 and 26.As a result, no request for authorization from the National Ethics Committees as required.

3. RESULTS

3.1 Viral Load quantification

Viral load for all samples was performed using the Abbott automated real-time system **(Table 1)**, Ninety-three percent of the patients (93%, n=136) showed a viral load ranging between 100 000 and 10,000,000 (IU/mL).

3.2. HCV Genotype identification using Abbott system

The samples were genotyped using the Abbott automated test. This technique provided typing results for all 100 samples. The determined genotypes were: Genotypes 1, 2, 3, 4, subtypes 1a and 1b and three cross-reactivities: between genotype 2 and 5, subtype 1a and genotype 2, and genotype 1 with 4.

Only subtypes 1a and 1b were detected using the Abbott test. Some samples (15%)were reported as genotype 1 with no subtype.,80% of the samples were typed 1d by the NS5B Sanger sequencing method. 4% (n=4) of the samples was not determined by the Abbott test method **(Table 3)**, but were detected by the sequencing method as 2i (60%), 3a (20%) 1d (20%) **(Table 2)**.

3.3. HCV Genotype identification by capillary sequencing of the NS5B region

Post-PCR visualization reveals 127/146 (87%) positive samples.

100 sequences out of the 146 sequence samples were about 350 bp in length (79%) **(Figure 1)**. A bioinformatic analysis revealed their close similarity (ranging from 96% to 99%) with strains isolated from HCV in South America, the United States, Canada, Europe (France, Italy, the Netherlands), in Asia and some African countries, including Tunisia and Ghana. Genotype 1 was the most predominant in all cases (53%), followed by genotype 2 (40%). Genotypes, 3 and 4 exhibited the lowest percentages; 6 % and 1 % respectively.

Different HCV subtypes were detected (2a, 2i, 1b, 1a, 3a, 2, 2q 4d). The subtypes 2i and 1b were predominant (29%) compared to the other subtypes.

3.4. NS5B sequencing method versus Abbott Real-time HCV genotype II (GT II) assay

Out of 100 samples, 91 (91%) were consistent. The Abbott test determined subtypes 1a and 1b. However 15% of sequences belonging to genotype 1 were not subtyped. On the other hand, 80% of the un-subtyped sequences were identified by the NS5B sequencing method as subtype 1d. were able to deduce that

The undetermined genotypes using Abbott assay (4%), were assigned genotypes 2i 3a (25%); 2i (50%); 1d (25%) by NS5B sequencing method **(Table 4)**.

In discordant cases, the Abbott test revealed a double reactivity for seven samples, mainly between genotypes 1a, 2, 4, and 5.

3.5.Phylogeny

The phylogenetic tree shows that our population belongs to four genotypes. 1, 2, 3, and 4 **(Figure 2)**.

Table 1: Viral load level during the confirmation of HCV infection (n= 146).

Viral loads (IU/mL)	Sample (n)	Sample %
1000–10,000	1	0,7
10,000–100,000	1	0,7
100,000–1,000,000	79	54,1
1,000,000–10,000,000	57	39
>10,000,000	8	5,5
Total	146	100

Table 2: Genotype Abbott Molecular Assay (N=100)

Genotypes identified by Abbott RealTime HCV Genotype II	Frequency	Percent
GT 1	15	15,00%
GT1a	3	3,00%
GT1b	27	27,00%
GT2	32	32,00%
GT3	6	6,00%
GT4	2	2,00%
RT (G1 /G4)	1	1,00%
RT (G1a/G2)	1	1,00%
RT (G2/ G5)	9	9,00%
UNDETERMINED	4	4,00%
TOTAL	100	100,00%

***GT: Genotype/ RT: Reactivity**

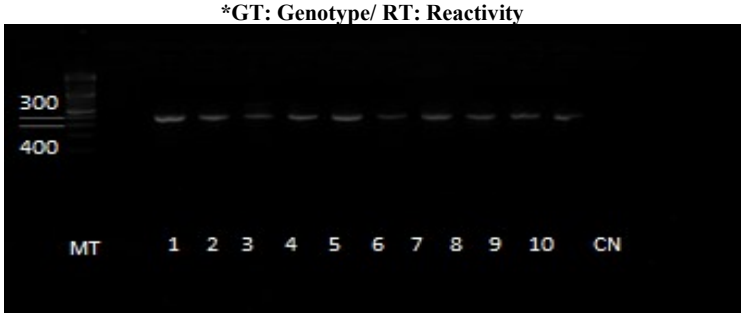

Figure 1: Example of post PCR bands after visualization

Table 3: Genotype determination by NS5B sequencing protocol (N=100)

Genotype (Subtypes) identified by NS5B sequencing	Frequency	Percentage
1a	3	3,00%
1b	31	31,00%
1d	19	19,00%
2	3	3,00%
2a	7	7,00%
2i	29	29,00%
2q	1	1,00%
3a	6	6,00%
4d	1	1,00%
TOTAL	100	100,00%

Table 4: Genotype determination comparison between NS5B sequencing and Abbott

	NS5B Sequencing	2	1a	1b	1d	2a	2i	2q	3a	4d
		0,03	0,03	0,31	0,19	0,07	0,29	0,01	0,06	0,01
Abbott RealTime HCV Genotype II										
GT 1										
0,15				0,2	0,8					
GT 1a										
0,03										
GT 1b										
0,27										
GT 2										
0,32		0,07				0,07	0,86			
GT 3										
0,06									1	
GT 4										
0,02										1
RT (G1 /G4)										
0,01					1					
RT (G1a/G2)										
0,01						1				
RT(G2/ G5)										
0,09						1				
UNDETERMINED										
0,04					0,25		0,5		0,25	

***G+T: Genotype/ RT: Reactivity**

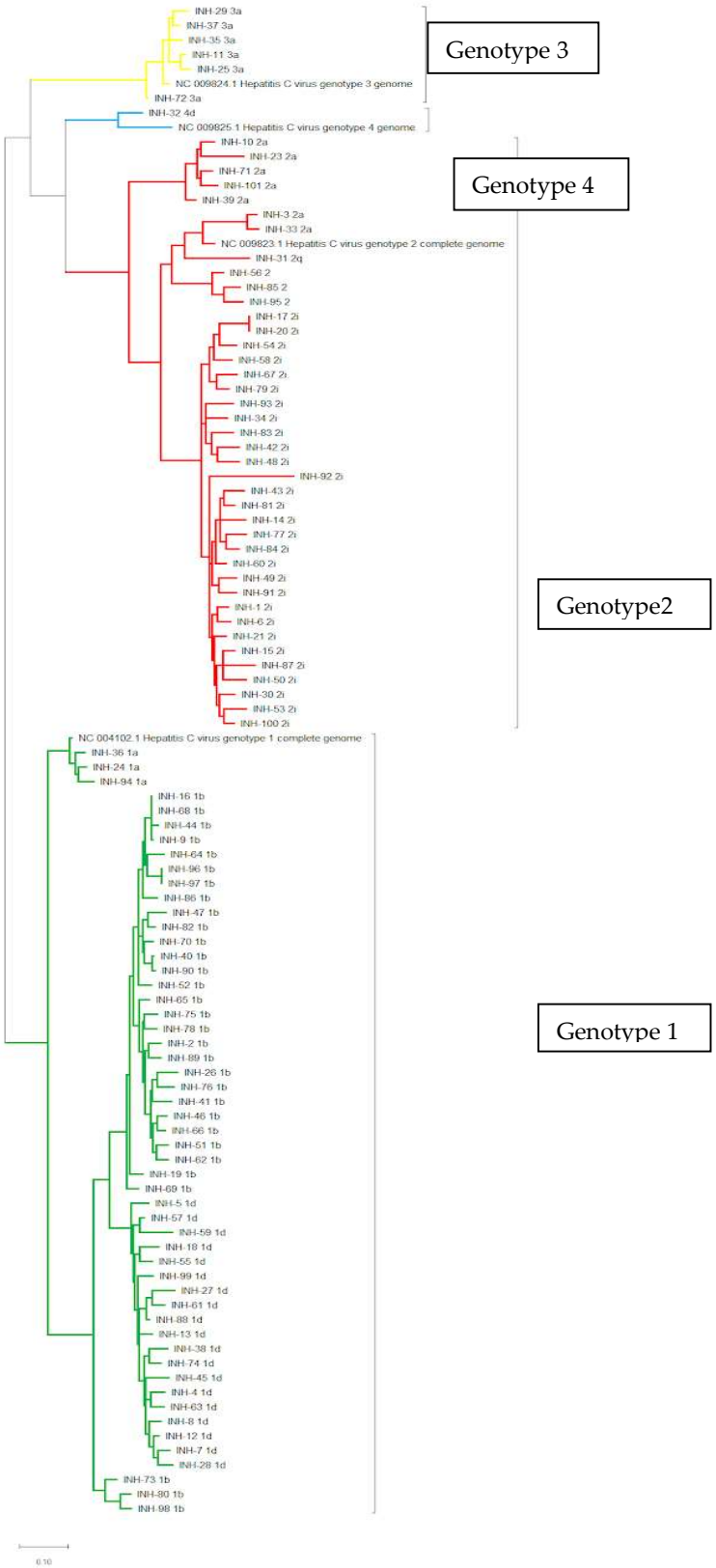

Figure 2: Phylogenetic tree

4. DISCUSSION

Hepatitis C virus (HCV) infection is one of the leading public health problems in the world, with high annual morbidity and mortality rates. In this context, the world health organization established a global action plan to eliminate HCV by 2030 (WHO 2015).

The Moroccan ministry of health joined this global vision, through a national program involving diagnosis, virological follow-up, and therapy. Data on viral circulation remains disparate and is dependent on the level of resolution of available techniques.

This work aims to assess the molecular variability of the circulating HCV subtypes among Moroccan patients using a combination of qRT-PCR automated technique and the Sanger sequencing of the NS5B region.in order to investigate the presence of naturally occurring resistance mutations in HCV NS5b region.

Analysis of the maximum likelihood of the phylogenetic tree of the NS5B sequence revealed that the sequences belong to four genotypes; 1,2,3 and 4 with different subtypes.

As part of the national HCV infection management program, the Abbott RealTime HCV Genotype II assay is used for genotype determination. Therefore, the present study compared the results obtained by using the sanger sequencing method and the Abbott test. The latter detected six genotypes and subtypes 1a and 1b. This high throughput automated method provides rapid results (5 hours) with high precision and easily interpretable results compared to the reference method, which relies on classical sequencing with high variability, including the NS5B protein.

In the present study, the Abbott automated test specificity was 91%. The determination of the viral subtypes (in particular genotype 1a versus 1b) is important because it influences the choice of therapeutic regimens and their duration **[20]**. The Abbott RealTime HCV Genotype II test targeted the 5 'UTR sequence for the detection of six genotypes, while the subtypes 1a and 1b were detected by primers amplifying a sequence of the NS5B region. 12 out of 15 (80%) of the subtype 1d were assigned genotype 1 using the Abbott test.

There was a concordance between the genotypes and subtypes of the 100 samples. Hybridization-based tests unrevealed reactivities by sequencing, which is why 9 samples (9%) were reported to be reactivity between genotypes 2 and 5.

Genotype 1 was the most prevalent genotype. These results corroborate the distribution reported by a study conducted between 2003 and 2010 at the Pasteur Institute of Morocco and the University Hospital Center of Casablanca **[21]**.

In the context of subtype distribution, subtypes 1b and 2i were the most predominant, followed by the subtype 1d. Other subtypes were less represented in this study. A similar distribution was also reported in a study by Brahim *et al* **[21]**, where subtypes 1b and 2i were the most predominant. This predominance of subtype 1b was also reported in a study conducted on 105 patients in 1997, however, genotype 2 showed a dominance of subtypes 2a and 2c **[21, 22]**.

In 2014, Gower *et al* published estimates of the prevalence and distribution of genotypes by continent, region, and country. The data published on Morocco underline the existence of genotypes 1, 2, 1a, and rarely genotype 4. In contrast, America and Europe host the six genotypes **[23]**. These estimates confirm the results of our study on prevalent genotypes across the Moroccan population.

Genotypes and sub-types determined after the bioinformatic analysis of our series, show similarities between the HCV strains isolated in South America, the USA, Canada, Europe (France, Italy, Netherlands), Asia, and some African countries, including Tunisia and Ghana. The prevalence of genotype 1 infection was not different for men and women.

In the present study, the frequency of patients with subtype 1b was significant; the most common risk factors were blood transfusion, those with an unknown mode of contamination, and the oldest subjects.

The subtypes 1a and 3a,generally related to intravenous drug addiction, were also present in the studied series **[24, 25]**.

The main route of HCV transmission within the Moroccan population is still attributed to age, dental treatment, use of glass syringes, and surgical history **[2]**.Therefore, nosocomial risk prevention and health education are the main interventions designed to limit the spread of HCV **[2]**.

This study also suggests that other HCV genotypes may exist in the Moroccan population due to increasing risk factors for contracting the virus, such as dental care, acupuncture, tattoos, piercings, intravenous drugs, and especially sexual transmissions.

This study also revealed the possibility of genotype determination discrepancies (9% of cases). Other studies evaluating the performance of the Abbott automated assay versus NS5B sequencing have shown that discrepancies may exist for cross-reactions between genotypes 1 and 6, and genotypes 2 and 5, which are recognized by the manufacturer **[19]**.

Conflicts of Interest: The authors declare no conflict of interest.

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REFERENCES

[1] M. J. Tong, N. S. el-Farra, A. R. Reikes, et R. L. Co, « Clinical outcomes after transfusion-associated hepatitis C », N. Engl. J. Med., vol. 332, nº 22, p. 1463-1466, juin 1995, doi: 10.1056/NEJM199506013322202.

[2] W. Baha *et al*., « Prevalence and risk factors of hepatitis B and C virus infections among the general population and blood donors in Morocco », BMC Public Health, vol. 13, p. 50, janv. 2013, doi: 10.1186/1471-2458-13-50.

[3] N. Echeverría, G. Moratorio, J. Cristina, et P. Moreno, « Hepatitis C virus genetic variability and evolution », World J. Hepatol., vol. 7, nº 6, p. 831-845, avr. 2015, doi: 10.4254/wjh.v7.i6.831.

[4] S. M. Borgia *et al*., « Identification of a Novel Hepatitis C Virus Genotype From Punjab, India: Expanding Classification of Hepatitis C Virus Into 8 Genotypes », J. Infect. Dis., vol. 218, nº 11, p. 1722-1729, oct. 2018, doi: 10.1093/infdis/jiy401.

[5] M. Keikha *et al*., « HCV genotypes and their determinative role in hepatitis C treatment », Virusdisease, vol. 31, nº 3, p. 235-240, sept. 2020, doi: 10.1007/s13337-020-00592-0.

[6] H. Barth, « Hepatitis C virus: Is it time to say goodbye yet? Perspectives and challenges for the next decade », World J. Hepatol., vol. 7, nº 5, p. 725-737, avr. 2015, doi: 10.4254/wjh.v7.i5.725.

[7] S. M. Borgia *et al*., « Identification of a Novel Hepatitis C Virus Genotype From Punjab, India: Expanding Classification of Hepatitis C Virus Into 8 Genotypes », J. Infect. Dis., vol. 218, nº 11, p. 1722-1729, oct. 2018, doi: 10.1093/infdis/jiy401.

[8] T. Asselah, M. Martinot, N. Boyer, et P. Marcellin, « Variabilité génétique du virus de l'hépatite C : implications cliniques », p. 10, 2010.

[9] Q. Cai, Z. Zhao, Y. Liu, X. Shao, et Z. Gao, « Comparison of three different HCV genotyping methods: core, NS5B sequence analysis and line probe assay », Int. J. Mol.

Med., vol. 31, nº 2, p. 347-352, févr. 2013, doi: 10.3892/ijmm.2012.1209.

[10] H. Zylberberg, M. L. Chaix, et C. Bréchot, « Infection with hepatitis C virus genotype 4 is associated with a poor response to interferon-alpha », Ann. Intern. Med., vol. 132, nº 10, p. 845-846, mai 2000, doi: 10.7326/0003-4819-132-10-200005160-00029.

[11] J. G. McHutchison *et al.*, « Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group », N. Engl. J. Med., vol. 339, nº 21, p. 1485-1492, nov. 1998, doi: 10.1056/NEJM199811193392101.

[12] J. Fung *et al.*, « Chronic hepatitis C virus genotype 6 infection: response to pegylated interferon and ribavirin », J. Infect. Dis., vol. 198, nº 6, p. 808-812, sept. 2008, doi: 10.1086/591252.

[13] E. Silini *et al.*, « Differential distribution of hepatitis C virus genotypes in patients with and without liver function abnormalities », Hepatology, vol. 21, nº 2, p. 285-290, 1995, doi: 10.1016/0270-9139(95)90082-9.

[14] L. Lu, T. Nakano, Y. He, Y. Fu, C. H. Hagedorn, et B. H. Robertson, « Hepatitis C virus genotype distribution in China: predominance of closely related subtype 1b isolates and existence of new genotype 6 variants », J. Med. Virol., vol. 75, nº 4, p. 538-549, avr. 2005, doi: 10.1002/jmv.20307.

[15] P. Trémeaux, « Étude du génome complet du virus de l'hépatite C par séquençage de nouvelle génération: mise au point et applications », p. 117.

[16] J. Aissa Larousse, « Genetic variability of NS3, NS5A and NS5B regions of hepatitis C virus in Tunisians naïve-patients », Theses, Université de Bordeaux, 2015. Consulté le: 16 avril 2018. [En ligne]. Disponible sur: https://tel.archives-ouvertes.fr/tel-01299032

[17] M. A. Mallory, D. X. Lucic, M. T. Sears, G. A. Cloherty, et D. R. Hillyard, « Evaluation of the Abbott RealTime HCV genotype II RUO (GT II) assay with reference to 5′UTR, core and NS5B sequencing », J. Clin. Virol., vol. 60, nº 1, p. 22-26, mai 2014, doi: 10.1016/j.jcv.2014.02.006.

[18] Q. Cai, Z. Zhao, Y. Liu, X. Shao, et Z. Gao, « Comparison of three different HCV genotyping methods: Core, NS5B sequence analysis and line probe assay », Int. J. Mol. Med., vol. 31, nº 2, p. 347-352, févr. 2013, doi: 10.3892/ijmm.2012.1209.

[19] P. Vaghefi, E. Marchadier, E. Dussaix, et A.-M. Roque-Afonso, « Génotypage du virus de l'hépatite C : comparaison du test Abbott RealTime HCV Genotype II au séquençage de la région NS5B », Numéro Spéc. Infect. - Virol. - Symp. Grippe Aviaire, vol. 58, nº 2, p. 175-178, avr. 2010, doi: 10.1016/j.patbio.2009.07.023.

[20] V. Leroy *et al.*, « Daclatasvir, sofosbuvir, and ribavirin for hepatitis C virus genotype 3 and advanced liver disease: A randomized phase III study (ALLY-3+) », Hepatol. Baltim. Md, vol. 63, nº 5, p. 1430-1441, mai 2016, doi: 10.1002/hep.28473.

[21] I. Brahim *et al.*, « Morocco underwent a drift of circulating hepatitis C virus subtypes in recent decades », Arch. Virol., vol. 157, nº 3, p. 515-520, mars 2012, doi: 10.1007/s00705-011-1193-7.

[22] A. Benani *et al.*, « HCV genotypes in Morocco », J. Med. Virol., vol. 52, nº 4, p. 396-398, août 1997.

[23] E. Gower, C. Estes, S. Blach, K. Razavi-Shearer, et H. Razavi, « Global epidemiology and genotype distribution of the hepatitis C virus infection », J. Hepatol., vol. 61, nº 1, Supplement, p. S45-S57, nov. 2014, doi: 10.1016/j.jhep.2014.07.027.

[24] J. B. Nousbaum, « Les sous-types génomiques du virus de l'hépatite C: épidémiologie, diagnostic et conséquences cliniques », Bull. Société Pathol. Exot., vol. 91, p. 29-33, 1998.

[25] M. Basaras, N. Lombera, B. de las Heras, C. López, E. Arrese, et R. Cisterna, « Distribution of HCV genotypes in patients infected by different sources », Res. Virol., vol. 148, nº 5, p. 367-373, sept. 1997, doi: 10.1016/S0923-2516(97)89133-9.

Data Availability Statement: The NS5Bs sequence data from our study has been published on Genbank. (https://www.ncbi.nlm.nih.gov/genbank/) with the following access numbers.

NCBI Accession Numbers				
MZ055881	MZ418138	MZ418158	MZ418178	MZ418198
MZ356313	MZ418139	MZ418159	MZ418179	MZ418199
MZ356314	MZ418140	MZ418160	MZ418180	MZ418200
MZ356315	MZ418141	MZ418161	MZ418181	MZ418201
MZ356316	MZ418142	MZ418162	MZ418182	MZ418202
MZ356317	MZ418143	MZ418163	MZ418183	MZ418203
MZ356318	MZ418144	MZ418164	MZ418184	MZ418204
MZ356319	MZ418145	MZ418165	MZ418185	MZ418205
MZ356320	MZ418146	MZ418166	MZ418186	MZ418206
MZ356321	MZ418147	MZ418167	MZ418187	MZ418207
MZ356322	MZ418148	MZ418168	MZ418188	MZ418208
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MZ356324	MZ418150	MZ418170	MZ418190	MZ418210
MZ356325	MZ418151	MZ418171	MZ418191	MZ418211
MZ356326	MZ418152	MZ418172	MZ418192	MZ418212
MZ356327	MZ418153	MZ418173	MZ418193	MZ418213
MZ356328	MZ418154	MZ418174	MZ418194	MZ418214
MZ356329	MZ418155	MZ418175	MZ418195	MZ418215
MZ356330	MZ418156	MZ418176	MZ418196	MZ418216
MZ356331	MZ418157	MZ418177	MZ418197	MZ418217