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Brief communication

Hepatitis B virus in the State of Alagoas, Brazil: genotypes characterization and mutations of the precore and basal core promoter regions

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ABSTRACT

The aims of this study were to investigate the genotypes of hepatitis B virus and to identify the precore G1896A and basal core promoter A1762T/G1764A mutations in HBsAg and anti-HBc-positive patients. Eighty-three asymptomatic individuals, three with acute hepatitis B and 33 with chronic hepatitis B referred to viral hepatitis centers in the State of Alagoas, Brazil were analyzed according to their viral load, HBeAg/anti-HBe profile and alanine aminotransferase serum level. The genotypes identified were: A (92.5%), C (5%), D (1.25%) and F (1.25%). The precore mutation was detected in 3.8% of sequences and basal core promoter mutation in 52.4%. These were identified in 45.45% of the asymptomatic individuals and 54.55% of the patients with chronic hepatitis, irrespective of viral load and alanine aminotransferase serum level. In genotype C, only the basal core promoter mutation was identified and no mutations were identified in genotypes D and F.

The hepatitis B virus (HBV) has a circular DNA genome with about 3200 nucleotides (nts), which consists of a full-length of negative strand and a shorter positive strand.[1] The lack of a revision mechanism involving DNA polymerase activity during the process of reverse transcription of pre-genomic RNA favors the appearance of specific mutations.[2]

HBV has been classified into ten genotypes (A–J). In Brazil, the genotype distribution is heterogeneous, reflecting the diversity of the Brazilian population. Type A is predominant,[3] while type F is most frequent among the indigenous population of the Amazon region.[4] Types B and C have been described most frequently among populations of Asian ethnicity,[5] while type D has been described among individuals of Italian descent, most frequently in the southeastern, southern and central-western regions of the country.[5,6]

The transition from guanine to adenine in the nucleotide 1896 creates a premature stop codon in the precore region that prevents synthesis of the HBV e antigen (HBeAg), thereby

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reducing virus replication.[6] On the other hand, the presence of thymine in nt 1858, forming a base pair with the adenine in nt 1896, makes the encapsidation signal of the pre-genomic RNA stronger. This increases the efficiency of the replication, despite the presence of anti-HBe. The genotypes B, C, D and E have thymine in nt 1858, F has cytosine or thymine and A generally has cytosine. This explains the greater frequency of this mutation in the genotypes B, C, D, E and F.[7]

Mutations in the basal core promoter (BCP) region (A1762T and G1764A) have been described independently of seroconversion of HBeAg and are common in different genotypes.[8]

The aims of this study were to investigate the genotypes of HBV and the presence of precore and BCP mutations among HBsAg and anti-HBc-positive patients, evaluated according to their viral load, HBeAg/anti-HBe profile and alanine aminotransferase (ALT) serum level.

Between September 2006 and October 2007, 119 patients referred to viral hepatitis centers in the State of Alagoas (74 men and 45 women) who were serologically positive for HBsAg and anti-HBc were selected. There were 83 asymptomatic individuals, three with acute hepatitis B, and 33 with chronic hepatitis B, with histological confirmation.

The patients signed a free and informed consent statement and filled out a questionnaire to provide demographic data. Blood was collected by means of venous puncture, without anticoagulant, in order to obtain serum.

This study was approved by the Research Ethics Committee of the Federal University of Alagoas (UFAL), in accordance with research protocol no. 000906/2005-98, on March 16, 2005.

ALT was assayed using a commercial kit in accordance with the manufacturer's instructions (Abbott Laboratories, Brazil). Assays were considered normal when the concentration was less than 1.5 times the normal upper limit (55 U/L) and high when it was greater than or equal to 1.5 times the normal upper limit.[9]

The HBeAg/anti-HBe markers were investigated by means of a commercial immunoenzymatic assay (Symbiosis Diagnóstica Ltda, São Paulo, Brazil).

Extraction of the viral genome and partial amplification of the S gene and precore region were performed in accordance with the protocol of Sitnik et al.[5]

The viral load was determined in accordance with the protocol for extraction followed by amplification of the core region of the virus genome that were developed by Kaneko et al.[10]

Analysis of the genotypes was performed by comparing the sequences obtained with the sequences for different HBV genotypes deposited in Genbank. The classification of the genotypes was confirmed using the genotyping tool available from the NCBI website (http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi). Mutations were identified by aligning the nt sequences obtained from the study compared with a sequence for the wild virus. For these analyses, the EditSeq and Megalign programs from the DNAstar software package were used (Lasergene Inc., Madison, WI, USA).

Results and discussion

In Brazil, the literature on the genotypes A, D and F is still sparse and, the present study is the first to describe genotypes and HBV mutations among patients from Alagoas, Brazil.

Among the 119 patients, the mean age was 35 ± 10.9 years and Asian ethnicity with 2.5% (3/119). The S gene fragment of HBV DNA was detected in 70.6% (84/119) patients and genotypes were identified in 95.2% (80/84) of the sequences. Genotype A was identified in 92.5% (74/80), C in 5% (4/80), D in 1.25% (1/80) and F in 1.25% (1/80). One patient with infection due to genotype A and other two infected by type C belonged to Asian ethnicity.

The greater frequency of infection due to genotype A found in this study has also been identified in Salvador,[11] Pernambuco[12] and the Amazon region.[4] Genotype A is always detected in Brazilian studies and sometimes is predominantly associated with genotype D[6,13] and sometimes with type F.[11,16] However, in Alagoas, genotype A was accompanied by genotype C, emphasizing that the infection due to genotype A was also found in a patient of Asian ethnicity.

The detection of type C was an unexpected finding, considering that this type predominates in Asia and that population of Asian ethnicity living in Alagoas is only 0.1% of the whole population, according to the Brazilian Institute for Geography and Statistics.[14]

The low frequency of genotype D in Alagoas is similar to data from Ribeiro et al.[11] who did not identify any infection by this genotype in 76 patients investigated in Salvador, Brazil. It is predominant in the southern and southeastern regions, which are areas where Italian immigration took place,[6,11,13] and its frequency is lower in the northeastern region.[11,12]

The genotype F did not follow the national distribution pattern, in which infections due to this type are more frequent in the northern and northeastern regions.[4,11]

One of the cases had the infection due to genotype D and another had the type F were identified in asymptomatic individuals with normal ALT serum level. The former was HBeAg negative and had a viral load of 3×10^{10} genomes/mL and the latter was anti-HBe positive and had a viral load 3×10^{2} genomes/mL.

The nt polymorphism that occurs naturally over the course of HBV infections is responsible for the appearance of specific mutations in the viral genome. The precore mutations G1896A/C1899A and BCP mutations A1762T/G1764A are the most frequent types and, for this reason, they have been intensively investigated.[8,15]

The presence of thymine/cytosine at position 1858 was investigated in 31 sequences, of which 26 were genotype A, three were C, one was D, and one was F. It was identified in 50% (13/26) of the sequences of genotype A and in 33.3% (1/3) of the genotype C sequences. Thymine was absent in the genotypes D and F.

The precore mutation G1896A was investigated in 31 sequences and was identified in one patient with chronic hepatitis due to genotype A. This individual presented a viral load of 3×10^{12} copies/mL, high ALT serum level, HBeAg-negative and anti-HBe-positive.

The BCP mutations A1762T/G1764A were investigated in 26 sequences, of which 21 were genotype A, three were C, one was D and one was F. These mutations were identified in 52.4% (11/21) of the infections due to genotype A, which 45.45% (5/11) were asymptomatic individuals and 54.55% (6/11) patients with chronic hepatitis. They were identified in 33.3%

(1/3) of the infections due to genotype C and no mutations were identified in genotypes D and F.

The genotype A generally has cytosine (C) at position 1858, according to the studies by De Castro et al.[16] and Sitnik et al.,[5] who found cytosine in 100% and 90% of the isolates of this genotype, respectively. However, in our study, cytosine was presented in 50% of the isolates. This is close to the frequency of 68.4% found by Rezende et al.[6]

Circulation of the precore mutation G1896A of genotype A is rare and shows a relationship with the presence of cytosine in nt 1858.[5,6,16] In our study, we also found a low frequency of this mutation, although cytosine was present in 50% of the sequences of genotype A. The identification of a precore mutation in one patient with high viral load and anti-HBe positive can be due to replacement of cytosine by thymine in nt 1858, although this substitution could not be identified.

The frequency of the BCP mutation A1762T/G1764A found in genotype A, i.e. 52.4% was comparable to the findings of Sitnik et al.,[5] who detected 62% (31/50). However, this rate differs from the results of De Castro et al.,[16] who found a frequency of 20% (2/10). The frequency of these mutations was similar among asymptomatic individuals and those with chronic hepatitis despite ALT serum level and viral load, and its presence seemed not to interfere with the clinical course of HBV infections.

However, in studies with other genotypes, BCP mutations have been correlated with greater activity and high frequency of chronic hepatitis B. These mutations are considered to be one of the independent predictive viral factors for the risk of developing hepatocellular carcinoma.[16]

In conclusion, in this first molecular study of HBV in Alagoas, we could demonstrate that investigation of HBV genotypes is important to both broadening the information about its geographical distribution and assessing its importance in the virus–host relation. Our results contributed to knowledge regarding the HBV genotypes in the State of Alagoas, Brazil, which can also be a reference for further investigations.

Conflicts of interest

The authors declare no conflicts of interest.

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