Prevalence and Molecular Diversity of Hepatitis B Virus and Hepatitis Delta Virus in Urban and Rural Populations in Northern Gabon in Central Africa

Maria Makuwa, Armel Mintsa-Ndong, Sandrine Souquière, Dieudonné Nkoghe, Eric M. Leroy, and Mirdad Kazanji

Unité de Virologie and Unité de Maladies Émergentes, Centre International de Recherches Médecinales de Franceville (CIRMF), BP 769 Franceville, Gabon; Service de Coopération et d’Action Culturelle, French Embassy, BP 2105 Libreville, Gabon; and Réseau International des Instituts Pasteur, Institut Pasteur, 28 rue du Dr Roux, Paris, France

Received 17 October 2008/Returned for modification 26 January 2009/Accepted 5 May 2009

The prevalence of hepatitis B virus (HBV) surface antigen was significantly higher in urban (12.9%) than in rural (7.6%) populations (P = 0.003), but no difference was found in the prevalence of hepatitis delta virus (HDV), which was high in both populations. Phylogenetic analysis showed the circulation of HBV-A3 and -E genotypes and the presence of HDV-1, HDV-7, and HDV-8 clades.

Hepatitis B virus (HBV) and hepatitis delta virus (HDV) are highly endemic in Africa (1, 2); however, little information is available on the origin, circulation, and genetic diversity of these viruses in central Africa.

We reported recently that the prevalence of HBV surface antigen (HBsAg) in pregnant women in Gabon was 9.2% and that 15.6% had antibodies to HDV. Furthermore, two genotypes of HBV (subgenotype A3 and genotype E) and HDV-1 and HDV-8 clades were found in this population (7). The previous study was, however, restricted to pregnant women, and we considered that more-extensive studies of HDV clades in the general population were needed in order to characterize the circulation of these viruses in indigenous African populations. With this aim, we assessed the prevalence and genetic diversity of HBV and HDV in urban and rural populations in Gabon and compared the distributions of the HBV genotypes and HDV clades in these two areas with those of neighboring African countries.

Two epidemiological surveys were conducted in the north part of the country, in the province of Woleu-Ntem (157,013 inhabitants), which is characterized by a high population density in the cities and a very low population density in rural areas (3). The rate of immigration from Equatorial Guinea, Cameroon, and Congo is high, representing more than 22% of the total immigration rate in Gabon (9). In the first survey, 394 samples (from 203 women and 191 men) were collected in the main city of the region, Oyem. In the second survey, 961 samples (from 565 women and 396 men) were collected in 34 villages in the same province. We used the cluster sampling method and obtained ethical clearance for the study from the local public health authorities; each person gave informed consent before blood was taken.

The presence of HBsAg was assessed with the Monolisa Ag HBs-Plus test (Bio-Rad, Marnes la Coquette, France). The presence of HDV total antibodies in all HBsAg-positive samples was determined with the Murex anti-Delta (total) assay (Abbott/Murex Diagnostic Division, Wiesbaden, Germany).

Molecular and phylogenetic characterizations of HBV and HDV were performed as described previously (7). To determine the HBV genotype and HDV clade of the new Gabonese strains, we amplified and sequenced a 377-bp fragment of the HBV-S gene and a 326-bp fragment of the HDV-sHD gene. These are the fragments usually used for phylogenetic analysis and are therefore the predominant sequences in the GenBank databases. One complete HBV genome was also sequenced and characterized.

As shown in Table 1, the overall prevalence of HBsAg was significantly higher in urban (12.9%) than in rural (7.6%) areas (P = 0.003). Conversely, the prevalences of antibodies to HDV among HBsAg carriers were extremely high in both the urban and the rural areas (P not significant). Persons aged 15 to 20 years in rural areas were more frequently HBV and HDV positive than those in urban settings (P not significant), and a significant difference was found between HBsAg-positive males and females living in rural areas (P = 0.04). The prevalence of HBsAg was significantly higher (P = 0.03) among men in the urban area (16.2%) than among those in the villages (9.8%). Interestingly, only in rural areas were men more frequently HBsAg carriers (9.8%) than women (6.0%) (P = 0.04).

A 377-bp fragment of the HBV-S gene was obtained from 13 persons (7 males and 6 females), 9 in the urban area and 4 in the villages. The neighbor-joining tree method showed that the HBV strains belonged to subgenotype HBV-A3 and genotype HBV-E (Fig. 1A).

A 326-bp fragment of the sHD gene of HDV was obtained from 17 HDV-infected individuals (8 males and 9 females), 7 in the urban area and 10 in the villages. The phylogenetic analyses indicated splitting of the HDV-1 clade into two subclades, one made up of HDV strains from Canada and the Central African Republic and the other subdivided into two
distinct groups (Fig. 1B). The first group contains strains from all over the world, including one rural strain from Gabon, and the second group was made up of only the Gabonese HDV strains (three urban and six rural strains). Two HDV strains originating from rural areas belonged to the HDV-7 clade, and the remaining strains from both urban and rural areas fell into the HDV-8 clade.

We present here the first analysis of the prevalences of HBV and HDV in urban and rural populations in north Gabon, by age and sex.

In conclusion, our data provide clear evidence that HBV and HDV are highly endemic in central Africa, with eight described clades (6, 7, 11). The first HDV strains were isolated and characterized from Africans living in France (6, 10). We showed recently that HDV-1 and HDV-8 clades are present in pregnant women in Gabon, and we provided the first evidence that HDV-8 is indigenous to Africa (7). In the present study, a large number of sequences were obtained, showing wide genetic diversity in the HDV-1, HDV-7, and HDV-8 clades, confirming that these HDV strains are endemic in the general population of Gabon. Two new strains from Gabon within the HDV-7 clade were closely related to HDV strains from Cameroon, indicating that this clade is also endemic in the country. In the HDV-1 clade, 9 of 10 newly characterized sequences and characterized from Africans living in France (6, 10).

More men than women were infected with HBV and HDV, and a significant difference in prevalence of HBsAg was observed between urban and rural populations. These findings are in accordance with previous results, showing that chronic carriage of HBsAg in Africa is usually related to gender, with statistically significantly higher carriage rates in males than in females (4, 12). Moreover, young males (aged 14 to 20) in rural settings were more frequently HBsAg positive than females. Information collected at blood sampling indicated no difference in cultural practices (such as scarification) among males and females, but young boys moved more frequently to the city than girls. The higher rate of chronic HBV infection among males may be due either to a prolonged replicative phase of the virus in boys or to differences in sexual behavior (4).

Many factors could be responsible for the transmission of HBV and HDV, including environmental, behavioral, and cultural factors (4). Moreover, HDV infection has a variable influence on the course of hepatitis B, and the clinical severity of dual infections probably depends on aspects such as the endemicity of HDV in the area, the degree of HBV viremia, and the genotypes of HBV and HDV (2).

We confirmed previous results from our group and others, showing that the HBV-A3 subgenotype and the HBV-E genotype are present throughout central and west Africa (5, 7, 8, 10). The new HBV strains described in this study were not restricted to a particular area, as HBV-A3 and -E strains were found in both rural and urban areas.

HDV is highly endemic in central Africa, with eight described clades (6, 7, 11). The first HDV strains were isolated and characterized from Africans living in France (6, 10). We showed recently that HDV-1 and HDV-8 clades are present in pregnant women in Gabon, and we provided the first evidence that HDV-8 is indigenous to Africa (7). In the present study, a large number of sequences were obtained, showing wide genetic diversity in the HDV-1, HDV-7, and HDV-8 clades, confirming that these HDV strains are endemic in the general population of Gabon. Two new strains from Gabon within the HDV-7 clade were closely related to HDV strains from Cameroon, indicating that this clade is also endemic in the country. In the HDV-1 clade, 9 of 10 newly characterized sequences from general population samples clustered with a strain previously described by our group, originating from pregnant Gabonese women. This Gabonese HDV-1 subclade is therefore widespread in the country. More-extensive studies are needed to confirm this clustering.

In conclusion, our data provide clear evidence that HBV and HDV are highly endemic in indigenous general populations of Gabon, with wide genetic diversity.

Nucleotide sequence accession numbers. Sequences were deposited in GenBank under the following accession numbers: for the new HBV-A3 and HBV-E strains, FJ349266 to FJ349277; for the complete genome sequence obtained for the

---

**TABLE 1. Prevalence of HBV and HDV in urban and rural populations in north Gabon, by age and sex**

<table>
<thead>
<tr>
<th>Population and age range (yr)</th>
<th>No. of positive samples/no. tested (%)</th>
<th>Value* for total population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Anti-HDV</td>
</tr>
<tr>
<td>Urban</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14–20</td>
<td>6/48 (12.5)</td>
<td>4 (66.7)</td>
</tr>
<tr>
<td>21–30</td>
<td>16/72 (22.2)</td>
<td>13 (81.3)</td>
</tr>
<tr>
<td>31–40</td>
<td>5/33 (15.1)</td>
<td>3 (60.0)</td>
</tr>
<tr>
<td>41–50</td>
<td>4/18 (22.2)</td>
<td>4 (100.0)</td>
</tr>
<tr>
<td>51–60</td>
<td>0/20</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>31/191 (16.2)</td>
<td>24/31 (77.4)</td>
</tr>
<tr>
<td>Rural</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15–20</td>
<td>4/9 (44.4)</td>
<td>4 (100.0)</td>
</tr>
<tr>
<td>21–30</td>
<td>7/39 (17.9)</td>
<td>6 (83.7)</td>
</tr>
<tr>
<td>31–40</td>
<td>9/65 (13.8)</td>
<td>7 (77.8)</td>
</tr>
<tr>
<td>41–50</td>
<td>7/60 (11.7)</td>
<td>4 (57.1)</td>
</tr>
<tr>
<td>51–60</td>
<td>5/93 (5.4)</td>
<td>2 (40.0)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>7/130 (5.4)</td>
<td>3 (42.9)</td>
</tr>
<tr>
<td>Total</td>
<td>39/396 (9.8)</td>
<td>26/39 (66.7)</td>
</tr>
</tbody>
</table>

*OR, odds ratio; CI, confidence interval.
29-Oym-10 sample, FJ349296; and for the HDV strains isolated from the general population of Gabon, FJ349279 to FJ349295.

Armel Mintsa Ndong is the recipient of a fellowship from the European Community (6th Framework program). We thank P. Roques and F. Simon for logistic help; Cindy Padilla for statistical analysis; and Marie-Thérèse Bedjabaga, Paul Ngari, and Philippe Enganga for technical help.

The CIRMF is funded by the Gabonese Government, Total-Gabon, and the French Foreign Ministry. Part of this study was also supported by funds from the Fonds de Solidarité Prioritaires (FSP no. 2002005700) from the French Foreign Ministry.

REFERENCES

hepatitis B virus and recombination between genotypes A and E in Came-
7. Makuwa, M., M. Caron, S. Souquière, G. Malonga-Muelet, A. Mahé, and M.
Kazanji. 2008. Prevalence and genetic diversity of hepatitis B and delta viruses in
pregnant women in Gabon: molecular evidence that hepatitis delta virus clade S
Mouinga-Ondeme, K. Onanga, P. A. Marx, M. Kazanji, P. Roques, and F.
Simon. 2006. Identification of hepatitis B virus subgenotype A3 in rural
analysis of the precore/core gene of hepatitis B virus genotypes E and A in
Molecular phylogenetic analyses indicate a wide and ancient radiation of
African hepatitis delta virus, suggesting a Deltavirus genus of at least seven
12. Richard-Lenoble, D., O. Traore, M. Y. Kombila, P. Roingeard, F. Dubois,
and A. Goudeau. 1995. Hepatitis B, C, D, and E markers in rural equatorial