MOLECULAR CHARACTERIZATION OF HEPATITIS C VIRUS STRAIN CIRCULATED IN CHRONICALLY INFECTED PATIENTS IN ABIDJAN (COTE-D’IVOIRE)

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ABSTRACT: Viral hepatitis C (HCV) is a public health problem. The therapeutic management and in particular the duration of treatment depends on the viral genotype. HCV is poorly documented in the population and there are few data on the different genotypes and subtypes of HCV circulating in Côte d’Ivoire. In this context, the main objective of this study was to study the genetic variability of the HCV virus in infected patients in Abidjan. We conducted a cross-sectional study at CIRBA from June 2015 to June 2017 which included adult patients with a Viral Load > 1000 IU/mL. HCV genotyping was performed by amplification of the NS5B region followed by sequencing with an ABI 3130 sequencer (Applied Biosystems, Courtaboeuf, France). Phylogenetic trees were produced using MEGA 7 software and genotypes were confirmed using online software (http://hcv.geno2pheno.org). In this study 94 subjects were included. The genotypes encountered were genotypes 1, 2 and 4 with a prevalence of 46%, 52% and 2% respectively. These strains were divided into 17 subtypes genotype 1 : 6 subtypes 1a, 1b, 1c, 1d, 1i, 1k, genotype 2 : 9 subtypes 2a, 2b, 2c, 2c/k, 2f, 2j, 2k, 2l, 2r and 2 subtypes 4f and 4r for genotype 4. The study allowed the implementation of a genotyping technique and monitoring showed that genotypes 1 and 2 are predominant in Côte d’Ivoire. The circulation of genotype 4 is noted.

KEYWORDS: HCV, Sequencing, Genotype, Treatment.

INTRODUCTION

The number of chronic carriers of the hepatitis C virus (HCV) is estimated at 3% of the world population, or 170 million individuals at a risk of 20% liver cirrhosis and 4% hepatocellular carcinoma (Cuypers et al., 2015). HCV infection is a public health problem. (Dimitris et al., 2015; Vilibic-Cavlek et al., 2015) with 3 to 4 million newly infected individuals each year Michael et al., 2015) In Côte d’Ivoire, prevalence in the population is over 5% (Enel et al., 2015).

HCV is classified into 7 genotypes rated from 1 to 7 (Ruta and Cernescu, 2015). The prevalence of HCV genotypes varies geographically and demographically throughout the world (Daw et al., 2015). Overall, genotype 1 is the most widespread, followed by genotypes 3, 2, 4 and 6. Genotypes 5 and 7 are less common (Chen et al., 2015) Genotype 1 is present in most countries
(Bukh, 2016), genotype 2 is dominant in West Africa. (Messina et al., 2015; James et al., 2014) Genotype 3 is more present in South Asia and Scandinavia (Messina et al., 2015) Genotype 4 reigns in Central and Northern Africa and the Middle East, but genotypes 5 and 6 are endemic in South Africa and Southeast Asia, respectively. (Chen et al., 2015; Cuypers et al., 2015) So far, only one genotype 7 infection has been reported. Genotype 7 has been isolated in Canada from an immigrant from central Africa, specifically Congo. (Messina et al., 2015; Wasitthankasem et al., 2015) The treatment offered to the hepatitis C patient is expensive and not specific. The duration and outcome of this treatment depends largely on the HCV genotype involved in the infection. (Mejri, 2012)

Indeed, HCV is poorly documented among the population living in Côte d'Ivoire and there are few data on the different subtypes of HCV circulating in Côte d'Ivoire. In this context, to characterize the circulating genotypes, the main objective of this study was to study the genetic variability of the hepatitis C virus in patients infected in Abidjan.

MATERIALS AND METHODS

Type of Study

This is a cross-sectional descriptive and analytical study conducted at Abidjan Integrated Bioclinical Research Center (CIRBA) between June 2015 and June 2017 to determine the prevalence of the different HCV genotypes circulating in Abidjan. All consecutive adult patients with chronic HCV antibody (HCV-Ac) and HCV viral load (HCV-VL) > 1000 IU/mL were eligible for the study. Not included in this study patients with HCV-VL < 1000 IU/mL and under 18 years of age. The study was approved by the National Committee of Ethics of Life Sciences and Health (CNESVS).

Biological Analysis

Quantification of HCV RNA

After the reagents and samples were prepared, a volume of 1000 µL of control and patient samples was collected and deposited in well identified barcoded tubes for automatic extraction of HCV RNA by Cobas AmpliPrep (ROCHE). The RNA extracts were subsequently removed from the automatic extractor and finally deposited in COBAS TaqMan 48 (ROCHE) to achieve amplification. The HCV viral load (HCV-VL) detection threshold was 15 IU/mL.

Determination of HCV genotypes

Viral RNA was extracted after lysis of viral particles and purification on filter columns (QIAamp viral RNA Mini KIT, Qiagen, Germany) according to the manufacturer's recommendations. After RNA extraction, The HCV sequences were specifically amplified using the Superscript III one Step RT kit (INVITROGEN) for the first PCR. The primer pairs used were 5'PR3/3'PR4. RT-PCR conditions were: 1 cycle at 50°C for 30 min; 1 cycle at 94°C for 2 min. Then : (30 sec at 94°C; 45 sec at 60°C; 1 min at 68°C) x 5 cycles followed by (-0.3°C/Cycle) (30 sec at 94°C; 45 sec/60°C at 49.5°C; 1 min at 68°C) x 35 cycles, (30 sec at 94°C; 45 sec/49.5°C, 1 min at 68°C) x 5 cycles and 1 cycle for 10 min at 68°C; storage at 8°C. The Qiagen High hot start kit (Hotstartaq® DNA Polymerase 1000 UNITS, Qiagen GmbH Germany) was used for the second PCR. The primer pairs used were 5'PR3/3'PR5. Nested
conditions were: 1 cycle at 95°C for 15 min, then (30 sec at 94°C; 30 sec at 55°C; 3 min at 72°C) x 35 cycles followed by 1 cycle at 72°C for 7 min; storage at 8°C. The amplicons obtained were purified with the QIAquick PCR Purification Kit (Qiagen, Germany). The sequence reaction was performed with the Bigdye Terminators V3.1 Sequencing Kit (Applied Biosystems, Courtaboeuf, France). The products of the sequence reaction were precipitated and purified by an ethanol purification process according to the manufacturer's recommendations (Applied Biosystems, Courtaboeuf, France). An electrophoretic migration of the purified products was done with the Genetic Analyser sequencer 3130 (Applied Biosystems, Courtaboeuf, France) for sequence determination. The sequences have been aligned with Seqscape software 3 (Applied Biosystems, Courtaboeuf, France). All HCV sequences obtained after sequencing were compared with reference sequences corresponding to genotypes 1-7 using BioEdit v7 and Mega 7 software (Kumar et al., 2016) on the online site http://hcv.geno2pheno.org.

Phylogenetic analyses

In order to specify the viral subtypes, the consensus sequences obtained were aligned with the reference sequences available in the online site http://hcv.geno2pheno.org. The sequences were aligned with BioEdit v7 software. The phylogenetic trees were made with Mega 7 software (Kumar et al., 2016).

RESULTS

Characteristics of the patients included in the study

94 HCV infected patients were enrolled in this study from June 2015 to June 2017. The male sex represented 61% (n= 57/94) and the median age was 59 years [28-81]. Patients had a median HCV-VL of 1.93.10^6 IU/mL [2.23.10^5 - 4.37.10^6] (Table 1).

Table 1. Social and biological characteristics of patients included in the study (N=94)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Men (N, %)</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Women (N, %)</td>
<td>37</td>
</tr>
<tr>
<td>AGE (year) (moyen, interval)</td>
<td>59</td>
<td>26-81</td>
</tr>
<tr>
<td>HCV-VL : (UI/mL) (median, interval)</td>
<td>1.93.10^6</td>
<td>2.23.10^5 - 4.37.10^6</td>
</tr>
</tbody>
</table>

Distribution of the different HCV genotypes encountered

The genotypes encountered were genotypes 1, 2 and 4 with a prevalence of 46% (n= 43/94), 52% (n=49/94) and 2% (n=2/94) respectively (Figure 1).
Phylogenetic analysis showed that the genotypes encountered were genotypes 1, 2 and 4 (Figure 2)

Figure 1. Distribution of the different HCV genotypes 1, 2 and 4 encountered with a prevalence in 94 subjects of study.

Figure 2. Phylogenetic trees and frequencies 1, 2 and 4 obtained from the HCV NS5B sequence after analysis of fragments produced using Mega 7 and Bio edit V7 software. The consensus sequences were aligned and compared with reference sequences. (http://hcv.geno2pheno.org)
Distribution of the different subtypes of HCV encountered

Analysis of the 94 HCV NS5B sequences from patients identified 17 subtypes. These strains were distributed as follows: genotype 1: 6 subtypes, genotype 2: 9 subtypes and 2 subtypes for genotype 4. The 6 subtypes of genotype 1 were: subtypes 1a, 1b, 1c, 1d, 1i, 1k, with a respective prevalence of 13.95% (6/43), 37.20% (16/43), 34.88% (15/43), 4.65% (2/43), 4.65% (2/43), 4.65% (2/43) (Figure 3). The 9 subtypes of genotype 2 were subtypes 2a, 2b, 2c, 2c/k, 2f, 2j, 2k, 2l, and 2r with respective prevalence 6.12% (3/49), 4.08% (2/49), 16.33% (8/49), 22.44% (11/49), 4.08% (2/49), 2.04% (1/49), 26.53% (13/49), 16.32% (8/49), 2.04% (1/49) (Figure 4).

![Figure 3](image_url)

Figure 3. Distribution of subtypes of HCV genotype 1 obtained from phylogenetic trees and an online molecular typing tool (http://hcv.geno2pheno.org).
Figure 4. Distribution of HCV genotype 2 subtypes obtained from phylogenetic trees and an online molecular typing tool (http://hcv.geno2pheno.org).

DISCUSSION

Phylogenetic analysis of the NS5b sequence of the 94 isolates obtained in this study indicated the presence of different genotypes 1, 2 and 4 and 17 HCV subtypes. This study showed that genotypes 1 and 2 were predominant in the Republic of Côte d'Ivoire with prevalences of 46%, 52%. These results confirm the circulation of genotypes 1 and 2 in Côte d'Ivoire as described by Forbi et al., 2014 in a retrospective study, the presence of genotypes 1 and 2 as the circulating genotypes in 18 HCV-PCR-positive plasma samples among 608 plasmas of pregnant women collected and preserved since 1995 in Côte d'Ivoire.

The presence of genotypes 1, 2 and 4 in Côte d'Ivoire could be justified by the circulation of these genotypes in West Africa as confirmed by the presence of genotypes 1, 2 and 4 in studies conducted by Candotti et al., 2003 and Jeannel et al., 1998 in HCV positive blood donors in West Africa, namely Guinea Conakry, Burkina Faso and Ghana. Umar et al., 2017 and Forbi et al., 2012 also described the circulation of genotypes 1 and 2 in northern Nigeria.

In other parts of Africa, the distribution of HCV genotypes showed that genotypes 1 and 2 were present in Kenya in studies conducted by Mwangi et al., 2015 In North Africa specifically in Libya Daw et al., 2015 and Elasifer et al., 2010 reported HCV genotypes 1, 2, 3 and 4 in positive patients in their studies.

This study highlighted the introduction of genotype 4 in Côte d'Ivoire. Several studies have described that genotype 4 is generally localized in central and northern Africa. These are the studies conducted in Cameroon by Ndjomou et al., 2003 who determined the presence of genotypes 1 and 4 in and by Ndong-Atome et al., 2008 who described only the presence of genotype 4 in pregnant women in this last study in Gabon.
This relatively high rate of genotype 2 (52%) compared to genotype 1 (46%) in our study is comparable to the different prevalences of genotypes determined in the studies conducted by Mwangi et al., 2015 and Candotti D et al., 2003 However, Umar et al., 2017 determined a high rate of over 92% of genotype 1 compared to genotype 2 which was approximately 8% in 173 HCV positive patients.

Among the 7 different HCV genotypes, we described 3 different genotypes circulating in Côte d'Ivoire, which were genotype 1, 2 and 4. Subtypes 1a, 1b, 1c, 1d, 1i, 1k, 2a, 2b, 2c, 2c/k, 2l, 2j, 2k, 2r 4r have been described with a predominance of subtype 2k. A study by Umar et al., 2017 described similar subtypes such as 1a, 1b, 1c, 2a, 2b with a high prevalence of subtype 1a at 54%. A total of 9 subtypes 1a, 1b, 2a, 2c, 2a/c, 3a, 4a, 4c/d were identified by Elasifer et al., 2010 for a high rate of subtype 1b.

The respective prevalences of genotypes 1, 2 and 4 were 46%, 52% and 2%. Among the subtypes of genotype 1 we determined 6 subtypes which were subtypes 1a, 1b, 1c, 1d, 1i, 1k, with a respective predominance of subtypes 1b, 1c, 1a. For the management of HCV positive patients, it should be noted that the duration and outcome of HCV treatment depends largely on the HCV genotype involved in the infection Mejri et al., 2012. We therefore reported that approximately 50% of HCV positive patients in Côte d'Ivoire would be under a long treatment time compared to the others. According to the 2014 Recommendation Report 2014, HCV treatment in naïve patients with genotype 1 and 4 was twice as long and therefore more expensive than genotype 2. Indeed for the same type of treatment with the combination of Sofosbuvir with ribavirin of these 3 different genotypes 1, 2 and 4, the duration of treatment that of genotype 2 would be between 12 and 16 weeks while that of genotypes 1 and 4 would be 24 weeks.

But the presence of circulating HCV subtype 1a would have an incident in the treatment of patients infected with this strain, because it has been demonstrated in several studies that subtype 1a is more resistant to treatment compared to other subtypes of the same genotype 1 hence the importance of knowing the subtype responsible for infection (Pelliceli et al., 2012). Not all antivirals currently recommended for the treatment of hepatitis C are equally effective on all genotypes, requiring pre-therapeutic determinations of genotypes and subtypes (Gepsi, 2016). This is a barrier in some low-income countries like ours and helps limit access to care. A pangenotypic treatment which would be effective whatever the genotype would make it possible to avoid these preliminary tests (AFEF, 2017) Hence the interest of using pangenotypic molecules that will effectively treat HCV regardless of the genotype in question.

CONCLUSION

The study allowed the implementation of a genotyping technique and monitoring showed that genotypes 1 and 2 are predominant in Côte d'Ivoire. Note the introduction of genotype 4. Among the subtypes, the 2k subtype was the most common. As HCV treatment is genotype-dependent, epidemiological surveillance is an important factor for better management in a resource-limited setting.
REFERENCES


