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RESEARCH ARTICLE

Seroprevalence and Molecular Biodiversity of Hepatitis C Virus in the Department of Lekoumou, Republic of the Congo

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ABSTRACT

Background: Viral hepatitis C is a major public health problem. The World Health Organization estimates that about 150-180 million people worldwide are chronic carriers of HCV. In Congo, its epidemiology is not well known. This study aimed to determine the seroprevalence and molecular biodiversity of HCV in the department of Lékoumou.

Methods: A cross-sectional descriptive study was conducted from January to July 2015. The study involved 171 adult patients attending health centers in 3 districts of the department. All samples collected were screened for the presence of HCV antibody with the "HEALTH MATE HCV Ab Plus assay" and ELISA kit. Viral RNA was reverse transcribed and amplified by the polymerase chain reaction using the virus 5'UTR region. Genotyping was performed by type-specific PCR.

Results: The average age of the study population was 45.0 ± 16.0 years. The overall seroprevalence of anti-HCV Ab was 14.6% (25/171). HCV RNA was detected in 22 patients, 20 of whom were seropositive and 2 were seronegative. Two genotypes were mainly identified: genotype 4 (G4), the most prevalent (22.7%) followed by G2 (13.6%). The co-infection of the two genotypes was observed in 45.5% of cases. The prevalence of infection was three times higher among women compared to men. Several risk factors have been identified. However, only the practice of scarification was significantly associated with carrying HCV ($p = 0.01$).

Conclusion: The results of this study suggest that the department of Lekoumou is a high prevalence area of hepatitis C in the country according to WHO criteria.

Background

Hepatitis C Virus (HCV) is a Ribonucleic Acid (RNA) virus of the family Flaviviridae. It has a hepatotropic tropism and causes inflammatory lesions resulting in acute or chronic hepatic infection. Without treatment, chronic infection progresses insidiously to serious complications such as cirrhosis and

Hepatocellular Carcinoma (HCC), hence the importance of early detection [1,2].

Diagnostic procedures include serum HCV antibody testing, HCV RNA measurement, viral genotype and subtype determination

Its diagnosis is based on the detection of HCV Antibody (Ab) serologically, the viral RNA, genotype and subtype by molecular biology techniques such as the Polymerase Chain Reaction (PCR) [2]. Currently, HCV is classified into seven (7) genotypes and 67 subtypes. Genotype identification is clinically important for determining treatment and duration [3,4]. Globally, viral hepatitis C is a real public health problem. Indeed, the World Health Organization (WHO) estimates that about 3% of the general population is infected with the virus and at least 170 million people are chronic carriers. This prevalence, however, is variable with 3 geographical areas: low, medium and high prevalence [5,6]. Africa is the continent with the highest prevalence rate of HCV at 5.3% [5]. In Congo-Brazzaville, no study has been conducted nationally. There is a diagnostic problem due to the scarcity of molecular biology laboratories. The current diagnostic conditions remain the search for anti-HCV antibodies in peripheral blood (serology). In this context, we present the first study on the epidemiology and biodiversity of genotypes of the hepatitis C virus in the department of Lekoumou whose capital is Sibiti. This department being one of the country's departments to have a high prevalence of Human Immunodeficiency Virus (HIV) [7].

Materials and Methods

A cross-sectional study was carried out from January to July 2015 at the Sibiti hospital and in two integrated health centers, Komono and Mayéyé. A total of 171 patients aged 18 years and older were included after obtaining their informed consent. A standardized questionnaire was administered to all patients in order to collect all the socio-demographic characteristics required and certain epidemiological risk factors. On each patient, 5 ml of venous whole blood were collected from the elbow folds in an EDTA tube for serological and molecular analyzes.

Serological analyzes were performed at the medical laboratory of the General Hospital of Loandjili in Pointe-Noire. After centrifugation of the blood, the plasma obtained was tested for anti-HCV antibody using the commercial "HEALTH MATE HCV one step Ab Plus" and confirmed by the Enzyme-Linked Immunosorbent Assay (ELISA) (Bio-Rad Laboratories, France) according to the manufacturer's instructions.

The molecular analyzes were carried out at the virology laboratory of the faculty of sciences and techniques of Hassan II University of Casablanca in Morocco. Total RNAs were extracted with Trizol reagent (Invitrogen). The reverse transcription and genic amplification of HCV was performed by a "One Step technique" using the "MyTaq ™ One-Step RT-PCR kit" (Bioline, UK). This technique consists of a single reaction in a single tube reverse transcription of RNA and polymerase chain reaction of the 5'UTR region of HCV. A 258 bp fragment of the 5'UTR region of HCV was amplified using the forward primer b1 (5'GCCATGGCGTTAGTATGAGT3 ') and the reverse primer b2 (5'TGCACGGTCTACGACCGCTC3'). Briefly, rt-PCR amplification was done in a final volume of 50 µL using: 25µL of My Taq Mix One Step, 14.5µL of DEPCH2O, 2µL of b1 and b2 primers, 1µL of RNase inhibitor, 0.5µL of reverse transciptase and 5µL of extacted RNA.

Reactions (Perkin Elmer 2400 GeneAmp® PCR thermal Cycler, Scientific Support, Inc, Hayward, CA) were cycled as follows: 48°C for 30 min for reverse transcription followed by 35 cycles of 95°C /30s for denaturation, +59°C / 30s for hybridization and 72°C /r 45s for elongation. Initial denaturation at 95°C/ 3 min and a final elongation phases at 72°C / 5 min were required. The amplified fragments were detected by electrophoresis on a 2 % agarose gel and revealed by UV transilluminators gel Doc (Life Science, Cambridge, UK) coupled to software UPV-Doc-It-LS Version 7.1 RC 3.54 after an Ethidium bromide staining (Bioline, UK).

The identification of HCV genotypes was performed using the type-specific PCR technique (TS-PCR). Two major genotypes prevalent in the sub region of Central Africa were searched: genotype 2 (G2) and genotype 4

(G4). Amplification was performed using the followed primers: b3 (5'AGGAAGACTTCCGAGCGGTC3')/ b4 (5'GAGCCATCCTGCCCACCCCA3') for amplified 144 bp of G4 and b3/b5 (5'ACCCTCGTTTCCGTACAGAG3') for amplified 123 bp of G2.

The statistical analysis was performed using the Pearson Chi2 test or the exact Fisher test from the epi-info software version 7.1.5.2. The significant p-value was <0.05.

Results

A total of 171 patients were included, of whom 42 (24.6%) men and 129 (75.4%) women. The mean age of the patients was 45.0 ± 16.0 years (range 18 - 85 years). The most represented age group was more than 50 years old with a frequency of 33.3% (57 patients). More than 70.2% (120/171) of the patients were workers with a secondary level of education. The majority of patients were married, 76.6% (131/171). All of the sociodemographic characteristics of the study population are shown in (Table 1).

Table 1: Socio-demographic characteristics of the study population.

Characteristics	Effectives (n)	Frequencies (%)
Age (years)		
≤20	10	5.8
21-30	27	15.8
31-40	36	21.1
41-50	41	24.0
>50	57	33.3
Profession		
Health workers	10	5.8
Workers	120	70.2
Public servants	11	6.4
Unemployed	30	17.5
Level of education		
University	2	1.2
Secondary	71	41.5
Primary	57	33.3
Illiterate	41	24.0
Marital status Married	131	76.6
Single	15	8.8
Divorced / widowed	25	14.6

Several risk factors were studied: Among factors related to sexual behavior, the lack of condom use and the multiplicity of sexual partners were the most observed risk factors with 86.5%; 70.8% respectively (Table 2).

Table 2: distribution of the study population according to risk factors.

Risk factors	Effectives (N=171)	Frequencies (%)
Blood transfusion	18	10.5
Surgery	31	18.1
Multiple sexual partners	121	70.8
lack of condom use	148	86.5
Scarification	90	52.6
Piercing	129	75.4
Tattoo	20	11.7
Accidental injury by dirty needle	81	47.4

Out of a total of 171 patients included, 25 were positive for anti-HCV antibody with a frequency of 14.6%. Among the 25 HCV-positive patients, the viral RNA was amplified in 22 patients, which represents a frequency of 88%. In reporting this prevalence in the study population, the relative frequency of hepatitis C viral infection was 12.9% (22/171). Type-specific PCR genotyping identified G2 (13.6%) and G4 (22.7%). Ten patients (45.5%) were co-infected with both genotypes. Four patients (18.2%) could not be typed as G2 or G4. Several risk factors have been identified. However, only the practice of scarification was significantly associated with carrying HCV (p = 0.01) (Table 3).

Discussion

The global epidemiological data have shown that the distribution of HCV genotypes varies by geographic region [8]. In the Republic of Congo, hepatitis C virus infections are poorly documented. We report here the first study of this kind in the department of Lekoumou, one of the departments with a high prevalence of HIV infection [7]. In our study population, we found a high level of HCV seroprevalence at 14.6%.

This prevalence is higher than that found in previous studies conducted in our country. Indeed, Elira-Dockekias

et al (2001) [9] found 3.2% in a study on blood donors at the Brazzaville Blood Transfusion Center. Cantaloube et al (2010), Atipo-Ibara et al, and Alidjinou et al (2014) found respectively a relative frequency of anti-HCV antibodies of 5.6%, 4.2% and 4.7% in blood donors in Brazzaville and Pointe-Noire [10-12]. Bossali et al (2012) found 6% of maternal women in Pointe-Noire [13] and Abena 9.5% of Sangha indigenous peoples in 2012 [unpublished].

Table 3: risk factors based on the porting of HCV viral RNA.

Characteristics	Frequencies n* (%)	p-value
Age (years)		0.65
≤20	2 (9.1)	
21-30	4 (18.2)	
31-40	2 (9.1)	
41-50	4 (18.2)	
>50	10 (45.4)	
Sex		0.83
Man	5 (22.7)	
Woman	17 (77.3)	
Profession		0.06
Health workers	5 (22.7)	
Workers	12 (54.6)	
Public servants	0 (0.0)	
Unemployed	5 (22.7)	
Level of education		0.83
University	0 (0.0)	
Secondary	11 (50.0)	
Primary	4 (18.2)	
Illiterate	7 (31.8)	
Marital status		0.35
Married	15 (68.2)	
Single	2 (9.1)	
Divorced / widowed	5 (22.7)	
Risk factors for transmission		
Blood transfusion	2 (9.1)	0.58
Surgery	4 (18.2)	0.59
Multiple sexual partners	15 (68.2)	0.77
lack of condom use	21 (95.5)	0.16
Scarification	17 (77.3)	0.01
Piercing	16 (72.7)	0.10
Tattoo	2 (9.1)	0.50
Accidental injury by dirty needle	12 (54.5)	0.52

The differences whatever the study could be explained by the type of population investigated. Indeed, blood donors as postpartum women constitute special populations because of the regular monitoring they receive with regard to this disease; which would justify the significantly lower rates found in these studies. Moreover, the particularity of the department of Lékoumou with a high prevalence of HIV infection partly explains the frequency found in our study. It is known that HIV infection is a major cofactor of viral hepatic infections in general. In Africa, similar prevalences have been reported in various populations. Indeed, Forbi et al (2012) in Nigeria found 15% in a natives population of two remote communities [14]; Iles et al (2013) in the Democratic Republic of Congo had found a prevalence of 13.7% [15]; Ntagirabiri et al. (2014) in Burundi reported a national prevalence of 8.2% [16] and Njouom et al (2012) in Gabon, found a prevalence of 11.2% of anti-HCV antibodies [17]. Considering the prevalence found in Lekoumou, Congo would be classified in a zone of high prevalence according to the WHO classification criteria. Amplification of the 5'UTR region of the viral genome detected HCV RNA in 22 of the 171 patients in the study population, with a molecular prevalence of 12.9%. The Forbi et al (2012) study also reported a molecular prevalence of HCV infection of 11.6% similar to ours among natives in Nigeria [14]. G4 and G2 are described in the literature as those circulating in the subregion of Central Africa. The identification of these two genotypes in our studies corroborates with the literature data [6,14,18]. In the present study, genotyping was investigated on the identification of genotypes 2 and 4. Molecular analysis of the results showed that 4 of the 22 positive HCV RNAs could not be identified. This rate shows the existence of other less prevalent HCV genotypes in our study population. Studies in Cameroon [19], Gabon [17] and Burundi [16] have identified two other genotypes (G1 and G3) outside G2 and G4; hence the need to search for other genotypes in addition to genotypes 2 and 4 but also to know the phylogeny of HCV in Congo. Scarification was the significant risk factor in our study, although other risk factors were present in our study

population. Our results contrast with those of Atipo-Ibara et al. in Congo who noted that tattooing was the first factor of transmission of HCV followed by sexual risk [12]. Ndong et al in Gabon [20] noted that ritual practices such as scarification and tattooing, which are common tribal customs, facilitated the transmission of HCV. On the other hand, Ndjomou et al in Cameroon had found transfusion as the first risk factor for HCV transmission [19].

In all case, scarification is still a common ritual practice in Congo in general, and in Lekoumou in particular. It is carried out on the one hand, for a traditional therapeutic purpose because some patients, for financial and economic reasons, do not have access to health care and fall back to traditional healers. On the other hand, it is done for a spiritual purpose because it would be a means of protection against evil spirits. The conditions for sterilization of the equipment used may not be optimal, which would justify the fact that this practice could be considered as a major source of contamination.

National mapping of the seroprevalence and molecular diversity of the HCV Virus is needed to better understand the molecular epidemiology of HCV infection in Congo.

Conclusion

This study showed that the department of Lekoumou is an area of high prevalence of HCV infection especially among older people. HCV Genotypes 4 and 2 were identified, with a predominance of genotype 4. Our results have contributed to the improvement of knowledge on the molecular epidemiology of viral hepatitis C in the department of Lekoumou. Future study is needed to confirm this trend at the national level.

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