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Brief Report

Common HBV Genotype in Southeastern Iranian Patients

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Asymptomatic hepatitis B infection is characterized as a type of hepatitis in which hepatitis B surface antigen is present in the patient's peripheral blood despite the absence of clinical symptoms. Previous studies have shown that a particular genotype may effect clinical manifestations of hepatitis B infection; hence, the aim of the current study was to determine the frequency of hepatitis B virus genotypes among asymptomatic carriers of hepatitis B.

In this experimental study, the plasma samples of 100 asymptomatic carriers were collected and tested for HBsAg and anti-HBs using ELISA. The genotype of hepatitis B virus was determined by the GAP-PCR technique.

The results of this study showed that all samples were positive for hepatitis B surface antigen and anti-hepatitis B core antigen was present in 60 (60%) cases. Our results also indicated that all patients had the D genotype of hepatitis B virus.

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Keywords: Asymptomatic hepatitis B infection • genotype • hepatitis B surface antigen • hepatitis B virus DNA • polymorphism

Introduction

Hepatitis B virus (HBV) is among the most important causes of hepatic infectious diseases in humans.[1] Hepatitis B has a worldwide distribution with a prevalence of 360 million cases of the chronic type of the disease.[1] Based on recent reports, Iran is among those countries with a low frequency of hepatitis B.[1] Sequence analysis of the viral isolates have indicated that the virus genome can be divided into eight separate genotypes, A to H. There is an eight to fifteen percent dissimilarity as well as certain serotype geographic patterns amongst the genotypes.[2] Genotypic determination of HBV is of particular importance in that it is helpful for detection of the virus's origin.[3] Secondly, it is useful for studying the course of evaluating HBV[3] and thirdly, genotype determination also plays a key role in the severity of the disease and its therapeutic process.[4] It is also suggested that a particular genotype may affect clinical manifestations during the course of the disease.[4] For example, patients affected by genotype A have a better prognosis, whereas genotype C is associated with a more severe disease. Genotype B enhances the possibility of hepatic malignancy and genotype D is associated with fulminant hepatitis.[4] Amini-Bavil-Olyaee and colleagues[5] have reported that the only genotype in Iran is genotype D and the other genotypes were not reported. Their study, however, was limited to only two provinces; Tehran and Fars. Therefore, the results of their study cannot be generalized for the other provinces in Iran. In order to shed more light on this subject, our study was designed to determine the frequency of HBV genotypes amongst asymptomatic carriers of chronic hepatitis B patients in Kerman Province, Iran.

Patients and Methods

Subjects

This study was approved by the Ethical

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Committee of Rafsanjan University of Medical Sciences and written informed consents were obtained from all participants.

Peripheral blood samples were collected from 100 asymptomatic carrier patients, three times within a six month period, in 5.5 mL ethylene diamine tetraacetic acid (EDTA) pre-coated tubes. Patients had no clinical and laboratory manifestation of hepatitis B, except serological HBV markers. The samples were centrifuged at 3500 rpm for 4 minutes and the plasma was separated within 24 hours after collection. The plasma samples were stored at -20°C for a maximum of two months or at -70°C in case the analysis took longer to conduct. Acute and chronic hepatitis patients who presented with symptoms such us elevated liver enzymes, elevated direct and total bilirubin, and with clinical symptoms of hepatitis B were excluded from the study.

Detection of serological HBV markers

All samples were screened for hepatitis B surface antigen (HBsAg) by ELISA (Behring, Germany). The anti-hepatitis B core antigen (anti-HBc) screening test was also performed (manual microplate enzyme immunoassay) with a commercial anti-HBc kit (RADIM, Italy).

Extraction of HBV-DNA from plasma samples

Viral DNA was purified from 200 μL of plasma as described in our previous study.[1] Briefly, each sample was incubated at 72°C for 10 minutes and allowed to cooled down at 4°C for 5 minutes in 200 μL proteinase K (200 μg/mL). After phenol/chloroform extraction (1:1), the viral DNA was precipitated with ethanol and the pellet was dissolved in DNase-free, deionized water and stored at -20°C.

HBV genotyping

HBV genotyping was performed as previously described by Amini-Bavil-Olyaee et al.[5] The sequences of forward and reverse primers used in this section were also the same as those mentioned by Amini-Bavil-Olyaee et al.[5] The primers that specifically distinguish genotype D from non-D are based on a specific deletion in the pre-S1 region that occurs naturally in genotype D. This can be a simple and cost-effective method for assessing the molecular epidemiology of HBV, especially in those areas of the world with a high prevalence of genotype D, such as the Middle East and Mediterranean countries. A pair of primers was used[5] at the two sides of the deletion in genotype D of the HBV pre-S1 region in order to discriminate genotypes D from non-D.

Results

Our results showed that all patients had detectable HBsAg at six months and anti-HBc was positive in 60 (60%) cases. Our results also indicated that all patients were positive for the D genotype of HBV. Other HBV genotypes were not seen.

Discussion

Hepatitis B disease can present with different clinical features.[4] The mortality rate of patients with acute hepatitis B is less than 1%. There are 5 – 15% of adults and 85 – 95% of neonates who suffer from the chronic form of the disease.[5] The reason why certain infections become chronic has not yet been fully understood; however, researchers have found that the genetic properties of HBV, including various genotypes may explain the prolonged course of this disease.[6] To date, investigators worldwide are involved in the detection of specific HBV genotypes within their own geographic area in order to determine a possible relationship between these genotypes and various clinical presentations and for epidemiologic studies of this disease.[5] For instance, genotypes B and C are prevalent in the Hunon and Guangxi states of China. In particular, genotype B has been reported with severe liver disease.[7] Genotypes B and C are more common in the northern and southern regions of Guangxi, respectively.[7] In Tunis the most frequent genotypes are A, D, and particularly E.[8] Interestingly, in India[3] and Pakistan[4] which are two neighbors of Iran, the most frequent genotype responsible for hepatitis B is genotype D. However, it is impossible to form a link between genotype D hepatitis B and the asymptomatic carrier state amongst infected Iranian people since all clinical variations of hepatitis B have been reported in Iran. Our unpublished data on occult HBV infected patients has also shown that all patients were infected by the D genotype of HBV. Additionally, other genotypes are also responsible for the carrier state in persons from other countries.[9,10] Therefore, based on our findings the link between the genotypes of hepatitis B and the clinical status of asymptomatic carriers is still weak. The results of

our study are in accordance with similar studies that have been performed in Iran.

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