HBs Ag and antibodies to hepatitis C virus in complicated chronic hepatic diseases in Gabon

A case control study

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SUMMARY

Background and objective — The prevalence of HBs Ag and anti-HCV antibodies are high in the general population in Gabon. The aim of this study was to perform a case control study to determine the role of hepatitis B and C viruses in decompensated cirrhosis and hepatocellular carcinoma.

Methods — Between October 1990 and June 1998, HBs Ag and anti-HCV antibodies were investigated in 1 204 newly hospitalized patients. Sixty-seven had decompensated cirrhosis, 38 had hepatocellular carcinoma and six an association of both diseases. Prevalences were compared with those in a group of 527 sex and age matched controls from the same cohort.

Results — HBs Ag prevalence among cases was 35.1% (decompensated cirrhosis: 34.2%; hepatocellular carcinoma: 40.5%) and 12.5% among controls. Anti-HCV were detected in 32.4% of cases (decompensated cirrhosis: 34.2%; hepatocellular carcinoma: 28.6%) and in 20.1% of controls. Complicated chronic liver disease was linked to HBs Ag (OR = 11.3; IC: 4.8-26.7; cirrhosis: OR = 18; IC: 5.3-61.5; hepatocellular carcinoma: OR = 8.3; IC: 2.5-27.8) in patients from 15 to 34 years old. Above 45 years, complicated chronic liver disease was linked to anti-HCV antibodies (OR = 2.9; IC: 1.4-5.3; hepatocellular carcinoma: OR = 3.2; IC: 1.1-9.5).

Conclusion — Both Hepatitis B and C viruses are linked to complicated chronic liver disease in Gabon in an age-dependent manner.
low serum prothrombin and albumin. The diagnosis of hepatocellular carcinoma was based on the presence of enlarged tumourous liver at ultrasonography, with or without biopsy, and elevated alpha-fetoprotein > 500 ng/ml.

The criteria for diagnosis of complicated chronic liver disease were met by 111 patients (71 men and 40 women), mean age 45 years (range, 15-83). The M/F ratio was 2.6 up to 44 years then 1.4 beyond 44 years. Decompensated cirrhosis was observed in 67 patients, hepatocellular carcinoma in 38, and both in 6.

**Controls**

Control patients free of chronic liver disease were selected to match the study population for sex, age (within 20 months), and period of hospitalization (less than 18 month difference). Three to 5 controls were identified for each patient, five for 92 patients, 4 for 10 patients and 3 for 9 patients. The controls had the following diseases: bacterial and parasitic infections (27%), diabetes mellitus (14%), malignant disease (12%), functional disorders (7%), cardiovascular disease (5%).

**Methods**

**SEROLOGY**

Blood samples were obtained from all patients and controls. Serum was separated and frozen immediately at −20 °C. Demographic data, final diagnosis and the principal results of complementary tests were recorded in an anonymous database at discharge. Frozen sera were sent to France several times a year where they were stored at −80 °C. EUSA was used to determine HBs Ag (Monolisa Ag HBs plus, Sanofi Diagnostics Pasteur SA, France) and anti-HCV Ab (Innotest HCV Ab III, Innogenetics N.V. Belgium); positive results of the latter were confirmed by an immunocapture test (Innotest HCV Ab III, Innogenetics N.V. Belgium).

**STATISTICAL ANALYSIS**

Ten-year age groups were constructed for analysis. Intervals of confidence were calculated with an alpha risk of 5%.

The MacNemar test was used for comparisons. Odds ratio (OR) and 95% intervals of confidence were calculated for correlations.

Multivariate analysis with logistic regression for matched variables was performed with SPSS version 10.0.5 (SPSS Inc. USA).

**Results**

**Overall prevalences**

**Ag HBs**

HBs Ag were found in 39/111 patients with complicated chronic liver disease (35.1%, CI 26.3-44%) and in 66/527 controls (12.5%, CI 9.7-15.3) (table I). The difference was significant (p < 10⁻⁷). Prevalence of HBs Ag was age-related, decreasing regularly with age, both in controls and even more clearly in the study population (22.8% in patients under 25 and 81.8% in patients over 65) (figure 1).

**Anti-HCV antibodies**

Anti-HCV antibodies were found in 36/111 patients in the study population (32.4%, CI 23.7-41.1%) and in 106/527 controls (20.1%; CI 16.7-23.5%) (table II). The difference was significant (p < 0.002). The prevalence of anti-HCV Ab increased with age, especially after 44 years, both in controls (7.6% in controls under 44 versus 41.5% in controls over 65) and in the study population (19% in patients under 44 and 62% in patients over 65) (figure 2).

**Prevalence by type of complicated chronic liver disease**

Among the 73 patients with decompensated cirrhosis, 25 were positive for HBs Ag (34.2%, CI 23.4-45.1%) and 25 for anti-HCV Ab (34.2%, CI 23.4-45.1%). These prevalences were higher than in controls, 12.5% and 20.1% (p < 0.000005 and p < 0.004), respectively. Among the 44 patients with hepatocellular carcinoma, 17 (40.5%, CI 25.6-55.3%) were positive for HBs Ag, a prevalence significantly higher than in controls (12.5%) (p < 0.0002). Twelve patients (28.6%, CI 14.9-42.2%) were positive for anti-HCV Ab, not significantly different from controls (20.1%).

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**Table 1.** Prevalence of HBs Ag according to age in patients with complicated chronic liver disease and controls. n(m): n = number of patients, m = positive patients; CI = 95% confidence interval.

<table>
<thead>
<tr>
<th>Age</th>
<th>Cancer</th>
<th>Cirrhosis</th>
<th>Mixed</th>
<th>Total</th>
<th>% positive Cl</th>
<th>Total</th>
<th>% positive Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-24</td>
<td>7 (5)</td>
<td>14 (12)</td>
<td>1 (1)</td>
<td>22 (18)</td>
<td>81.8 [65.7-97.9]</td>
<td>92 (21)</td>
<td>22.8 [14.2-31.4]</td>
</tr>
<tr>
<td>25-34</td>
<td>11 (5)</td>
<td>8 (5)</td>
<td>2 (2)</td>
<td>21 (12)</td>
<td>57.5 [36-78.3]</td>
<td>105 (14)</td>
<td>13.3 [6.8-19.8]</td>
</tr>
<tr>
<td>35-44</td>
<td>4 (1)</td>
<td>6 (1)</td>
<td>1 (0)</td>
<td>11 (2)</td>
<td>18.2 [0-41]</td>
<td>55 (9)</td>
<td>16.4 [6.6-26.1]</td>
</tr>
<tr>
<td>45-54</td>
<td>2 (1)</td>
<td>18 (2)</td>
<td>0</td>
<td>20 (3)</td>
<td>15 [0-30.6]</td>
<td>91 (7)</td>
<td>7.7 [2.2-13.1]</td>
</tr>
<tr>
<td>55-64</td>
<td>10 (2)</td>
<td>9 (1)</td>
<td>2 (0)</td>
<td>21 (3)</td>
<td>14.3 [0-29.3]</td>
<td>105 (11)</td>
<td>10.5 [4.6-16.3]</td>
</tr>
<tr>
<td>&gt; 64</td>
<td>4 (0)</td>
<td>12 (1)</td>
<td>0</td>
<td>16 (1)</td>
<td>6.3 [0-18.1]</td>
<td>79 (4)</td>
<td>5.1 [0.2-9.9]</td>
</tr>
<tr>
<td>Total</td>
<td>38 (14)</td>
<td>67 (22)</td>
<td>6 (3)</td>
<td>111 (39)</td>
<td>35.1 [26.3-44]</td>
<td>527 (66)</td>
<td>12.5 [9.7-15.3]</td>
</tr>
</tbody>
</table>

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Co-infections

Among the 70 patients with complicated chronic liver disease who were positive for at least one of the viral markers, 5 (7.1%) were positive for both anti-HCV Ag and HBs Ab: a 21-year-old man and a 55-year-old woman with hepatocellular carcinoma, and three men aged 37, 50, and 60 years with decompensated cirrhosis. Among the controls, HBV-HCV co-infection was identified in 11/161 (6.8%).

Relationship between complicated chronic liver disease and viral markers by age

Three patterns were distinguished by age:

1) From 15 to 34 years, complicated chronic liver disease was strongly correlated with HBs Ag (p < 10\(^{-7}\), OR = 11.3, CI 4.8-26.7). For patients with decompensated cirrhosis and hepatocellular carcinoma, the ORs were 18 (CI 5.3-61.5) and 8.3 (CI 2.5-27.8), respectively. In contrast, there was no correlation between complicated chronic liver disease and anti-HCV Ab (p = 0.3, NS).

2) From 35 to 45 years, there was no significant correlation between complicated chronic liver disease and anti-HCV Ab (p = 0.2, NS) or HBs Ab (p = 0.1, NS).

3) For patients aged over 44, there was a significant correlation between complicated chronic liver disease and prevalence of anti-HCV Ab (p < 0.001, OR = 2.9, CI 1.6-5.3). The ORs for decompensated cirrhosis and hepatocellular carcinoma were 2.8 (CI 1.4-5.8) and 3.2 (CI 1.1-9.5). In contrast, there was no correlation between complicated chronic liver disease and HBs Ag prevalence.

Multivariate analysis

There was a significant correlation between complicated chronic liver disease and HBs Ag (OR = 4.6, CI 2.8-7.5) and between complicated chronic liver disease and anti-HCV Ab (OR = 2.5, CI 1.5-4). There was no co-infection interaction.

Table II. – Prevalence of anti HCV according to age in patients with complicated chronic liver disease and controls. n(m): n = number of patients, m = positive patients; CI = 95% confidence interval.

<table>
<thead>
<tr>
<th>Age</th>
<th>Cancer</th>
<th>Cirrhosis</th>
<th>Mixed</th>
<th>Total</th>
<th>% positive CI</th>
<th>Total</th>
<th>%positive CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-24</td>
<td>7 (1)</td>
<td>14 (0)</td>
<td>1 (0)</td>
<td>22 (1)</td>
<td>4.5 [0-13.2]</td>
<td>92 (1)</td>
<td>1.1 [0-3.2]</td>
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<tr>
<td>25-34</td>
<td>11 (0)</td>
<td>8 (0)</td>
<td>2 (0)</td>
<td>21 (0)</td>
<td>0 [0-0]</td>
<td>105 (8)</td>
<td>7.6 [2.5-13]</td>
</tr>
<tr>
<td>35-44</td>
<td>4 (0)</td>
<td>6 (2)</td>
<td>1 (0)</td>
<td>11 (2)</td>
<td>18.2 [0-41]</td>
<td>55 (4)</td>
<td>7.3 [0.4-14.1]</td>
</tr>
<tr>
<td>45-54</td>
<td>2 (0)</td>
<td>18 (10)</td>
<td>0</td>
<td>20 (10)</td>
<td>50 [28.1-71.9]</td>
<td>91 (23)</td>
<td>25.3 [16.4-34.2]</td>
</tr>
<tr>
<td>55-64</td>
<td>10 (6)</td>
<td>9 (5)</td>
<td>2 (1)</td>
<td>21 (12)</td>
<td>57.1 [36-78.3]</td>
<td>105 (39)</td>
<td>37.1 [27.9-46.3]</td>
</tr>
<tr>
<td>&gt; 64</td>
<td>4 (4)</td>
<td>12 (7)</td>
<td>0</td>
<td>16 (11)</td>
<td>68.8 [46-91.5]</td>
<td>79 (31)</td>
<td>39.2 [28.5-50]</td>
</tr>
<tr>
<td>Total</td>
<td>38 (11)</td>
<td>67 (24)</td>
<td>6 (1)</td>
<td>111 (36)</td>
<td>32.4 [23.7-41.1]</td>
<td>527 (106)</td>
<td>20.1 [16.7-23.5]</td>
</tr>
</tbody>
</table>

Discussion

This study demonstrated that in Gabon there is a strong correlation between HBV and complicated chronic liver disease in subjects under the age of 35 years and that HCV is clearly linked to the presence of complicated chronic liver disease in subjects over the age of 44 years. These two associations with chronic liver disease have been reported in other studies in several countries in Africa [11-20], but many were not case-control studies with age-matched controls so several were unable to demonstrate any age relationship. In our data, the demonstrated correlation of HCV with hepatocellular carcinoma in patients over 44 would be masked if all patients were considered independently of age. We chose to study a clinically identifiable entity, complicated chronic liver disease, because of the cost and also the availability of complementary examinations for liver disease, which can be intermittent in Gabon. In a healthcare system with limited means for treatment, the short- or mid-term fatal outcome of complicated chronic liver disease is a major public health problem.

The control population used here is not necessarily representative because of hospital recruitment bias. In addition, certain parameters could not be measured. This recruitment would probably explain why the observed prevalence of anti-HCV Ab was higher than that found in the general Gabonese population [5]. On the other hand, the prevalence of HBs Ag observed in our controls is the same as found in the general population [1-5]. The difference between HCV prevalence in our study population and hospital control population would suggest possible iatrogenic contamination, particularly among controls and patients with complicated chronic liver disease aged over 44 years, similar to data reported for Egypt [21]. Nevertheless, the stability of anti-HCV Ab over a 10-year period [22] suggests a certain degree of persistence in the modes of transmission involved.

The absence of information on other hepatotoxic parameters (alcohol, aflatoxin, anabolizing steroids, oral contraceptives, iron overload) probably explains why 36.9% of the complicated chronic liver disease patients were negative for both viral markers studied. Special attention should be given to alcohol, which is widely consumed in Gabon, because of its direct effect on liver function and also its accelerating effect on viral disease;
Table III

Table III. Prevalence of HBsAg, HCV Ab among patients in Africa. HCC hepatocellular carcinoma.

<table>
<thead>
<tr>
<th>Author [ref]</th>
<th>Country (year)</th>
<th>Chronic liver disease</th>
<th>HCC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HBs Ag</td>
<td>HCV Ab</td>
</tr>
<tr>
<td>Weinig [17]</td>
<td>Zimbabwe (1997)</td>
<td>42.6</td>
<td>23.8</td>
</tr>
</tbody>
</table>

* Chronic liver disease and hepatocellular carcinoma grouped together.

The morbidity of viral infection is known to be higher in patients with both factors [23]. These causal diseases factors could be the reason why no correlation was found between complicated chronic liver disease and HBV or HCV among patients aged 35-44 years.

Despite these limitations, our data demonstrate the causal implication of hepatitis B and C viruses in severe liver disease in Gabon. There is an age-related pattern which could be useful in developing an appropriate public health policy. The risk of HBV infection is particularly high in the young population, an expression of neonatal and infantile contamination. This observation points out the importance of early vaccination which is not subsidized in this country and thus inaccessible to the majority of the population. The consequences of severe liver disease related to HCV infection are particularly severe for the older population. However, the lack of precise knowledge concerning the mechanisms involved in iatrogenic transmission of HCV in Africa prohibits any valid prognosis concerning the longevity of this situation. For the time being, elementary prophylactic measures — more strict application of the rules of parenteral injections, advise on temperance in alcohol consumption among infected patients — must be strongly encouraged.

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REFERENCES