International Journal of Research in Medical Sciences
Bajjou T et al. Int J Res Med Sci. 2015 Mar;3(3):617-621
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: 10.5455/2320-6012.ijrms20150316

Research Article

Occult hepatitis B virus infection in Moroccan HIV infected patients

Tahar Bajjou[1,2], Hicham Elannaz[1,3], Mohamed Rida Tagajdid[1,3]*, Hicham Elghafouli[2], Abdelilah Laraqui[2], Rachid Abi[1,3], Farida Elhilali[2], Mimoune Zouhdi[4], Idriss Lahlou[1], Yassine Sekhsoukh[1,2], Saad Mrani[1,3]

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

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/mm^3 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/mm^3 (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.[1,2] OBI has been reported worldwide and its frequency varies with the prevalence of HBV infection in the studied population.[1-4,5-7] The analysis of liver HBV-DNA extracts represents the gold standard for OBI testing.[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.[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".[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.[15-18] There is still a debate on the consequences of occult HBV infection in patients with HIV.[10,19-21]

Morocco is low and intermediate endemic area for HIV and HBV infections respectively.[22-24] The estimated prevalence of HIV-infected individuals is under 1%,[20] while is ranging between 1.5 and 3,3% for HBV infection.[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 CTG 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 $MgCl_2$, 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

comparison between variables was performed using the unpaired t-test, χ^2-test, Fisher exact test, or Mann-Whitney test, as appropriate. P values were considered significant at a level lower than 0.05.

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 and Anti-HBs. No HBsAg or anti-HCV markers were detected on these screening samples.

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 ± 8.2 (29-56), the median HIV-1 viral load and CD4 cell count were 127500 (54108-325325) copies/ml and 243 (80-385) respectively and 27.1% (13/48) of these patients were found to have isolated anti-HBc.

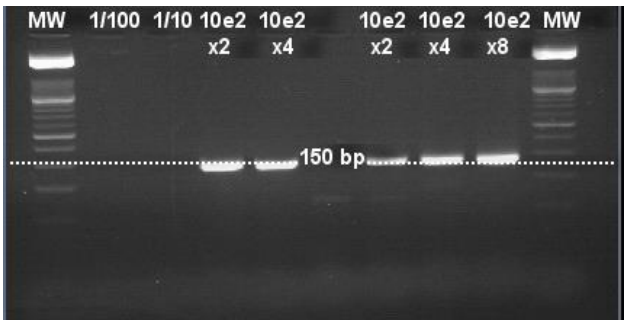

Figure 1: Serial dilutions of HBV positive control containing 40000 copies/ml.

1/100 and 1/10 represent 10-fold serial dilutions of the control specimen. 10e2 x2 to 10e2 x8 are 2-fold serial dilutions. MW represent molecular weight marker. The detection limit was 10 copies/ml.

Univariate analysis of the associations between DNA HBV and categorical variables using ki^2-test showed, a significant correlations with, HIV viral load higher than 100000 copies/ml (P = 0.004), CD4 cell count lower than 400 cells/mm^3 (P = 0.013, P = 0.006) and isolated anti-HBc samples (P <0,005). However there was no significant association with age, sex and clinical stage (Table 1).

Table 1: Baseline characteristics of patients and summary of results.

Characteristic	HBV DNA positive (N=48)	HBV DNA negative (N=34)	Total of patients (N=82)
Gender, No. (%)			
Men	28 (58)	19 (56)	47 (58)
Women	20 (42)	15 (44)	35 (42)
Age (years)			
Mean ± SD*	38 ± 8.2 (29-56)	39.7 ± 7.4 (31-60)	39 ± 9.5 (29-60)
Transmission risk group, No. (%)			
Heterosexuals	44 (88)	28 (88)	72(88)
Unknown	04 (12)	06 (12)	10(12)
WHO classification, No. (%)			
Class 1	06 (12.5)	04 (11.8)	10 (12.2)
Class 2	10 (20.8)	09 (26.4)	19 (23.2)
Class 3	15 (31.2)	11 (32.4)	26 (31.7)
Class 4	17 (35.5)	10 (29.4)	27 (32.9)
Plasma viral load (copies/ml), median (percentile 25-75)	127500 (54108 - 325325)	61850 (13850 - 225250)	90550 (27450 - 336000)
Plasma viral load category (copies/ml), No. (%)			
40-9999	18 (37.5)	19 (55.9)	37 (45.1)
10000-99999	10 (20.8)	05 (14.7)	15 (18.3)
≥100000	20 (41.7)	10 (29.4)	30 (36.6)
CD4 cell count (cells/mm^3) median (percentile 25-75)	243 (80-385)	345.5 (195-495)	204 (141-401)
CD4 cell count category (cells/ml), No. (%)			
0-200	22 (45.8)	15 (44.1)	37 (45.1)
200-400	13 (27.1)	02 (05.9)	15 (18.3)
>400	13 (27.1)	17 (50.0)	30 (36.6)
HBsAg	0	0	0
Isolated anti-HBc	13 (27.1)	6 (17.6)	19 (23)
Anti-HBc and Anti-HBs	06 (08.3)	02 (05.8)	07 (8.5)
Anti-HCV	0	0	0

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.[8,9] 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.[2-6] 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,[12] 20% in Italy[11] 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.[1-5] 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.[35]

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 quanlitative 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

1. Torbenson M, Thomas DL. Occult hepatitis B. Lancet Infect Dis. 2002;2:479-86.
2. Chemin I, Trépo C. Clinical impact of occult HBV infections. J Clin Virol. 2005;34(Suppl 1):S15-21.
3. Soriano V, Vispo E, Labarga P, Medrano J, Barreiro P. Viral hepatitis and HIV co-infection. Antiviral Res. 2010,85:303-15.
4. Soriano V, Puoti M, Peters M, Benhamou Y, Sulkowski M, Zoulim F, et al. Care of HIV patients with chronic hepatitis B: updated recommendations from the HIV-Hepatitis B Virus International Panel. AIDS. 2008;22:1399-410.
5. Skiest DJ. The importance of co-morbidity in older HIV-infected patients. AIDS. 2003;17:1577.
6. Nabil Ahmed Said Z. An overview of occult hepatitis B virus infection. World J Gastroenterol. 2011;17:1927-38.
7. Fabris P, Giordani MT, Tositti G, Rassu M, de Lalla F. Occult hepatitis B virus infection in HIV/hepatitis C virus co-infected patients. AIDS. 2003;17:1581-2.
8. Chan HL, Leung NW, Lau TC, Wong ML, Sung JJ. Comparison of three different sensitive assays for hepatitis B virus DNA in monitoring of responses to antiviral therapy. J Clin Microbiol. 2000;38:3205-8.
9. Gilbert N, Corden S, Ijaz S, Grant PR, Tedder RS, Boxall EH. Comparison of commercial assays for the quantification of HBV DNA load in health care workers: calibration differences. J Virol Methods. 2002,100:37-47.
10. Marque-Juillet S, Benghalia K, Monnier S, Fernand-Laurent C, Mazeron MC, Harzic M. Faut-il rechercher une hépatite B occulte chez les patients infectés par le virus de l'immunodéficience humaine (VIH)? Pathologie Biologie. 2010;58:e39-42.

11. Filippini P, Coppola N, Pisapia R, Scolastico C, Marrocco C, Zaccariello A, et al. Impact of occult hepatitis B virus infection in HIV patients naive for antiretroviral therapy. AIDS. 2006;20:1253-60.
12. Pogany K, Zaaijer HL, Prins JM, Wit FW, Lange JM, Beld MG. Occult hepatitis B virus infection before and 1 year after start of HAART in HIV type 1-positive patients. AIDS Res Hum Retroviruses. 2005;21:922-6.
13. Núñez M, Rios P, Perez-Olmeda M, Soriano V. Lack of 'occult' hepatitis B virus infection in HIV-infected patients. AIDS. 2002;16:2099-101.
14. Piroth L, Grappin Ml, Buisson M, Duong M, Portier H, Chavanet P. Hepatitis B Virus seroconversion in HIV-HBV co-infected patients treated with highly active antiretroviral therapy. JAIDS J Acquir Immune Defic Syndr. 2000;23:356-7.
15. Puoti M, Torti C, Bruno R, Filice G, Carosi G. Natural history of chronic hepatitis B in co-infected patients. J Hepatol. 2006;44:S65-70.
16. Raimondo G, Navarra G, Mondello S, Costantino L, Colloredo G, Cucinotta E, et al. Occult hepatitis B virus in liver tissue of individuals without hepatic disease. J Hepatol. 2008;48:743-6.
17. Sulkowski MS. Viral hepatitis and HIV co-infection. J Hepatol. 2008;48:353-67.
18. Bonacini M, Louie S, Bzowej N, Rock Wohl A. Survival in patients with HIV infection and viral hepatitis B or C: a cohort study. AIDS. 2004,18:2039-45.
19. Mrani S, Chemin I, Menouar K, Guillaud O, Pradat P, Borghi G, et al. Occult HBV infection may represent a major risk factor of non-response to antiviral therapy of chronic hepatitis C. J Med Virol. 2007;79:1075-81.
20. Kay A, Datta, Shetal. Paths to leadership for people living with HIV in the Middle East and North Africa. In: Kay A, Datta, Shetal, eds. Health Policy Initiative, Task Order 1. Washington, DC: Futures Group; 2010.
21. Konopnicki D, Mocroft A, de Wit S, Antunes F, Ledergerber B, Katlama C, et al. Hepatitis B and HIV: prevalence, AIDS progression, response to highly active antiretroviral therapy and increased mortality in the EuroSIDA cohort. AIDS. 2005,19:593-601.
22. Cacoub P, Ohayon V, Sekkat S, Dumont B, Sbai A, Lunel F, et al. Etude épidémiologique et virologique des infections par le virus de l'hépatite C au Maroc. Gastroenterol Clin Biol. 2000;24:169-73.
23. Atitar I, Achour J, Amrani L, Serraj I, Acharki M, Amrani N. Prevalence of hepatitis B and C markers in high risk hospitalized patients in Morocco. Arab J Gastroenterol. 2009;10:AB62-5.
24. Elharti E, Alami M, Khattabi H, Bennani A, Zidouh A, Benjouad A, et al. Some characteristics of the HIV epidemic in Morocco. East Mediterr Health J. 2002;8(6):819-25.
25. Ezzikouri S, Chemin I, Chafik A, Wakrim L, Nourlil J, Malki AE, et al. Genotype determination in Moroccan hepatitis B chronic carriers. Infect Genet Evol. 2008;8:306-12.
26. Rebbani K, Ouladlahsen A, Bensghir A, Akil A, Lamdini H, Issouf H, et al. Co-infections with hepatitis B and C viruses in human immunodeficiency virus-infected patients in Morocco. Clin Microbiol Infect. 2013 Apr;19(10):E454-7.
27. Singh S. Occult hepatitis B virus infection in ART-naive HIV-infected Indian patients. Int J Infect Dis. 2011;14:e225-7.
28. Cohen Stuart JW, Velema M, Schuurman R, Boucher CA, Hoepelman AI. Occult hepatitis B in persons infected with HIV is associated with low CD4 counts and resolves during antiretroviral therapy. J Med Virol. 2009;81:441-5.
29. Raimondo G, Pollicino T, Cacciola I, Squadrito G. Occult hepatitis B virus infection. J Hepatol. 2007;46:160-70.
30. Lo Re Iii V, Wertheimer B, Localio AR, Kostman JR, Dockter J, Linnen JM, et al. Incidence of transaminitis among HIV-infected patients with occult hepatitis B. J Clin Virol. 2008;43:32-6.
31. Marino N, Lo Caputo S, Pierotti P, Blè C, Riccardi MP, Trezzi M, et al. Prevalence of occult hepatitis B virus infection in a cohort of HIV infected subjects. Utrecht, the Netherlands: Virology Education; 2004: 186.
32. Sellier P, Schnepf N, Jarrin I, Mazeron MC, Simoneau G, Parrinello M, et al. Description of liver disease in a cohort of HIV/HBV co-infected patients. J Clin Virol. 2010,47:13-7.
33. Firnhaber C, Viana R, Reyneke A, Schultze D, Malope B, Maskew M, et al. Occult hepatitis B virus infection in patients with isolated core antibody and HIV co-infection in an urban clinic in Johannesburg, South Africa. Int J Infect Dis. 2009;13:488-92.
34. Harania RS, Karuru J, Nelson M, Stebbing J. HIV, hepatitis B and hepatitis C co-infection in Kenya. AIDS. 2008;22:1221-2.
35. Benouda A, Boujdiya Z, Ahid S, Abouqal R, Adnaoui M. Prévalence de l'infection par le virus de l'hépatite-C au Maroc et évaluation des tests sérologiques de dépistage pour la prédiction de la virémie. Pathologie Biologie 2009;57:368-72.

DOI: 10.5455/2320-6012.ijrms20150316
Cite this article as: Bajjou T, El annaz H, Tagajdid MR, Elghafouli H, Laraqui A, Abil R, Zouhdi M, Lahlou I, Sakhsoukh Y, Mrani S. Occult hepatitis B virus infection in Moroccan HIV infected patients. Int J Res Med Sci 2015;3:617-21.