Characterisation of hepatitis C virus genotype among blood donors at the regional blood transfusion centre of Ouagadougou, Burkina Faso

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Introduction

Hepatitis C virus (HCV) is a major risk factor for liver cancer and causes about 900 deaths per year in Burkina Faso1. The prevalence of HCV in the general population is high and has a wide local variation2. In Burkina Faso, systematic screening for HCV is done only in blood donors by searching for antibodies against the virus using enzyme immunoassays. Different prevalences of HCV in Burkina Faso have been reported depending on the type of diagnostic test used, such as enzyme-linked immunoassay (ELISA) or polymerase chain reaction (PCR) analysis, and the population studied (blood donors or pregnant women)3-6. Indeed, most studies which used reverse transcription PCR as the method for detecting HCV RNA found a lower prevalence of HCV in the studied populations.

Background. Hepatitis C virus (HCV) is responsible for about 900 deaths every year in Burkina Faso. In this country, serological screening for hepatitis B and C viruses is only carried out systematically among blood donors. The aim of this study was to determine the prevalence and genotypes of HCV among blood donors using reverse transcription polymerase chain reaction (PCR) and real-time PCR, respectively.

Materials and methods. Serum samples were screened for antibodies to HCV using an enzyme-linked immunosorbent assay (ARCHITECT-i1000SR-ABBOTT). All the reactive samples for HCV antibodies were re-tested using a second enzyme-linked immunosorbent assay (Bio-Rad, Marnes la Coquette, France) for confirmation. RNA was detected in all the reactive samples for antibodies to HCV. HCV RNA positive samples were genotyped using the HCV Real-TM Genotype kit (Sacace Biotechnologies, Italy).

Results. Among 2,200 blood donors, the prevalences of antibodies to HCV and viral RNA were 4.4% (95% confidence interval=3.5-5.3) and 1.5% (95% confidence interval=1.0-2.0), respectively. Among HCV RNA carriers, genotyping showed that HCV genotypes 2 and 3 were the most prevalent as they were detected in 18 (56.3%) and 5 (15.6%) individuals, respectively. HCV genotypes 1a and 4 were the least frequent among the blood donors. HCV mixed genotypes 2/3 and 2/4 were also detected among the blood donors.

Conclusion. The prevalence of HCV found in this study is lower than previously reported prevalences. Large-scale studies are needed to obtain a better picture of the molecular epidemiology of HCV in Burkina Faso.

Keywords: HCV, RNA, genotype, blood donors.
broadly distributed worldwide\textsuperscript{10}, whereas genotypes 5\texttrademark and 6\texttrademark are common in South Africa and South-East Asia\textsuperscript{11,12}. Genotype 4 is predominant in Central Africa\textsuperscript{13,14} and in North Africa\textsuperscript{15}. Genotypes 1 and 2 are more prevalent in West Africa (Benin, Guinea)\textsuperscript{16,17}.

The molecular epidemiology of HCV is less documented in Burkina Faso. The aim of this study was to confirm HCV positive serology by detection of viral RNA and to characterise HCV genotypes among blood donors.

Materials and methods

Blood donors

Blood samples were collected using standard procedures from 2,200 voluntary donors at the National Blood Transfusion Centre of Ouagadougou, in July 2011. Voluntary donors were all healthy subjects, selected after responding to a panel of questions comprising a medical history. Apparently healthy individuals aged 17-65 years with a weight $>50$ kg were eligible for blood donation. All donors answered questions intended to identify and then exclude recipients of a previous transfusion, individuals who had had jaundice or signs of hepatitis, pregnant women and people having experienced high risk sexual behaviour within the 2 weeks preceding the intended donation.

All the samples were screened for hepatitis B virus surface antigen (HBsAg), antibodies to human immunodeficiency virus (HIV), \textit{Treponema pallidum} and HCV. All the reactive samples for HCV antibodies were kept at $-20$ °C for further analysis.

Serological analysis

Antibodies to HCV were detected using a fourth generation ELISA (ARCHITECT-i1000SR-ABBOTT, Santa Clara, California, United States of America). This is a two-step sandwich chemiluminescent microparticle immunonassay (CMIA) for the qualitative detection of antibodies against HCV in human serum or plasma.

All the samples reactive for HCV were re-tested for confirmation using a second ELISA (Bio-Rad, Marnes la Coquette, France). A result was considered positive if both the first and second tests were positive.

HBsAg and antibodies to HIV types 1 and 2 were screened using Hepanostika HBsAg Ultra (Biomérieux, Boxtel, The Netherlands) and Vironostika HIV Uniform II Ag/Ab (Biomérieux, Boxtel, The Netherlands), respectively. Antibodies to \textit{Treponema pallidum} were detected using a rapid plasma reagin (RPR) test (Cypress Diagnostics, Langdorp, Belgium) and confirmed with a \textit{Treponema pallidum} haemagglutination (TPHA) test (Cypress Diagnostics).

Hepatitis C virus RNA extraction and reverse transcription

Viral RNA was extracted from 140 μL of plasma using the QI Amp viral RNA extraction kit (Qiagen, Hilden, Germany) following the manufacturer's instructions and was reverse transcribed using the Reverta-L reverse transcription protocol (Sacace Biotechnologies, Como, Italy). Briefly, 10 μL of viral RNA and 10 μL of reaction mix were placed into a thermocycler (GeneAmp PCR System 9700, Applied Biosystems, Foster City, California, United States of America) and incubated at 37 °C for 30 min then at 95 °C for 5 min. The cDNA obtained were stored at $-20$ °C.

Hepatitis C virus genotyping

HCV RNA positive samples were genotyped using the HCV Real-TM Genotype kit (Sacace Biotechnologies) able to detect HCV genotypes 1a, 1b, 2, 3 and 4, following the manufacturer's instructions with minor modifications. Briefly, 5 μL of a sample of cDNA, 4 μL of TaqF Polymerase, and 6 μL of each PCR mix: (PCR-mix-1-FRT HCV 1b/3, PCR-mix-1-FRT HCV 1a/2 and PCR-mix-1-FRT HCV 4/IC) were distributed on a MicroAmp Optical 96-Well Reaction Plate (Applied Biosystems, Foster City, California, United States Of America). The PCR reactions were done in a 7500 Fast Real-Time PCR System (Applied Biosystems). Fluorescence curves were analysed with Fast 7500 Sequence Detection Software v2.1 (Applied Biosystems).

Statistical analysis

Data were analysed using EPI-Info version 6.04 dfr (CDC, Atlanta, United States of America). A chi-square test was applied to compare proportions. P-values $<0.05$ were considered statistically significant.

Results

As shown in Table I, among a total of 2,200 blood donors, 97 (4.4%; 95% CI=3.5-5.3) were reactive to HCV antibodies. Among these 97 blood donors, 62 (63.9%) were male and 35 (36.1%) were female. An
Discussion

The aim of this study was to determine the prevalence of HCV infection using detection of viral RNA and to characterise HCV genotypes among blood donors from the Regional Blood Transfusion Centre of Ouagadougou in Burkina Faso.

Previous studies found that the serological prevalence of HCV in Burkina Faso ranged from 1 to 3% in pregnant women\(^5,6,18\), whereas it was reported to be higher than 7% in blood donors\(^3,19\) (Table III). In the present study, the prevalence of HCV antibodies among blood donors was 4.4%. Viral RNA was detected in 1.5% of blood donors.

The difference between the prevalence of anti-HCV antibodies and the prevalence of viral RNA detectable in blood donors could be related to the persistence of anti-HCV antibodies after months or years in individuals in whom the virus has been spontaneously cleared\(^20\). Despite the accuracy of third-generation immunoassays in detecting antibodies and the high reliability of automated equipment, false-positive anti-HCV antibody results occur at unacceptable frequencies (15% to 62%)\(^21\).

The prevalence of HCV found in this study was lower than that found by Nagalo \(^3\), Ruggieri \(^22\) or Walusansa and Kagimu\(^23\) who reported prevalences of about 9%, 6.5% and 3.3%, respectively, but it was higher than the prevalences reported in Indian\(^24\) and Chinese blood donors\(^25\). These discrepancies could be due to the difference of the screening algorithm used\(^26\). In the present study, a third-generation enzyme immunoassay was used to detect anti-HCV antibodies in blood donors. The specificity and the sensitivity of third-generation immunoassays are higher than 98% and the mean time of seroconversion is reduced to 2-3 weeks compared to 3-4 weeks for the second-generation immunoassays\(^27\). The use of new combined assays for HCV antigen and antibody could be an

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total blood donors</td>
<td>2,200</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>62</td>
<td>63.9</td>
</tr>
<tr>
<td>Female</td>
<td>35</td>
<td>36.1</td>
</tr>
<tr>
<td>HCV infection and co-infections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total anti-HCV positive</td>
<td>97</td>
<td>4.4</td>
</tr>
<tr>
<td>Anti-HCV only</td>
<td>65</td>
<td>3.0</td>
</tr>
<tr>
<td>HCV/HBV</td>
<td>14</td>
<td>0.6</td>
</tr>
<tr>
<td>HCV/syphilis</td>
<td>12</td>
<td>0.5</td>
</tr>
<tr>
<td>HCV/HIV</td>
<td>1</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Legend: N: Number of individuals.

isolated HCV infection was detected in 65 (3.0%) individuals. HCV co-infections with HBV, syphilis and HIV were detected in 14 (0.6%), 12 (0.5%) and 1 (0.05%) individuals.

Among the 97 blood donors with anti-HCV antibodies, viral RNA was detected in only 32 (1.5%) (95% CI=1.0-2.0) individuals (Table I).

HCV genotyping among the 32 blood donors with detected viral RNA showed that the most prevalent HCV genotypes were genotypes 2 and 3, accounting for 56.3% (18/32) and 15.6% (5/32) of the infections, respectively. The HCV genotypes 1a and 4 were less represented, with a prevalence of 3.1% (1/32) among the blood donors. HCV mixed infections between genotypes 2/3 (9.4%) and genotypes 2/4 (3.1%) were also detected, as shown in Table II.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Mixed infections</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subtype 1a</td>
<td>1b</td>
<td>2/3</td>
<td>2/4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>1</td>
<td>3</td>
<td>18</td>
<td>5</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Percentage</td>
<td>3.1</td>
<td>9.4</td>
<td>56.3</td>
<td>15.6</td>
<td>3.1</td>
<td>9.4</td>
</tr>
</tbody>
</table>

Table III - Prevalence of HCV in Burkina Faso according to the detection method used and the populations studied.

<table>
<thead>
<tr>
<th>Populations</th>
<th>Prevalence (%)</th>
<th>Methods</th>
<th>Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood donors</td>
<td>1.5</td>
<td>ELISA and PCR</td>
<td>This study</td>
</tr>
<tr>
<td>Pregnant women (Zeba et al., 2011)</td>
<td>2.1</td>
<td>ELISA and PCR</td>
<td>2011</td>
</tr>
<tr>
<td>Pregnant women (Serme et al., 2006)</td>
<td>2</td>
<td>ELISA and PCR</td>
<td>2004</td>
</tr>
<tr>
<td>Blood donors and pregnant women (Collenberg et al., 2006)</td>
<td>1.5-2.2</td>
<td>PCR</td>
<td>2004</td>
</tr>
<tr>
<td>Blood donors (Nagalo et al., 2011)</td>
<td>6.5-8.7</td>
<td>ELISA</td>
<td>2011</td>
</tr>
<tr>
<td>Pregnant women (Simpore et al., 2006)</td>
<td>5.4</td>
<td>ELISA</td>
<td>2005</td>
</tr>
<tr>
<td>Urban and suburban (jeannel et al., 1998)</td>
<td>2.2-8.3</td>
<td>ELISA</td>
<td>1998</td>
</tr>
</tbody>
</table>
advantage by reducing the discrepancies between serological and viral RNA detection observed among blood donors.

In this study, the prevalence of HBV/HCV co-infection was 0.64% and was higher than the 0.18% found by Vardas et al.28

The global distribution of HCV genotypes is well-documented7,14,15,29-31, but there are few data on the molecular epidemiology of HCV in Burkina Faso. In our study, genotype 2 was most prevalent, accounting for 56.3% of the HCV infections, followed by genotype 3. This is in accordance with previous findings that genotype 2 was the most prevalent HCV genotype in West Africa16,17,22. According to the literature, genotypes 1 and 2 are predominant in West Africa, but we found the genotype 1 in 12.5% (4/32) of the HCV-positive blood donors.

The low prevalence of HCV genotype 1 could be explained by the fact that subtypes 1a and 1b are mostly found among intravenous drug users and multiple transfused subjects, respectively; these types of blood donors were excluded from blood donation and, unlike in some countries in the sub-region such as Nigeria, intravenous drug use is not widespread in Burkina Faso30,32,33. Genotype 4 was the least prevalent type found among the HCV-infected blood donors. This was not surprising because HCV genotype 4 is mainly found in Central Africa10,13 and North Africa15,31. Mixed infection with two genotypes was observed in 12.5% (4/32) of the HCV-infected blood donors. HCV mixed genotype infections provide essential information that can be used to determine type and duration of therapy needed and to predict disease outcome19. In this study, the genotyping kit used was not able to detect the subtypes of genotypes 2 and 3.

In conclusion, the present study provided data on HCV infection in blood donors in Burkina Faso. This investigation was the first on HCV molecular epidemiology in Burkina Faso and is a springboard for further studies on a large scale, in order to draw up the molecular profile of HCV in Burkina Faso.

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The Authors declare no conflicts of interest.

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