High prevalence and predominance of Hepatitis D genotype 1 infection in Cameroon

Foupouapouognigni Yacouba¹, Noah Noah Dominique², Tagni Sartre Michèle³, and Njouom Richard¹*.

1 ; Service de Virologie, Centre Pasteur du Cameroun, P.O.Box 1274 Yaounde, Cameroon.  
2 ; Hôpital Central de Yaoundé, Yaounde, Cameroon  
3 ; Cabinet Médical de la Cathédrale, Yaounde, Cameroon.

*Corresponding author  
Richard Njouom, PhD  
Service de virologie  
Centre Pasteur du Cameroun  
P.O.Box 1274 Yaounde, Cameroon  
Tel: (237) 22 23 10 15 / (237) 79 87 36 04  
Fax: (237) 22 23 15 64  
E-mail: njouom@pasteur-yaounde.org or njouom@yahoo.com
Abstract

Antibodies to the hepatitis delta virus (HDV) were found in 17.6% of the 233 Hepatitis B virus surface antigen positive subjects in Cameroon. Phylogenetic analyses showed the presence of HDV-1, HDV-5, HDV-6 and HDV-7 genotypes. These results enrich the limited data on HDV prevalence and molecular diversity in Cameroon.

Text

Hepatitis delta virus (HDV) is associated with hepatitis B virus (HBV) infection and is frequently related to more severe disease than that due to the underlying HBV mono-infection (5, 10). HDV infection has a worldwide distribution but its frequency largely varies throughout different geographic regions. It is highly endemic in the Middle East, in the Mediterranean area, in the Amazonian region, and in several African countries (3). Genomic analysis of HDV isolates from different regions of the world reported at least eight phylogenetically distinct genotypes with dissimilar geographic distributions. Apart from HDV genotype 1 (HDV-1), which is ubiquitous, each virus clade is geographically localized: HDV-2 is found in Japan, Taiwan, and Russia; HDV-4 in Taiwan and Japan; HDV-3 in the Amazonian region; and HDV-5, HDV-6, HDV-7 and HDV-8 in Africa (6). The role of these HDV genotypes is not yet well determined, but some studies have suggested an association between the severity of disease and infection with different HDV genotypes (4, 8). The two studies conducted two decades ago on HDV infection in Cameroon have reported the prevalence of antibodies against HDV (HDV-Ab) of 6.5% and 27.3%, respectively (9, 12). In spite of this high prevalence, no data is available about HDV genotype diversity in Cameroon. Therefore, in the present study, we investigated the current HDV seroprevalence and the genotype diversity in Cameroonian patients with chronic HBV infection.
This study was performed on the plasmas from 233 Hepatitis B virus surface antigen (HBsAg) carriers (mean age, 34.5 years; 79 women and 154 men) seen at two medical care centres in Yaounde, Cameroon from May 2008 to May 2009. As recommended by the Cameroonian Society of Gastroenterology, in addition to the HBV DNA quantification, and the assessment of biochemical liver enzyme, HDV screening should be part of the screening of HBsAg carriers considering the endemicity of this infection in Central Africa. The analyses included in HDV screening are HDV-Ab and HDV RNA quantification for HDV-Ab positive patients. During the medical consultation, HBsAg carriers were screened for HDV infection and were asked to participate to this study. In case they agree written informed consent was obtained for HDV genotyping since this analysis is not part of the original protocol in routine work-up of liver disease.

The presence of HDV-Ab was determined by the Murex anti-delta assay (Abbott, Wiesbaden, Germany) and HBV DNA viral load by the Abbott RealTime HBV quantification kit (Abbott Molecular Inc., Rungis, France) according to manufacturer’s protocol. During routine work-up of the liver disease, the biochemical liver enzyme alanine aminotransferase (ALT) (upper normal limit 45 IU/L) was also assessed by standard laboratory tests. In order to detect HDV, RNA was extracted from 140 µL HDV-Ab-positive plasma using QIAamp® RNA Mini kit (Qiagen, Courtaboeuf, France) and HDV RNA was detected by RT-PCR amplification of a 360-bp fragment coding for the small hepatitis delta antigen (HDV-sHD) gene as described previously (4). HDV-Ab was found in 41 (17.6%; 95%CI: 13.1%-23.2%) of the 233 tested plasmas, and then HDV RNA was detected in 25 (61%; 95%CI: 44.5%-75.4%) of the 41 HDV-Ab positive plasma. The percentage of patients with abnormal serum ALT was significantly higher in HBsAg carriers with HDV-Ab than in those without HDV-Ab. Remarkably; the proportion of HBsAg carriers with undetectable HBV DNA was higher in HDV-Ab positive than in HDV-Ab negative patients. (Table 1). The Phylogenetic analyses,
performed as described elsewhere (11), revealed that 22 (88%) out of the 25 HDV Cameroonian strains were classified as genotype 1 (HDV-1) with a 100% bootstrap. The other three strains clustered with the previously described HDV-5, HDV-6, and HDV-7 genotypes with bootstrap values of 100%, 99%, and 97%, respectively (Fig. 1). Although they were not supported by significant bootstrap values (less than 70%), the phylogenetic analyses indicated splitting of the Cameroonian HDV-1 genotypes into five probable “sub-genotypes” (Fig. 1).

The average of nucleotide differences between the 25 Cameroonian HDV genotypes was 15.5%, whereas the average of nucleotide differences within the 22 Cameroonian HDV-1 strains was 12.9% (data not shown). The average of nucleotide differences among the Cameroonian HDV-1 strains seems to be higher than the 11% average reported among other worldwide HDV-1 strains (6). This result highlights the extent of intra-genotypic variation for the Cameroonian HDV-1 isolates.

The prevalence of HDV-Ab obtained in this study is as high as that previously reported by other groups from Cameroon (9, 12). HDV RNA was found in 61% of the HDV-Ab patients, indicating the low prevalence of HDV clearance in this population. Natural history of HDV infection shows that coinfection evolves to chronicity only in a small number of patients and patients recover from both hepatitis B and D, while superinfection of HDV leads to progressive disease and cirrhosis in approximately 60-80% of cases (1, 2). It can thus be suggested that most of our patients acquired the infection as a superinfection on hepatitis B. It is known that coinfection or superinfection of HBV and HDV may cause severe liver diseases (1). Our observations showed that the median of serum ALT level was significantly higher in carriers with HDV-Ab than in those without HDV-Ab (Table 1). In our study, the proportion of HBsAg positive patients with undetectable HBV DNA was higher in patients with HDV-Ab than in those without HDV-Ab (61% vs 35.4%, p<0.05). This result indicates, as reported by previous studies (2), the suppression/inhibition of HBV replication by HDV.
infection and highlights the importance of the screening of HDV infection in HBV carriers. We showed the presence of four different genotypes HDV-1, HDV-5, HDV-6, and HDV-7. HDV-1 was found to be the predominant genotype. Three new strains from Cameroon were closely related to HDV-5, -6, and -7 genotypes previously described from African patients (6), indicating that these genotypes are circulation in Africa. However, although recently reported to originate and to be endemic in Central Africa (7), the newly describe HDV-8 was not found in our study. As claimed by other authors (6), the great genetic diversity of HDV found in Cameroon, with at least 4 different genotypes suggests a wide and ancient radiation of African HDVs. In contrast to previous studies (13), our results showed no significantly well-defined sub-genotytes within genotype 1. Further studies with full genome characterization of these strains are required.

In conclusion, our results point to a high endemicity of HDV in the Cameroonian populations which is in sharp accordance with data reported from Cameroon 20 years ago and to the situation in neighbouring Central African countries. Therefore, practitioners and health care managers should be made aware of the risk of dual infection with HBV and HDV, especially when the HBV viral load is low or undetectable with abnormal serum ALT. HDV infection in Cameroon is characterized by a wide genetic diversity with the circulation of four different genotypes 1, 5, 6, and 7, HDV-1 being predominant.

Nucleotide sequence accession numbers. The GenBank accession numbers of the new HDV strains isolated from the population in this study are HQ013330 to HQ013354.

Acknowledgments: The authors are grateful to the HBsAg carriers who agreed to participate in this study. We are also grateful to Jean-Marc Reynes for critical reading of the manuscript.
This work was supported by funds from the Centre Pasteur of Cameroon.

References


Table 1: Demographic and laboratory characteristics of the 233 HBV carriers according to HDV antibody (HDV-Ab) status

<table>
<thead>
<tr>
<th></th>
<th>HDV-Ab positive (n = 41)</th>
<th>HDV-Ab negative (n = 192)</th>
<th>P-level(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>35.7</td>
<td>35.4</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(% male)</td>
<td>65.9</td>
<td>66.1</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Serum ALT</td>
<td></td>
<td></td>
<td>0.0004</td>
</tr>
<tr>
<td>(median, IU/L)</td>
<td>96.75</td>
<td>58.5</td>
<td></td>
</tr>
<tr>
<td>HBV DNA Level</td>
<td></td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td>(% undetectable)</td>
<td>61</td>
<td>35.4</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Statistical differences were evaluated using the Chi-square test or Fisher’s exact test to compare qualitative data. The Mann–Whitney U-test was used to compare quantitative variables. Differences were considered statistically significant for P values less than 0.05.
Fig. 1: Phylogenetic analysis of a 360-bp fragment of the *HDV-sHD* gene from different HDV strains using the NJ method. Twenty-five HDV sequences from Cameroonian patients (highlighted in boldface; name of the isolate and GenBank accession number are indicated) were aligned with 41 previously published HDV sequences of clades 1–8 (HDV-1 to HDV-8) available from GenBank as reference genes (Genotype and GenBank accession number are indicated) by using CLUSTAL X v1.81 software program and imported into MEGA v.4.0 software to perform phylogenetic analysis. Numbers next to the nodes of the tree represent bootstrap values (1000 replicates). Only bootstrap values above 70% are presented. Genetic distances were calculated using Kimura two-parameter method.