International Scholars Journals

African Journal of Virology Research ISSN 2756 - 3413Vol. 15(1), pp. 001-002, May, 2021. Available online at www.internationalscholarsjournals.com© International Scholars Journals

Short Communication

Division of hepatitis B virus genotypes in 50 patients with chronic hepatitis B in Abidjan–Côte d'Ivoire

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Accepted 13 May, 2021

Hepatitis B virus (HBV) strains isolated worldwide have been classified into eight genomic groups deduced from genome comparisons and designated as genotypes A to H. In order to investigate the prevalence of HBV genotypes in Abidjan-Cote d'Ivoire, a total of 50 chronic hepatitis B patients from two teaching hospitals were enrolled. Patients who were HBs Ag positive in serum at least for 6 months, who had HBV- DNA in serum and elevation of ALT levels more than 1.5 times upper limits of normal, and who had percutaneous liver biopsy within 6 months were included and genotyped. Three genotypes (A, B and C) out of total eight reported genotypes so far were identified; genotype A was the most predominant in this collection appearing in 22 samples (55%). Mixed genotypes were seen in 12 (25.3 %) HBV isolates. Ten of these were infected with genotypes AB whereas one with genotypes BC. One patient was infected with 3 genotypes (A/B/C).

Key words: Hepatitis B virus, Genotypes, Nested-PCR, Cote d'Ivoire.

INTRODUCTION

Chronic hepatitis B virus (HBV) infection affects 350 millions people worldwide. HBV strains isolated world-wide have been classified into eight genotype; A to H. Epidemiological studies suggest that these serotypes are common in different parts of the world with various frequencies (Halfon et al., 2002) . Data on the distribution of genotypes for patients with chronic hepatitis B are limited in Africa in particularly in Cote d'Ivoire. The present study aims to investigate the prevalence of distribution of genotypes in Abidjan, Cote d'Ivoire.

MATERIAL AND METHODS

Patient selection

Fifty adults patients with chronic HBV infection who were admitted in two teaching hospital of Abidjan (CHU Yopougon and CHU Cocody), in 2003, were prospectively included into the study. The patients included in this study were 16 to 65 years old. They were HBS Ag positive for at least a six months period and their sera were HBe-Ag or anti-HBe and -HBV DNA positive. Their serum alkaline aminotransferase levels were 1.5 fold or more than normal range upper limit, at least for a six months period. Patients with chronic active hepatitis in percutaneous liver biopsy but that had no previous antiviral therapy were also included in the study.

Biochemistry and serologic markers

Liver biochemical tests, ALT (0-46 IU/L), aspartate aminotransferase (AST) (8-46 IU/L), and gamma-glutamyl -transpeptidase (GGT) (7-49 IU/L) were measured in all patients at initial examination, six months and during the follow-up. Serological markers HBsAg, hepatitis B e antigen (HBe Ag), anti-HBe, anti-HBc IgG, anti-HBs, anti-HCV and anti-HDV total (Ig M+Ig G) were tested during first evaluation at Pasteur Institute (CI) by commercially available kits (MONOLISA, Biorad Laboratories).

Determination of HBV genotypes

Serum samples from each subject were stored at -70°C. The nucleic acids were extracted from 100 µl serum samples using phenol chloroform. The resulting pellet was resuspended in RNase-free water and then subjected to nested PCR. HBV genome was amplified by nested PCR using first the universal primers (P1 and

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S1-2), followed by two different mixtures containing type-specific nested primers (Naito et al., 2001). The first PCR was carried out in a tube containing 40 µl of a reaction buffer made up of the following components: 50 ng of each primer, a 200 µM concentration of each of the four deoxynucleotides, 1 U of Ampli Taq Gold DNA polymerase (Perkin-Elmer, Norwalk, Conn.), and 1 x PCR buffer containing 1.5 mM MgCl2. We used Ampli Taq Gold DNA polymerase to obtain an automatic hot- start reaction. The thermocycler (Gene Amp PCR system 9600; Perkin-Elmer) was programmed to first incubate the samples for 10 min at 95°C, followed by 40 cycles consisting of 94°C for 20 s, 55°C for 20 s, and 72°C for 1 min. Two second round PCR were performed with 1 µl aliquot for each sample of the first reaction, with the common universal sense primer (B2) and mix A for types A to C and the common universal antisense primer (B2R) and mix B for types D to F. These were amplified for 40 cycles with the following parameters; at 95°C for 10 min, 20 cycles of 94°C for 20 s, 60°C for 20 s, and 72°C for 30 s. Genotypes of HBV for each sample were determined by identifying the genotype-specific DNA signals. The two different second – round PCR products from one sample were separately electrophoresed on a 3% agarose gel, stained with ethidium bromide, and evaluated under UV light. The sizes of PCR products were estimated according to the migration pattern of a Smart ladder SF (Pharmacia Biotech, Uppsala, Sweden).

Statistical analysis

Differences between groups were examined by X2 test, Fisher's exact test. A P value of less than 0.05 was considered to be significant.

RESULTS AND DISCUSSION

The study included 50 patients with chronic hepatitis B, 19 males (38 %) and 31 females (62 %). The mean age was 32.1 ± 8.9 years. Three genotypes (A, B and C) out of total eight reported genotypes so far were identified. However, the genotypes of 9 (18%) samples could not be determined with the present genotyping system. Genotype A was the most prevalent appearing in 22 samples (55%) genotype B in 7 samples (17.1%). Mixed genotypes were seen in 12 (29.2 %) HBV isolates. Ten of these were infected with genotypes A/B whereas one was with genotypes B/C. One patient was infected with 3 genotypes (A/B/C). Comparison DNA detection versus Ag HBe and Ac anti-HBe positive seen that HBV DNA was present in 29 sera (72.5%) with Ac anti HBe positive and in 11 sera (27.5%) with Ag HBe positive.

The implement of mass HBV immunization program is recommended by the WHO since 1991, and has dramatically decreased the prevalence of HBV infection in many countries. Genotype A was predominant in Cote d'Ivoire as Southern Africa and Europe (Micalessi et al., 2005; Hannoun et al., 2005). But genotypes D and E were not detected as in East Africa (Kew et al., 2005). Mixed genotypes AB, BC and ABC were also identified. In Taiwan, authors identified high prevalence of mixed genotypes in 325 HBV intravenous drugs users (Chen et al., 2004) . We used nested PCR method as described by Naito et al. (2001). This nested PCR method was limited by the detection of only six major genotypes A to F. In this study, genotyping failed in nine samples; these may probably be genotypes G and/or H.

In this study, 29 (72, 5 %) patients with anti-Hbe positive, have also HBV DNA. Pena Lopez, in Spain identified that one hundred patients (92.6%) were HbeAg-negative and 90 (83.3%) had detectable viral DNA (Pena-Lopez et al., 2005). These results revealed the poor correlation between serological indirect marker of viral replication and detection of DNA. It is preferable to assess hepatitis B infection by detection of HBV DNA. The clinical significance of the different genotypes isolated should be further evaluated.

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