Occult hepatitis B virus infection in Moroccan HIV infected patients

Tahar Bajjou1,2, Hicham Elannaz1,3, Mohamed Rida Tagajdid1,3*, Hicham Elghafouli2, Abdelilah Laraqui1, Rachid Abi1,3, Farida Elhilali2, Mimoune Zouhdi4, Idriss Lahlou1, Yassine Sekhsoukh1,2, Saad Mrani1,3

1Unit of Research in Molecular Virology and Oncobiology, Faculty of Medicine and Pharmacy of Rabat, Mohamed V University, Rabat, Morocco
2Laboratory of Research and Biosafety P3, Mohamed V Military Teaching Hospital of Rabat, Hay Riad, Rabat, 10100, Morocco
3Laboratory of Virology, Mohammed V Military Teaching Hospital of Rabat, Hay Riad, Rabat-10100, Morocco
4Laboratory of Bacteriology, Ibn Sina Hospital BP 6527, Rabat, Morocco

Received: 8 January 2015
Revised: 21 January 2015
Accepted: 4 February 2015

*Correspondence:
Dr. Mohammed Reda Tagajdid,
E-mail: reda.tagajdid@laposte.net

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: The purpose of this study is to assess the prevalence of Occult hepatitis B virus Infection (OBI) among antiretroviral treatment naïve HIV-1 infected individuals in Morocco and to determine factors favouring its occurrence.

Methods: The retrospective study was conducted in the Mohammed V military teaching hospital in Rabat between January 2010 and June 2011. It included patients with confirmed HIV infection, tested negative to serological detection of HBV surface antigen (HBsAg) and did not received antiviral treatment or hepatitis B vaccine. All samples were tested for anti-HBc, anti-HBs and anti-HCV antibodies using enzyme immunoassay (ELISA). The detection of HBV DNA was performed by real-time PCR using two specific primers for a gene in the region C of the viral genome. The sensitivity of the technique was 20 copies/ml.

Results: A total of 82 samples were analyzed, 19 (23 %) were found to have isolated anti-HBc, 07 (8.5%) with associated anti-HBc and Anti-HBs. No anti-HCV marker was detected on these screening samples. The HBV DNA was detected in 48 (58%) samples, of which, males constituted 58% (28/48). The mean age of these patients was 38 ± 8.2 (29-56), the median HIV-1 viral load and CD4 cell count HIV-1 infected patients were 127500 (54108-325325) copies/ml and 243 [80-385] cells/mm³ respectively and 27.1% (13/48) of these patients were found to have isolated anti-HBc. A significant correlations between DNA HBV and HIV viral load higher than 100000 copies/ml (P = 0.004), CD4 cell count lower than 400 cells/mm³ (P = 0.013, P = 0.006) and isolated anti-HBc samples (P <0.005) were founded. However there was no significant association with age, sex, transmission mode and clinical stage.

Conclusion: The consequences of this high prevalence of OBI in Morocco need to be considered in laboratory diagnosis of HBV infection in HIV infected patients and the PCR seems to be inevitable for a better diagnosis and therapy.

Keywords: Occult hepatitis B, HIV patients, Real-time PCR
INTRODUCTION

Occult Hepatitis B Virus (HBV) Infection (OBI) has been defined by the presence of HBV-DNA in the liver tissue of HBV surface antigen (HBsAg) negative individuals, with or without serological markers of previous viral exposure.\textsuperscript{1,2} OBI has been reported worldwide and its frequency varies with the prevalence of HBV infection in the studied population.\textsuperscript{1,4-5} The analysis of liver HBV-DNA extracts represents the gold standard for OBI testing.\textsuperscript{1} However, the execution of a needle liver biopsy cannot be performed in the great majority of the subjects. Alternatively, many real time PCR based assays for serum (or plasma) HBV-DNA detection are sufficiently sensitive to detect many (but not all) OBI cases.\textsuperscript{8,9}

The prevalence of OBI in Human Immunodeficiency Virus (HIV) infected patients remains controversial and the available data are widely divergent. Published studies report a prevalence from nil to 89% in HIV-1 infected patients with “anti-HBc alone”.\textsuperscript{10-14} This high prevalence in HIV infected patients, probably because both HBV and HIV virus share the same way of transmission. Despite evidence that co-infection with HIV and HBV may accelerate the progress to liver disease, be related to the development of hepatocellular carcinoma (HCC) and influence the response to HCV and HBV treatments.\textsuperscript{15-18}

There is still a debate on the consequences of occult HBV infection in patients with HIV.\textsuperscript{10,19-21}

Morocco is low and intermediate endemic area for HIV and HBV infections respectively.\textsuperscript{22-24} The estimated prevalence of HIV-infected individuals is under 1%,\textsuperscript{20} while is ranging between 1.5 and 3.3% for HBV infection.\textsuperscript{22,23,25} However, there are no data available on OBI in HIV infected patients in Morocco. The purpose of this study is to assess the prevalence of OBI among antiretroviral treatment naïve HIV-1 infected individuals in this country and to determine factors favouring its occurrence.

METHODS

Study design and sampling

The patients included in this retrospective laboratory-based study were HIV infected patients followed-up at the Mohammed V Military Teaching Hospital (MVMTH) in Rabat from January 2010 to June 2011. The MVMTH is a 1000 bed university hospital with about 80000 inpatient admissions and 200000 outpatients per year. It is intended for active or retired military personnel as well as their families, but it also treats civilians thereby serving about 5 million people living in the north of the country.

The inclusion criteria were as follows: patients with confirmed HIV infection, tested negative to serological detection of HBsAg and did not received antiviral treatment or hepatitis B vaccine. Demographic, clinical data including (age, sex, mode of HIV transmission, WHO clinical classification) and measurements of CD4 T cell count, analysed by flow cytometer (Coulter® EPICS XL™, Beckmann Coulter) and HIV viral load determined by real-time PCR (Cobas TaqMan HIV-1 Test, Roche Diagnostics Systems) were collected from the MVMTH database.

Serological assays

All samples which met the inclusion criteria and received in the period of the study were included and tested for anti-HBc, anti-HBs antibodies using enzyme immunoassay (ELISA) (Enzygnost®, Dade Behring) and anti-HCV antibodies using ELISA(Geenscreen® ULTRA HIV Ag-Ab, BIO-RAD Laboratories). We repeated HBsAg test using ELISA (Enzygnost®, Dade Behring) on the same sample in which HBV DNA testing was performed.

HBV DNA detection

Viral DNA was extracted from 200 µl of plasma using High Pure Viral Nucleic Acid Kit (Roche Diagnostics Systems). HBV DNA detection was performed by real-time qualitative PCR using the Light Cycler 2.0 (Roche Diagnostics). PCR primers, forward primer CSB (5’-TCG GAG TGT GGA TTC GCA CTC CTC 3’, nucleotide position 2265-2288), and Reverse primer CASB (5’-GAT TGA GAC CTT CCT CGA GGC-3’, nucleotide position 2322-2415) were used [19]. PCR reactions were performed in a 20 µl total volume using 2 µl of light Cycler DNA Master Hybridization Mixture SYBR Green (Roche Diagnostics), 3.2 µl of 25 mM MgCl₂, 0.3 µl each of the 20 µM primers (forward primer CSB and Reverse primer CASB) and 2 µl of DNA.

PCR conditions for HBV detection were as follows: 1 cycle of 95°C for 10 min, slope 20°C/s, followed by 45 cycles of 95°C for 15 s, annealing at 58°C for 5 s, and extension at 72°C for 15 s. The programmed temperature transition rate was 20°C/s. For melting curve, a single melt cycle was generated by holding the reaction at 65°C for 15 s, followed by cooling at 40°C for 30 s. The melting curve and qualitative analysis were conducted by using light Cycler software 3.5 following the manufacture’s instruction (Roche Diagnostics Applied Science, Penzberg, Germany). In addition, we have tested serial dilutions of a control specimen (HBV viral load = 40000 copies/ml). Doubling dilutions were tested until a dilution of 20 copies/ml (Figure 1). Positive and negative controls were included in all runs of extraction and amplification. Positive results were repeated for confirmation in another run. PCR products were confirmed on 2% agarose gel electrophoresis.

Statistical methods

A descriptive study in percentage terms of all recorded variables was done using the SPSS software (V10.0). The
RESULTS

According to the mentioned criteria, a total of 82 Moroccan patients were analyzed in the present study, 47 (58%) were men and 35 (42%) women, with a mean age of 39 ± 9.5 years (range: 29-60). For the mode of transmission, 72 (88%) patients reported heterosexual contacts, and for 10 patients (12%) the mode of infection were not available. The duration of HIV-1 infection was not known. The WHO clinical classification indicated that 12% of these patients were at stage 1, 23% at stage 2, 32% at stage 3 and 33% at stage 4. The median HIV-1 viral load and CD4 cell count HIV-1 infected patients was 90550 (27450-336000) copies/ml and 189 (80-385) cells/mm3 respectively. The baseline characteristics of the population are summarized in Table 1.

A total of 19 (23%) patients were found to have isolated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc. 07 (8.5%) patients with associated anti-HBc.

The detection limit of our in-house PCR assay was 20 copies/ml for specimens tested individually (Figure 1). Of the 82 analyzed samples, 48 (58%) were positive for HBV DNA, of which, males constituted 58% (28/48).

The mean age of these patients was 38 ± 9.5 years (range: 29-60). Plasma viral load category (copies/ml), median (percentile 25-75)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HBV DNA positive (N=48)</th>
<th>HBV DNA negative (N=34)</th>
<th>Total of patients (N=82)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>28 (58)</td>
<td>19 (56)</td>
<td>47 (58)</td>
</tr>
<tr>
<td>Women</td>
<td>20 (42)</td>
<td>15 (44)</td>
<td>35 (42)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD*</td>
<td>38 ± 8.2 (29-56)</td>
<td>39.7 ± 7.4 (31-60)</td>
<td>39 ± 9.5 (29-60)</td>
</tr>
<tr>
<td>Transmission risk group, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterosexuals</td>
<td>44 (88)</td>
<td>28 (88)</td>
<td>72 (88)</td>
</tr>
<tr>
<td>Unknown</td>
<td>04 (12)</td>
<td>06 (12)</td>
<td>10 (12)</td>
</tr>
<tr>
<td>WHO classification, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class 1</td>
<td>06 (12.5)</td>
<td>04 (11.8)</td>
<td>10 (12.2)</td>
</tr>
<tr>
<td>Class 2</td>
<td>10 (20.8)</td>
<td>09 (26.4)</td>
<td>19 (23.2)</td>
</tr>
<tr>
<td>Class 3</td>
<td>15 (31.2)</td>
<td>11 (32.4)</td>
<td>26 (31.7)</td>
</tr>
<tr>
<td>Class 4</td>
<td>17 (35.5)</td>
<td>10 (29.4)</td>
<td>27 (32.9)</td>
</tr>
<tr>
<td>Plasma viral load (copies/ml), median (percentile 25-75)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40000</td>
<td>127500 (54108 - 325325)</td>
<td>61850 (13850 - 225250)</td>
<td>90550 (27450 - 336000)</td>
</tr>
<tr>
<td>40,000-99999</td>
<td>10 (20.8)</td>
<td>05 (14.7)</td>
<td>15 (18.3)</td>
</tr>
<tr>
<td>≥100000</td>
<td>20 (41.7)</td>
<td>10 (29.4)</td>
<td>30 (36.6)</td>
</tr>
<tr>
<td>CD4 cell count (cells/mm³), median (percentile 25-75)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>243 (80-385)</td>
<td>345.5 (195-495)</td>
<td>204 (141-401)</td>
<td></td>
</tr>
<tr>
<td>CD4 cell count category (cells/ml), No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-200</td>
<td>22 (45.8)</td>
<td>15 (44.1)</td>
<td>37 (45.1)</td>
</tr>
<tr>
<td>200-400</td>
<td>13 (27.1)</td>
<td>02 (05.9)</td>
<td>15 (18.3)</td>
</tr>
<tr>
<td>&gt;400</td>
<td>13 (27.1)</td>
<td>17 (50.0)</td>
<td>30 (36.6)</td>
</tr>
<tr>
<td>HBsAg</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Isolated anti-HBc</td>
<td>13 (27.1)</td>
<td>6 (17.6)</td>
<td>19 (23)</td>
</tr>
<tr>
<td>Anti-HBc and Anti-HBs</td>
<td>06 (08.3)</td>
<td>02 (05.8)</td>
<td>07 (8.5)</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

DISCUSSION

Serological markers have been the standard method of diagnosis of HBV infection for more than 30 years. The wider availability during recent years of very sensitive molecular biology techniques for detecting HBV DNA in serum and liver tissue has increased attention to OBI.9 Occult HBV infection has important implications when considering the transmission of the virus and complications of chronic HBV infection such as cirrhosis and hepatocellular carcinoma. Further investigation of
occult HBV DNA infection in HIV-seropositive patients is needed given the more rapid progress towards chronic liver disease in people with HIV/HBV co-infection.15-18

In Morocco, the prevalence of HIV/HBV co-infection has been reported recently, using serological markers, showed that 5.2% of HIV patients were HBV surface antigen positive.26 However, Occult HBV infection needs to be considered when establishing the prevalence of HIV/HBV co-infection. To date, this is the first study of occult HBV infection in HIV positive patients in Morocco.

Here, we report the detection of OBI in 58% of HIV-infected patients using a nested in-house PCR assay with a sensitivity of 20 copies/ml. According to the literature, the prevalence of OBI in HIV positive patients ranged from nil to 89% [10-14]. These variations are explained by differences in sensitivity of PCR techniques used and the number of samples tested.1,10,27 Moreover, the prevalence of OBI also depends on the prevalence of HBV and the characteristics of the study population.28,29 Our results differ from other studies using the same or similar in-house PCR, showing 0% in Spain,13 4% in Netherlands,15 20% in Italy11 and 35% in France.14 Other factors may account for this founding, unfortunately, data on OBI in HIV-positive or negative patients from Morocco are lacking, and there are no studies for comparison. In addition, we ask if hepatitis B is well estimated in the general population in this country.

Analysis of our data revealed that 23% (19/82) of patients were with isolated anti-HBc, of which 68.4% (13/19) were positive for HBV DNA. The significant correlation between HBV-DNA and isolated anti-HBc antibodies founded was in agreement with some previous study.13,14 In addition, a statistically significant correlation between HBV DNA and low CD4 cell count and between HBV DNA and HIV viral load founded in our results was reported by many authors.11,13,32,34

Many studies have reported co-infection with HCV as a risk factor for OBI.15 In our patients, the absence of detection of anti-HCV can be explained by the fact that we did not analyze HCV RNA in our study, that the mode of transmission was heterosexual contacts in more than 88% of our patients and that the prevalence of anti-HCV in general population is low (2%) in Morocco.15

This study had several limitations. First, we included the small sample size. Second, the median of CD4 count of 45% of patients recruited was below 200 cells/mm3 and 65% of them were in stage 3 and 4 of WHO clinical classification. This is due to late stage of HIV diagnosis of our patients. So it is unclear what the OBI prevalence might be in patients who are more immune competent in this country. Third, the use of real time PCR would have been superior for HBV-DNA detection used in this study, as compared to quantitative PCR methods. Finally, other large studies are needed to confirm our results.

CONCLUSION

This study demonstrates that the HIV/HBV co-infection rate is significantly underestimated with the current serology testing methods alone. The consequences of this high prevalence need to be considered in laboratory diagnosis of HBV infection in HIV infected patients and the PCR techniques can be usually used. With the high prevalence of HIV/HBV co-infection in Morocco, treatment with the nucleoside analogue reverse transcriptase inhibitors as first-line therapy would not only improve the safety and simplify HIV treatment, but would also treat the patients co-infected with HBV.

ACKNOWLEDGEMENTS

The authors thank Dr. Abdellatif Allami for his help in carrying out statistical analysis in this study.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

REFERENCES


