


Characterization of Hepatitis C (HCV) Virus Genotypes and Genomic Subtypes in Burkina Faso

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Abstract

Background: Hepatitis C virus (HCV) causes about 900 deaths per year in Burkina Faso. In the absence of a vaccine, HCV infection remains a major public health problem. In addition to prevention efforts, an important strategy in the fight against HCV is therapy. Knowledge of the different genotypes circulating can allow for better treatment tailoring. This study aimed to 1) determine the HCV genotypes among blood donors and among patients, and 2) to assess the performance of the rapid diagnostic tests used extensively in sub-Saharan Africa by comparing their results to those of molecular methods. **Methods:** A total of 85 anti-HCV-positive samples were tested for viral RNA and sequenced. The analysis was performed in three centers: The Molecular Biology and Genetic Laboratory/PIETRO Annigoni Biomolecular Research Centre (CERBA/LABIOGENE), the University of Ouagadougou, the National Blood Transfusion Centre (CNTS), Burkina Faso, and the National Blood Transfusion Institute (INTS), Paris, France. Data were analyzed using the software CLC and Mega. **Results:** Nineteen (19) samples from 85 (22.35%) were confirmed positives by Monolisa; 9 from patients and 10 blood donors, but only 47.36% (9/19) of these were RNA positive. The median viral load was 204,807 IU/ml. The nine RNA-positive samples were found through sequenc-

ing to be 88.88% of genotype 2 and 11.11% of genotype 1. The identified Subtypes of genotype 2 are subtypes 2f and 2d; subtype 1d for genotype 1. **Conclusion:** There is an important number of false positive results from serology. The HCV genotype 2 is the most frequent in Burkina Faso.

Keywords

Hepatitis C Virus, Genotypes, Blood Donors, Patients, Burkina Faso

1. Background

About 180 million people are infected worldwide with the hepatitis C virus (HCV) [1]. In Africa in general, HCV remains a public health problem; its prevalence varies from one region of the continent to another. A recent study on the prevalence of HCV among migrants from North and West Africa to Europe gives an average prevalence of 7.6% with a West African prevalence between 2.1% and 14.1% [2]. The HCV infection has long existed in West Africa [3]. Sub-Saharan Africa has about 20% of global infections [4]. In Burkina Faso, its prevalence is around 1% [5]. Compared to HBV (14.7%), this prevalence appears low. However, the absence of a vaccine and the high cost of treatment make HCV a major health problem in the country. It is responsible for over 900 deaths per year in Burkina [6]. In general, because of the lag between HCV infection and complications that ensue (cirrhosis, hepatocellular carcinoma, decompensating hepatitis), morbidity related to HCV may increase in the next decades [7].

Until recently, HCV has had six identified genotypes. A new genotype was recently identified, which brings the number of genotypes of the virus to 7, with 67 subtypes [8]-[10]. This genetic diversity is a challenge for vaccine development. Also, the dosage and duration of treatment depend on the genotype implicated in the infection [11]. For example, sofosbuvir (SOF) and ribavirin (RBV) are safe and effective drugs for the treatment of patients with HCV genotype 2 infection [12], while the direct-acting antivirals (DAAs) with pan-genotypic activities (Simeprevir, sofosbuvir, and Daclatasvir) are recommended in triple regimens with PEG-IFN/RBV for the treatment of HCV genotype 4 [13]. Identification of the genotype implicated in an infection is of great importance.

The objective of this study was to determine the current genotypes of HCV in Burkina Faso and to compare the results of rapid tests to those of molecular methods.

2. Methods

2.1. Study Design and Population

This was a cross-sectional study with a population involving patients diagnosed HCV positive after medical consultation at the Pietro Annigoni Centre for Biomolecular Research (CERBA) and 75 first-time blood donors positive for HCV antibodies recruited from 31st January to 28th February 2016 at the blood transfu-

sion national Centre, Burkina Faso. The patients were treatment naïve. The inclusion criteria were adults with an HCV seropositive result without any exposure to antiviral agents active against HCV.

2.2. Sample Size Estimation and Sampling Procedure

Eighty-five (85) participants were enrolled. Participants were included in the study based on their hepatitis C seropositivity, without other considerations. The low number of participants included in the study is due to the fact that the prevalence of hepatitis C in our country is quite low, around 1%. This justifies our consideration of both patients and blood donors.

2.3. Study Procedures

The socio-demographic data were collected following the standard panel of questions for blood donors and by consulting patient health records. Eight milliliters of venous blood were collected in EDTA tubes, centrifuged, and aliquots made. HCV status was determined using a rapid diagnosis test TDR (code 172-025/S, anti-map HCV) Cypress diagnostics at the National Blood Transfusion Centre, CNTS, and at CERBA/LABIOGENE, Burkina Faso. A drop of serum is placed in the deposition zone of the test strip, and the results are read after migration. The emergence of two distinct red lines indicates the presence of anti-HCV antibodies, and the sample is then considered positive. The appearance of a single line in the control area indicates the absence of anti-HCV antibodies (negative sample) but validates the test.

The samples were then sent to Paris, France, at the National Institute of Blood Transfusion (INTS), French National Reference Laboratory for hepatitis and HIV. All samples were then tested again using Monolisa HCV Ag-Ab ULTRA Version 2. Viral RNA was extracted from 200 µl of serum using the extraction kit High Pure Viral Nucleic Acid Kit Roche, following the manufacturer's instructions. Quantitation of HCV viral RNA was performed using the Cobas Taqman method.

RT was performed with pdN6 Random primer. It was followed by a nested PCR targeting the NS5B region of the genome (the expected size of approximately 382 bp). The PCR products were purified using the QIAprep spin miniprep kit (QIAGEN). Eurofins performed sequencing using PR3 and PR5 primers. Multiple sequence alignment was performed with the CLC software on the sequences of genotypes 1-6 available in GenBank

(<http://www.ncbi.nlm.nih.gov/genbank/index.html>). Phylogenetic analysis was performed using the neighbor joining method with Kimura-2 algorithm, and the phylogenetic tree illustrating this analysis was constructed with the Tree Explorer program contained in MEGA 5.03 because of its availability, its adaptation to the Windows platform, and above all the fact that its tree inference algorithms compare favorably with other software in terms of accuracy and efficiency. A reconstruction bootstrap was conducted 1000 times, and the values > 70% were considered significant.

3. Results

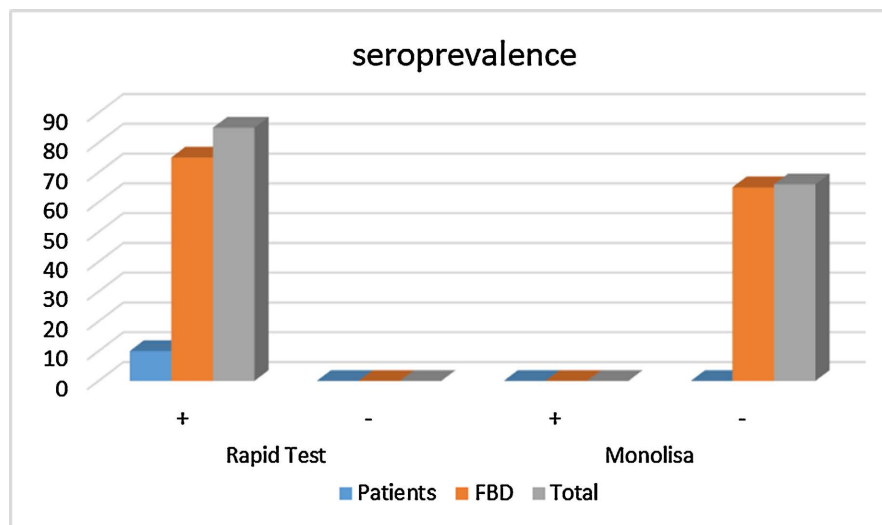
3.1. Patient's Characteristics

A total of 85 participants were enrolled, of which 67 (78.80%) were men and 18 (21.2%) women. Their age is between 18 and 87 years old. Most of the population (48.20%) was composed of pupils/students. 51.80% are distributed among traders, self-employed, officials, and faithful believers. Blood donors and patients all live in Ouagadougou and its suburbs. Because the capital is home to our collection centers, which are the Blood Transfusion Center and CERBA. This explains why participants come mainly from the capital and its suburbs for obvious reasons of proximity.

3.2. Serological and Molecular Analysis

Monolisa confirmation

Figure 1 shows that in total, 22.35% of the study population was confirmed positive for HCV antibodies.



TDR: Rapid tests; FBD: First-time blood donors.

Figure 1. HCV seroprevalence.

Molecular prevalence

A total of 19/85 (22.35%) were positive using the Monolisa test, while the nested PCR targeting the NS5B region of the genome was positive in 47.36% (9/19) of the individuals (**Table 1**).

Viral loads

Eight viral loads between 10,542 and 2,057,042 IU / ml were determined (**Table 2**). The median is 204,807 IU/ml. A sample gave an invalid result despite repeated testing.

Genotypes and subtypes:

One patient 1/9 (11%) had the genotype 1 (HCV-1) subtype 1d. Five (5) patients and 3 first-time blood donors 8/9 (89%) were of the genotype 2 (HCV-2). **Table**

3 shows the different genotypes.

Table 1. Molecular prevalence.

	Monolisa+	NS5B ARN
Patients	09	06 (66.66%)
Blood donors	10	03 (30%)
Total	19	09 (47.36%)

Table 2. Viral loads for RNA-positive samples (N = 9).

	Participant N°	Viral Loads (UI/ml)
Patients	77	2,057,042
	79	331,026
	80	invalid
	81	674,262
	83	1,543,816
	85	63,381
Blood donors	21	78,589
	48	37,761
	65	10,542

Table 3. Identified genotypes of VHC.

	NS5B ARN HCV Positive	Genotypes	
		1	2
Patients (N)	06	1	5
Blood donors (N)	03	-	3
Total	09	1 (11.11%)	8 (88.88%)

The identified Subtypes of genotype 2 are subtypes 2f and 2j, observable in **Figure 2**. The subtypes of the two samples (circled in blue in **Figure 2**) could not be clearly determined with regard to their position on the phylogenetic tree.

4. Discussion

Previous studies have reported that HCV genotypes circulate among blood donors in Burkina Faso. Genotypes 2 and 3 were found to be the most common [6]. Apart from blood donors, HCV genotypes in Burkina Faso have not been well investigated. This study sought to identify genotypes in first-time blood donors and in patients found to be HCV seropositive at the clinics, and take a critical look at the rapid tests used extensively in sub-Saharan Africa by comparing their results with those of molecular methods. The results of confirmation tests by Monolisa gave

diagnostic performance of these antibody tests is low, and false positives are more likely to occur due to previous HCV infection in the individuals. A total of 47.36% of HCV antibody-positive individuals were confirmed RNA positive (**Table 2**), a disagreement of about 52% between HCV sero-positive by Monalisa and the results of molecular analyses. This discordance is, however, a big surprise. Indeed, Zeba *et al.*, in 2013, had found a 1.5% positive RNA, which is a difference of nearly 99% between serological and molecular results. This could be explained by the fact that most patients have not yet detectable viral RNA. Mullis and colleagues reported in Uganda in 2013 that 7.6% of undetectable viremia in 1000 HCV-infected people [15]. However, it is not possible to consider this as a fact because NTAGI-RABIRI and colleagues in 2014 achieved 100% positive RNA from HCV antibodies positive in Burundi [16]. Response to treatment, long-term prognosis, and seroconversion profile are influenced by genotype. This is why it is very important to know the genotype of an infected person. In this study, we report genotypes 1 and 2 (**Table 3**) as those encountered in Burkina Faso. Among blood donors, the identified genotype is genotype 2. Zeba *et al.* have also found this, mainly (56.3%) among blood donors, in addition to genotypes 3 and 4. Genotypes 2 and 3 are the most virulent and account for the majority of infections worldwide. Our results corroborate those of other studies. Indeed, genotype 2 is considered to be from West Africa [17]. Candotti *et al.* reported in 2003 that, in West Africa, genotype 2 (2a) is responsible for the majority of infections [18]. For samples for which the subtypes could not be clearly determined, sequencing of the core region may be able to remove the ambiguity.

Viral loads that range from 10,542 to 2,057,042 IU/ml are high. For treatment, we suggest the use of the combination pegylated interferon alfa 2a and ribavirin, which gave a sustained virological response (SVR) of 92.3% for genotype 2 and 81.2% for all genotypes combined in patients in Burkina Faso in 2010 [19]. Nevertheless, further studies on the efficacy of treatment for genotype 2 remain indicated [20].

Limitations of the study

The small number of patients does not allow for the study of the correlation between socio-demographic characteristics and HCV infection. This small number constitutes a limit to the statistical strength of the study.

5. Conclusion

Genotype 2 is the most frequent both in blood donors and patients. The identified Subtypes of genotype 2 are subtypes 2f and 2d; subtype 1d for genotype 1. We can conclude that genotype 2 is the most common genotype in Burkina Faso. The genotype 1, although rare in West Africa, is present in our country (11.11%). There is a high percentage of false positives by RDT; the causes of this situation need to be known to avoid harmful consequences. In the meantime, we suggest to the health authorities that particular attention be paid to the conditions of transport and storage of rapid diagnostic tests in particularly hot countries.

Ethics Approval and Consent to Participate

The Ethics Committee for Health Research (CERS) of Burkina Faso has approved this study (Reference No. 2013-7-065 of July 11, 2013). All participants provided a written informed consent to participate in the study. Anonymity and confidentiality with respect to the information collected were scrupulously respected.

Availability of Data and Materials

All relevant data supporting the findings are contained within the manuscript. The dataset is available from the corresponding author upon a reasonable request.

Authors' Contributions

IT and EY collected samples; IT and DC performed molecular analysis and data analysis; IT and ATY drafted the manuscript; FWD and CB contributed to the study design and revised the manuscript; OAK revised the manuscript; DOY and JS designed the study and revised the manuscript for important intellectual content.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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List of Abbreviations

HCV	Hepatitis C Virus
RNA	Ribonucleic Acid
CNTS	Centre National de la Transfusion Sanguine
INTS	Institut National de la Transfusion Sanguine
DAAs	Direct-Acting Antivirals
HOSCO	Hôpital Saint Camille de Ouagadougou