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*Full Length Research Paper*

# Hepatitis C virus infection in Abidjan Cote d Ivoire: heterogeneity of genotypes

**Alphonsine Kouassi-M Bengue[1]*, Marie Jeanne Lohoues Kouacou[2], Euloge Ekaza[1], Liliane Siransy-Bogui[3], Daniel Coulibaly N'Golo[1], Patrick Labonté[4] and Mireille Dosso[1]**

[1]Laboratory of bacteriology-virology, Institute Pasteur of Côte d'Ivoire, CHU of Cocody Abidjan, Côte d'Ivoire.
[2]Gastro enterology department, CHU of Cocody, Abidjan, Côte d'Ivoire.
[3]Centre national de transfusion sanguine, Abidjan, Côte d'Ivoire.
[4]INRS-Institute Armand-Frappier, Laval, Canada H7V 1B7.

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**Whereas the distribution of HCV genotypes in many countries is well documented, very little is known about the genotypes prevalent in Cote d' Ivoire, in West Africa. This study was undertaken to clarify molecular characteristics of HCV isolated in Cote d' Ivoire. Plasma samples from 2866 blood donors and 100 patients hospitalized at the teaching hospital of Cocody in Medical and Pediatrics' services were collected and screened for HCV antibodies, hepatitis B surface antigen (HBsAg) and HIV antibodies by enzyme immuno assays (EIA). Viral RNA was extracted from anti HCV antibodies positive samples using a one step RT-PCR (Qiagen one-step RT-PCR kit). From blood donors and patients, detection of HCV antibody was positive for 43 (1.5%) and 23 (23%), respectively. Only 27 samples were positive for RNA detection. Concerning genotyping, we observed predominance of genotype 1 (77.8%), followed by genotype 2 (18.5%) and genotype 5 (3.7%). This study revealed heterogeneity of HCV genotypes in Cote d' Ivoire.**

**Key words:** HCV, antibodies, RT-PCR, genotypes, Cote d' Ivoire.

## INTRODUCTION

Hepatitis C virus (HCV), an enveloped positive-stranded RNA virus of the family *Flaviviridae,* is recognized as a major cause of chronic liver disease. According to World Health Organization (WHO 1999) estimates, approximately 3% of the world population may be infected with HCV. The prevalence of HCV infection varies widely according to the location and the population studied (Memon et al., 2002). In sub-Saharan Africa, HCV prevalence has been reported to be less than 1% in southern African countries (Vardas et al., 1999) ranged between 1.7 and 27.5 in Central Africa (Ndjomou et al., 2002) and between 1.4 and 7% in West and East Africa (Tess et al., 2000; Sarkodies et al., 2001). Because of its high genetic heterogeneity, HCV has been classified into six genotypes groups and a large number of subtypes (Simmmonds et al., 2005). Genotype identification is clinically important for prognosis, and determining antiviral therapy duration (Zein 2000) as genotypes 1 and 4 are more resistant to treatment with pegylated alpha interferon and ribavirin than genotypes 2 and 3 (Zylberberg et al., 1996). In addition, the geographical distribution and diversity of HCV may provide important clues regarding the origin of HCV (Salemi et al., 2002). Whereas the distribution of HCV genotypes in many countries is well documented, very little is known about the genotypes prevalent in Cote d' Ivoire, in West Africa. This study was undertaken to clarify the molecular characteristics of HCV isolated in Cote d' Ivoire.

## MATERIALS AND METHODS

### Plasma samples

Plasma samples from 2866 blood donors and 100 patients hospitalized at the teaching hospital of Cocody in Medical and Pediatrics' services were collected and screened for anti HCV antibodies by enzyme immuno assay (EIA) at the National blood

*Corresponding author. E-mail: kouassimbengue@yahoo.fr.

**Table 1.** Demographical, HCV antibodies and genotypes for the two groups.

| Parameter | Blood donors | | Patients | |
|---|---|---|---|---|
| | N | % | N | % |
| **Total tested for HCV antibodies** | 2866 | | 100 | |
| HCV antibodies positive | 43 | 1.5 | 23 | 23 |
| Men | 35 | 81.4 | 17 | 74 |
| Women | 08 | 18.6 | 6 | 26 |
| Mean age; range | 31 (19-46) | | 55 (10-70) | |
| Reported transfusion | 0 | 0 | 2 | 8.6 |
| HIV antibodies positive | 2 | 4.6 | 0 | 0 |
| HBSAg positive | 1 | 2.3 | 0 | 0 |
| HCV RNA positive (27) | 12 (44.4%) | | 15 (55.6%) | |
| Genotype 1 | 10 | 83.3 | 11 | 73.3 |
| Genotype 2 | 2 | 6.7 | 3 | 20 |
| Genotype 5 | 0 | 0 | 1 | 6.7 |

transfusion center of Abidjan and at the Institute Pasteur of Cote d 'Ivoire. Patients had symptoms like icterus and asthenia. Reactive samples were stored at -20℃ and shipped in dry ice to the Laboratory of Molecular Virology, Institute Armand Frappier, Laval, Canada for molecular studies. All these studies were approved by the Ethics committee of the Ministry of Health of Cote d' Ivoire.

#### Serological screening

Plasma samples were tested by Monolisa anti-HCV plus (Biorad-Sanofi Diagnostics Pasteur) for detection of HCV antibodies. The test was performed according to the manufacturer's instructions. Positive samples were confirmed by PCR, as described below. The presence of hepatitis B surface antigen (HBsAg) was determined by the HBs Ag version EIA (Abbott Laboratories, Abbott Park, North Chicago, IL) on each HCV antibodies positive sample. HCV anti bodies positive sera were screened for HIV antibodies by an enzyme-linked immunosorbent assays (ELISA; Vironostika HIV Uni-Form II Plus 0; Biomérieux by Bosenid 15, 5281 RM Boxtel. NL). Positive sera were confirmed by a synthetic peptide test (PeptiLAV 1-2, Biorad-Sanofi Diagnostics Pasteur). Each test was performed according to the manufacturer's instructions.

#### RNA preparation

RNA was extracted from 140 µl of plasma using a commercial reagent (QIAamp viral RNA mini kit, Qiagen).

#### Methods of amplification

Molecular detection was done by amplification by RT-PCR according to the protocol of the Qiagen kit One-Step RT-PCR (Qiagen): primers sequences were those used by Chan et al. (1992) (DM50/DM51 262 bp for the 5'-UTR region), and Murphy et al. (2007) (11) (DM100/DM101 573 bp for NS5B region and DM110/DM111 584 bp for E1/C1 region).

Three RT-PCR methods were applied for the detection of three regions. Each RT-PCR is a single-step combined RT-PCR amplification. The method was performed in a Gene Amp PCR system 9700 (Applied Biosystem, Foster City, CA). The reaction mixture contained 12.5 µl of 2X reaction mix (a buffer containing 0.4 mM of each dNTP, 2.4 mM $MgSO_4$), 10 µM of each of the 2 primers, 1 µl of the RT/*Taq* mix (QIAGEN) and 2.5 µl of RNA extract was added to give a final volume of 25 µl. The cycling conditions for the three RT-PCRs were: an initial cycle at 50℃ for 30 min and 95℃ for 15 min; followed by 45 cycles at 95℃ for 30 s, 50℃ for 30 s for 5'UTR region (55℃ for NS5B region), and 72℃ for 1 min; and a final extension at 72℃ for 10 min. For E1/C1 region we used the same step but the cycle number were 40 and annealing was at 57℃. The RT-PCR products were visualised after electrophoresis on an ethidium-bromide stained 1.5% agarose gel.

#### Sequencing of NS5B and E1/C1

The RT-PCR products of NS5B and E1/C were purified with the QIA-quick PCR purification kit (QIAGEN, Inc., Chatsworth, Calif.). The Purified DNA fragments were sent to be sequenced at Mc Gill University and Genome Quebec Innovation Centre.

#### Statiscal analysis

The associations between gender, population group and HCV seroprevalence and genotypes were tested using the chi squared and Fisher exact tests.

## RESULTS

### Prevalence of HCV antibodies

Samples from 2866 Ivorian blood donors were initially tested for HCV antibody, and 43 were reactive (1.5%) (Table 1). These 43 blood donors were mainly males (81.4%) ranging in age from 19 to 46 years (median = 31). Among these 43 blood donors, detection for HIV antibody and HBs Ag were reactive for 2 (4.6 %) and 1 (2.3%) specimens, respectively. Of the samples from 100 hospitalized patients tested for HCV antibodies, 23 were reactive (23 %). There were 3 children with a median age of 11 and 20 adults ranging in age from 45 to 70 years (median = 55). No HIV or HBV infection was detected in this group. There was a significant difference observed (P = 0.44) between our two groups for the HCV seroprevalence.

### HCV-RNA detection and genotyping

Overall, 66 HCV antibodies positive serum samples from the two groups were tested by RT-PCR, and 27 (41%) were HCV-RNA positive (Table 1). Among these 27 HCV-RNA positive samples, 12 were blood donors (44.4%) and 15 were hospitalized patients (55.6%). Within the two groups, the genotypes distribution was the following: In the blood donors' group, 10 were genotype 1 (83.3%) and two were genotype 2 (16.7%). In the patients' groups, 11 were genotype 1 (73.5%), three were genotype 2 (20 %) and one was genotype 1 (6.5%) (Table 1). The two groups did not differ significantly regarding the relative frequencies of genotype 1 (Fisher exact test, P = 0.87) and genotype 2 (P = 0.78); however the numbers were too small to draw firm conclusion.

## DISCUSSION

Seroprevalence of HCV antibodies in the blood donors in our study was identical to those of Wiwanitkit (2005) in Thailand and Dieye et al (2006) in Dakar with 1.37 and 1.4%, respectively. On the other hand, our results were higher than those reported by Thakral et al. (2006) in India and Chalbicz et al. (2005) in Poland with 0.4 and 0.5%, respectively.

We observe similar results among the hospitalized patients; in fact, our observed figures were higher than those reported by Saigal et al. (2002) who found a seroprevalence of 9.5% in 210 alcoholic cirrhotic patients and 0.6% was observed by Utkan et al. (2006) in a Department of Surgery in Turkey. HCV seroprevalence difference between our two groups could be explained by the fact that the hospitalized patients had viral hepatitis symptoms; whereas the blood donors were asymptomatic.

Co-infection of HCV with HIV or HCV with HBV was found only among blood donors' group, although the in-patient population participants was small. However the seroprevalence of co-infection HCV/HIV and HCV/HBV was 11.1 and 20.6%, respectively, in Nigeria (Forbi et al., 2007). This is much higher than what we observed. This difference could be related to the criteria of selection used by the authors. In effect, all the 180 persons included in their study were HIV-1 infected, before being tested for the presence of HBS Ag and HCV antibodies. Whereas, we only tested the positive samples for HCV.

With regard to the detection of HCV RNA genome, our figures were lower than those of Silva et al. (2006) (73.6%) in hemodialysis patients. This difference could be accounted for by the type of population studied; indeed hemodialysis patients are population at risk of viral blood infection such as hepatitis C or B virus.

Besides our low figure of HCV RNA can be explained by degradation of RNA during samples' transport. Nevertheless, it is known that among HIV positive patients, some may not have sufficient viral replication despite the fact that they serology remains positive. But in our study, HCV RNA was detected in the two persons coinfected with HIV with HCV. According to Candotti et al. (2003) in Ghana, 26. 4% of HCV positive patients did not have a detectable level of viral RNA.

As far as the genotype distribution of HCV is concerned, our results differed from that reported from Cameroon in Central Africa (Njouom et al., 2003), which showed a high frequency of genotype 4. On the other hand in West Africa, in Nigeria, Agwale et al. (2004) reported a prevalence of genotype 1 of 75% and genotype 2 of 25%; Candotti et al. (2003) reported a prevalence of genotype 2 with 87% in Ghana followed by genotype 1. Although, previously, genotype 5 has been reported only in South Africa (Chamberlain et al. 1997). We observed genotype 5 in one patient, a 52 years old woman with a medical history of blood transfusion. Henquell et al. (2004) reported a high frequency (14.2%) genotype 5a in France and Verbeeck et al. (2006) reported 57.4% of genotype 5a.

### Conclusion

Seroprevalence of HCV was low in blood donors in Abidjan. While genotype 2 has been described by many authors as predominant in West Africa, we observed more cases of genotype 1 than genotype 2 and one case of genotype 5 in Cote d' Ivoire. So, it would be necessary to investigate more about the distribution of HCV genotypes by other studies in the whole country, in order to draw a firmer conclusion.

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