

HBV and HCV Seroprevalence and the Predominant HCV Genotypes in a Hospital Setting in Cameroon

Djuidge Ngounoue M^{1,2*}, Fepa Kwesseu AG¹, Nwobegahay J³ and Moundipa Fewou P¹

¹University of Yaoundé I, Cameroon

²Yale Boyer Center for Molecular Medicine, New Haven, CT, US

³Military Health Research Center (CRESAR), Yaoundé, Cameroon

*Corresponding author: Djuidge Ngounoue Marceline, Fulbright Visiting Scholar, Yale University Boyer Center for Molecular Medicine, New Haven, CT, US; Senior Scientist, University of Yaoundé I; Tel: (237) 699 12 88 74; E-mail: djngmarceau@yahoo.fr

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Abstract

Infectious diseases are a substantial threat to global health. Hepatitis viral infections are life threatening infections, responsible for most liver cirrhosis and cancers. In order to determine the seroprevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infections; as well as the HCV genotypes of the collected isolates, a study was conducted at a hospital setting in Cameroon. Enzyme immunoassays were used for HBV and HCV screening tests; HCV genotypes and subtypes were determined using standard molecular biology and genomic techniques that involved RT-PCR, gene cloning, DNA sequencing, and bioinformatics tools. The results showed that 14.15% of the study populations were screened HBV positive; whereas 24.82% were diagnosed HCV positive. The seroprevalence was higher in men: 12.75% and 16.1% respectively for HBV and HCV infections. The majority of HBV infected were younger, whereas HCV infected people were older. HCV genotypes 1 and 4 were identified. These findings are useful for policy making in Cameroon. Furthermore, the HCV clones generated in the present study might constitute useful and representative molecular tools for vaccine and drug development.

Keywords Hepatitis B virus; Hepatitis C virus; Seroprevalence; Genotypes; Subtypes

Background

Hepatitis viral infections are life threatening infections of the liver. Five types of viruses are known to be responsible of hepatitis: A, B, C, Delta and E. Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the most virulent types, with infections among the top 10 causes of death worldwide; they are responsible for most liver cirrhosis and cancers. HBV, HCV and human immunodeficiency virus (HIV) are blood-borne pathogens but it is reported that the risk of contracting HBV is 50 to 100 fold higher than that of HIV, because HBV has a higher degree of infectivity [1]. HBV immunization is available worldwide. Although acute HBV infection does not require treatment, chronic infection is not yet curable and therefore involves a continuous treatment. Conversely, no vaccine is currently available against HCV. Antiviral medicines can cure approximately 90% of people with HCV, thereby reducing the risk of death from liver cirrhosis and cancer [2]. The introduction of direct acting antivirals (DAAs) like “Daclatasvir” (NS5A inhibitor), “Symeprevir” (NS3 inhibitor), and “Sofosbuvir” (NS5B inhibitor), against HCV is revolutionizing the field of HCV as HAART did in 1996 with HIV, and therefore improving the prognosis of HCV infection as well as co-infections with other viruses like HIV [3]. However, due to their cost, DAAs are unavailable to the vast majority of patients in sub-Saharan African countries. In addition, the duration and the success of HCV treatment depend on the stage of the infection (acute or chronic), but also on the HCV genotypes/subtypes. Hence, there is a need to always perform HCV genotyping prior to the initiation of the treatment. The previous national survey indicates that Cameroon is highly endemic for HBV infection (11.9%), but slightly

endemic for HCV infection in the general population (1.03%). However, HCV infection is higher in the elder populations: from 3% between 45-55 years to 7% in people above 55 years [4]. Moreover, studies showed that HCV infection is characterized by a greater genetic diversity in Cameroon [5-7]. However, most studies focused on the general population, while more studies involving the most at risk populations are also needed for policy making, as recommended in the Cameroon national strategic plan. UNAIDS reports in 2015 indicates that rates of sexually transmitted infections in Armed Forces that are considered a risk group are 2 to 5 times higher compared to the general population in peace time, and could be 50 fold or more in the periods of conflicts. Whether HCV strains circulating worldwide and/or genotypes previously identified in the general population in Cameroon also circulate in the key populations is not known. The great heterogeneity of infections commonly identified in hospital settings in Cameroon suggests that Yaoundé Military Hospital might be a suitable site for such evaluation. The overall goal of this study was to determine the seroprevalence of HCV and HBV, and HCV genotypes among individuals visiting the Yaoundé Military Hospital.

Methods

Blood samples were collected from 149 randomly selected participants; aged between 18-64 years, and screened for HBV surface antigen (HBsAg), as well for capsid antigen and antibodies associated with HCV infection using the enzyme-linked immunosorbent assay. Cameroonian positive HCV isolates were transferred to Yale University, US, for molecular characterization and further study on cell culture systems' development. Following RNA isolation, the HCV core and Nonstructural protein 5B (NS5B) genes were subsequently amplified using reverse transcription coupled to polymerase chain

reaction (RT-PCR), semi-nested and nested PCR. PCR products were resolved in 1.5% agarose gel electrophoresis, purified, cloned into a TOPO vector, and clones were used to transform chemically competent DH5- α cells. Plasmid DNA obtained was used for DNA sequencing BigDye Terminator cycle sequencing (Applied Biosystems)

as described by Iles et al. [5]. The nucleotide sequences obtained (FASTA) and chromatograms were analyzed using the Geneious version 12.2.3 software. Consensus/FASTA sequences were used for phylogenetic analysis that included genotyping (Table 1).

Primer name	Sequence (5'-3')	Nucleotide position	Orientation
NS5B region (semi-nested PCR)			
YO 2774	TATGATACCCGCTGCTTTGACTC	8259-8278	Outer/sense
YO 2775	GCAGAGTACCTCGTCATAGCCTC	8641-8622	Outer/antisense
YO 2776	GCTAGTCATAGCCTCCGT	8633-8619	Inner/antisense
Core Region(Nested PCR)			
YO 2777	ACTGCCTGATAGGGTGCTTGCGAG	291-311	Inner/Sense
YO 2778	ATGTACCCCATGAGGTCGGC	748-732	Outer/Antisense
YO 2779	AGGTCTCGTAGACCGTGCATCATG	324-344	Inner/Sense
YO 2780	CATGTGAGGGTATCGATGAC	721-705	Outer/Antisense

Table 1: Primers for the various PCR.

Results and Discussion

Out of the 149 collected samples, 27 (14.15%) were screened HBV positive whereas 37 (24.82%) were diagnosed HCV positive. The seroprevalence was higher in men: 19 (12.75%) and 24 (16.1%) respectively for HBV and HCV infections. The majority of HBV infected were younger (51% between 20-39 years), whereas HCV infected people were older, with 35% of patients above 55 years. No HBV-HCV co-infection was observed. These seroprevalence results are unusually higher and different, compared to the previous studies and the WHO data. In fact, previous findings' figures display a high HBV seroprevalence but a low HCV incidence [6,8-10]. The relatively low HBV prevalence in the study group might be due to the fact that they are more likely to undergo regular medical check-up and are well follow-up. Conversely, the unusual high HCV seroprevalence might be explained by the high number of participant aged above 55 years. This confirms that HCV infection is prevalent in older people. Moreover,

the high HCV seroprevalence observed might be in part due to the robustness of the screening test/early detection: In the present study, the 4th generation ultra-enzyme immunoassay for the detection of HCV core antigen and anti-HCV antibodies was used.

Successfully analyzed HCV samples were characterized as belonging to genotypes 1 and 4 (1b and 4a). These findings corroborate previous investigations on the circulating genotypes in Africa. WHO data indicate that the genotype 1 (e.g. subtypes 1a and 1b) circulates worldwide, especially in the western nations whereas molecular epidemiology studies in Cameroon describe a predominance of HCV genotypes 1 and 4, though infection due to genotype 2 has also been reported in the country [5,8]. A study conducted in Gabon showed a high prevalence of HCV genotype 4 [7]. Since infection due to the named genotypes is also known to be virulent, this might explain the great challenge of a chronic hepatitis progression in these countries of the central Africa sub-Regions (Table 2).

Initial denaturation	PCR amplification-35 cycles of	Final extension and cooling
95°C for 5 min	Denaturation: 93°C for 30 sec	72°C for 10 min
	Annealing: 60°C for 30 sec	12°C forever
	Extension: 72°C for 1 min	-
Semi-nested and nested PCR		
Initial denaturation	PCR amplification-35 cycles of	Final extension and cooling
95°C for 5 min	Denaturation: 95°C for 30 sec	72°C for 10 min
	Annealing: 55°C for 30 sec	12°C forever
	Extension: 72°C for 30 sec	-

Table 2: Cycling conditions/amplification program on the BIORAD C1000 Touch Thermal cycler.

RT-PCR

First step (reverse transcription)

50°C for 30 min

5 cycles of: 93°C for 30 sec, 60°C for 45 sec, 72°C for 1 min

Conclusion

HBV and especially HCV seroprevalence is higher in the study population; The virulent HCV genotypes 1 and 4 identified confirm that these genotypes circulate in Cameroon, and are predominant even among vulnerable population like military. These results express the current epidemiological state of HBV and HCV infection in the country. The current data shall be of interest for diagnostic and management policies, as well as for the therapeutic decision in Cameroon. Indeed, the HCV clones generated through the present study might constitute useful and representative molecular tools for further investigations regarding the life cycle of the virus, the virus persistence, as well as vaccine and drug development.

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Conflict of Interest

Authors declare not having any conflict of interest associated with the present manuscript.

Ethical Consideration

The present study satisfied the national and international ethical standards: Ethical clearance was obtained from the Cameroon National Research Ethics Committee for Human Health, prior to the

study implementation, and informed consent was obtained from each enrolled participant. The study was conducted according to the CIOMS guidelines, and complied with the Declaration of Helsinki, 2013. Participants gave their authorization that samples are transferred from Cameroon to United States, for future investigations in the field of genomics and cell biology.

Limitations of the Study

Obtaining administrative authorization from hospital settings is not obvious, reason why the study was limited to one site, reducing the total number of positive isolates collected.

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