International Journal of Infectious Diseases 45 (2016) 18–23

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Molecular characterization of hepatitis B virus from chronically-infected patients in Niamey, Niger

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ARTICLE INFO

Article history:
Received 13 December 2015
Received in revised form 8 February 2016
Accepted 10 February 2016

Corresponding Editor: Eskild Petersen, Aarhus, Denmark.

Keywords:
Hepatitis B
genotype
hepatitis B surface antigen
recombinant
Niger
Niamey

SUMMARY

Objectives: In Niger, 65% of hepatocarcinoma and 75% of cirrhosis cases were due to hepatitis B virus (HBV). We studied the genotypic characteristics of HBsAg in chronically HBV-infected patients in Niamey.

Methods: We studied prospectively HBV genotypic patterns among hospitalized patients with HBV infection in the National Hospital of Niamey, Niger. Patients were screened for hepatitis B surface antigen (HBsAg) and HBV genotyping was performed on the HBsAg-positive patients.

Results: In this study, we have confirmed the predominance of the HBV genotype E (HBV-E) in Niger and have identified 2 recombinant forms including HBV-E/D and HBV-A3/E reported previously among blood donors in Niger and Ghana, respectively. Amino acid substitutions found in HBV sequences obtained here included P120T, S143L, G145A and A194T. These substitutions were characterized as being associated with modified antigenicity and, notably, with impaired serological detection of HBsAg, while the A194T variant was found to have a controversial role in reduced susceptibility to tenofovir.

Conclusions: We have identified two recombinant HBV forms and rare genotypic patterns in Niger that may affect hepatitis B surface antigen antigenicity, and improve current knowledge of epidemiological, clinical and virological patterns of hepatitis B in this country.

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1. Background

Hepatitis B virus (HBV) is a public health problem worldwide.[1] An estimated 2 billion people have been in contact with the virus, of whom 240 million are chronically infected (http://www.who.int/mediacentre/factsheets/fs204/en/).

Chronic HBV infection is responsible for 60-80% of liver cancers worldwide.[1] Chronic HBV infection is spread very unevenly across regions: in Asia, the Pacific region, Sub-Saharan Africa, the Amazonia region, Alaska, Egypt and Arabian Peninsula approximately 8-20% of the population has chronic HBV infection while in North America, Eastern Europe and the Mediterranean basin 2-7% of the population is infected. Finally, less than 2% of the population has chronic hepatitis B in North America, Northern and Western Europe and Australia. Through mass vaccination campaigns, countries such as Singapore, Malaysia, Bahrain, Israel and Iran have joined the group of countries with a low prevalence of hepatitis B.[2–4]

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http://dx.doi.org/10.1016/j.ijid.2016.02.009
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Approximately 70–140 million of the chronic HBV infections and 250,000 of the 1.3 million HBV-related deaths recorded each year around the world occur in Africa.[5,6] The West African country of Niger has a high prevalence of HBV. Among the 238 asymptomatic young students living on the campus of the University of Niamey in 1985, 18% carried hepatitis B surface antigen (HBsAg).[7] Studies conducted by Cenac et al. in 1985 and 1995 have shown that cases of chronic liver disease in Niger including cirrhosis and hepatocellular carcinoma are associated with HBV infection (HBsAg pos) in 65%[8] and 73%[9] of cases, respectively. In addition, Mayaki et al. found HBsAg in 15% of blood donors[10] and Mamadou et al. found a 16% prevalence rate among the 495 pregnant women screened in 2008.[11] To date, only one paper has described HBV genotypic patterns in Niger, and it suggested the occurrence of recombinant forms between genotypes D and E and a novel subgenotype D (D8) strain in blood donors.[12] The aim of the present study was to describe the genotypic characteristics of HBsAg in chronically HBV-infected patients in the National Hospital of Niamey (NHN).

2. Study design

2.1. Patients

The first part of this prospective study was conducted in the National Hospital of Niamey (NHN), Niger, with authorization from this institution. Serum samples were collected from patients older than 18 years who were hospitalized in the internal medicine, infectious diseases and hepato-gastroenterology units between August 1, 2013 and February 30, 2014. After explaining the aims of the study and the methods to be utilized, oral consent was obtained from the patients. All patient data were reported without divulging personal health information.

2.2. Methods

2.2.1. Data collection

Socio-demographic and clinical data were collected from the records of hospitalized patients. The variables analyzed included age, gender, marital status, vaccine immunization status, aspartate aminotransferase level (AST), alanine aminotransferase level (ALT), alpha fetal protein level (AFP), HBsAg, and HBV genotype.

2.2.2. Blood sample collection and processing

Blood samples collected in dry tubes were centrifuged and aliquots were frozen at -40 °C at the bacteriology-virology laboratory of the NHN. Aliquots of HBsAg-positive samples were forwarded to Marseille for further analysis.

2.2.3. HBsAg testing

HBV surface antigen (HBsAg) detection was performed at the NHN bacteriology-virology laboratory using the Virus Combo Rapid test (Abon Biopharm, Co. Ltd, Hangzhou, China). HBsAg-positive serum samples were sent to the bacteriology-virology laboratory of Timone University Hospital in Marseille, France.

2.2.4. Determination of HBV genotype and amino acid patterns based on the HBsAg/reverse transcriptase (RT) encoding gene

DNA extraction, amplification and direct sequencing of the full-length HBsAg and the reverse transcriptase (RT) genes were performed at the bacteriology-virology laboratory of Timone university hospital using in-house protocols as previously described.[13] Sequencing was performed using the BigDye Terminator Cycle sequencing kit v1.1 (Applied-Biosystems, Branchburg, NJ, USA) on the ABI Prism 3130 genetic analyzer (Applied-Biosystems). HBV genotypes were determined by phylogenetic analysis using published HBV sequences available from the genotyping reference set available on the NCBI website (http://www.ncbi.nlm.nih.gov/projects/genotyping/view.cgi?db=2). Sequence alignment was generated using the MUSCLE software[14] and phylogeny reconstruction was performed by the MEGA v.5 software;[15] evolutionary history was inferred using the neighbor-joining method and evolutionary distances was determined using the Kimura 2-parameter method. The HBsAg/RT nucleotide sequences obtained were translated into amino acid sequences, aligned and compared with HBV sequences of the same genotype available in the NCBI genotyping reference set using the Microsoft Excel software program. Amino acid patterns were analyzed using the HBV tools from the HIV grade website (http://www.hiv-grade.de/cms/grade/explanations/hbv-tool/). In addition, reverse transcriptase or HBsAg sequences obtained from sub-Saharan blood samples were retrieved from GenBank using the following keywords: "hepatitis B"[Ti] AND ("hepatitis B surface antigen"[Ti] OR "polymerase"[Ti] OR "reverse transcriptase"[Ti] OR "complete genome"[Ti]) AND (Africa OR Algeria OR Angola OR Benin OR Botswana OR Burkina Faso OR Burundi OR Cameroon OR Cape Verde OR Central African Republic OR Chad OR Comoros OR Congo OR Congo Democratic Republic OR Cote d'Ivoire OR Djibouti OR Egypt OR Equatorial Guinea OR Eritrea OR Ethiopia OR Gabon OR Gambia OR Ghana OR Guinea OR Guinea-Bissau OR Kenya OR Lesotho OR Liberia OR Libya OR Madagascar OR Malawi OR Mali OR Mauritania OR Mauritius OR Morocco OR Mozambique OR Namibia OR Niger OR Nigeria OR Rwanda OR Sao Tome & Principe OR Senegal OR Seychelles OR Sierra Leone OR Somalia OR South Africa OR South Sudan OR Sudan OR Swaziland OR Tanzania OR Togo OR Tunisia OR Uganda OR Zambia OR Zimbabwe).

3. Results

3.1. Epidemiological and clinical observations

A total of 31 serum samples from 31 different patients tested positive for HBsAg. The patients included 17 men and 12 women (sex ratio, 1.3) whose mean age was 42 years [range, (21, 70)] (Table 1); 23 were married and 7 were single, and none reported having received the HBV vaccine. The clinicians who were taking care of these patients were hepatogastroenterologists (for 13 patients); infectious diseases specialists (for 9 patients) and internists (for 9 patients). End-stage liver disease including cirrhosis and hepatocarcinoma was detected in 17 patients (61%), was absent in 11 patients and was not documented in 3 patients. Transaminase measurement in 22 patients showed a mean AST of 40 IU/ml [range, 8-98 (usual values: 4-45 IU/L)] and a mean ALT of 47 UI/ml [range, 10-105 (usual values: 5-45 UI/L)]. AFP levels were found to be elevated in 5 of the 6 patients evaluated (509 ng/ml, 456 ng/ml, 514 ng/ml, 89 ng/ml, 65 ng/ml; normal values < 10 ng/mL).

3.2. HBV genotypes

Of the 23 HBV sequences obtained from these 31 serum samples, 21 were classified as belonging to genotype E, one was clustered with an A3/E recombinant (GH2537) described in Ghana in 2000 (GenBank identifier (GI): 255653196)[16] and the last one was clustered with a D/E recombinant form isolated in Niger in 2009 (isolate bne442; GI: 283466950)[12] (Figure 1).

Overall, mean (± standard deviation (SD)) nucleotide identity between the 23 HBV sequences characterized in this study and their best match available in GenBank was 96.8±2.2% (range, 91.3-99.8). The mean nucleotide identity of the 21 genotypes E HBV sequences with their best match in GenBank was 98.8±0.6% (97.7-99.8) and mean identity between each other was 97.7±0.08 (95.2-99.8). Among

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