Saudi Journal of Biological Sciences (2016) xxx, xxx–xxx

King Saud University

Saudi Journal of Biological Sciences

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ORIGINAL ARTICLE

Sequence analysis of sub-genotype D hepatitis B surface antigens isolated from Jeddah, Saudi Arabia

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Received 15 November 2015; revised 16 February 2016; accepted 5 March 2016

KEYWORDS

Hepatitis B virus;
HBV sub-genotypes;
HBV/D;
HBsAg;
Viral isolates;
Population studies

Abstract Little is known about the prevalence of HBV genotypes/sub-genotypes in Jeddah province, although the hepatitis B virus (HBV) was identified as the most predominant type of hepatitis in Saudi Arabia. To characterize HBV genotypes/sub-genotypes, serum samples from 15 patients with chronic HBV were collected and subjected to *HBsAg* gene amplification and sequence analysis. Phylogenetic analysis of the *HBsAg* gene sequences revealed that 11 (48%) isolates belonged to HBV/D while 4 (18%) were associated with HBV/C. Notably, a HBV/D sub-genotype phylogenetic tree identified that eight current isolates (72%) belonged to HBV/D1, whereas three isolates (28%) appeared to be more closely related to HBV/D5, although they formed a novel cluster supported by a branch with 99% bootstrap value. Isolates belonging to D1 were grouped in one branch and seemed to be more closely related to various strains isolated from different countries. For further determination of whether the three current isolates belonged to HBV/D5 or represented a novel sub-genotype, HBV/DA, whole HBV genome sequences would be required. In the present study, we verified that HBV/D1 is the most prevalent HBV sub-genotype in Jeddah, and identified novel variant mutations suggesting that an additional sub-genotype designated HBV/DA should be proposed. Overall, the results of the present *HBsAg* sequence analyses provide us with insights regarding the nucleotide differences between the present *HBsAg*/D isolates identified in the populace of

Abbreviations: HBV, hepatitis B virus; HAV, hepatitis A virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; P, HBV polymerase gene; C/pre C, HBV core/pre Core gene; X, HBV X gene; *HBsAg*, HBV surface antigen; IFN, interferon; *Pre S1*/*Pre S2*/*S*, *HBsAg* genes; PCR, polymerase chain reaction; DDBJ, DNA Data Bank of Japan; EMBL, European Molecular Biology Laboratory.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2016.03.003

Jeddah, Saudi Arabia and those previously isolated worldwide. Additional studies with large numbers of subjects in other areas might lead to the discovery of the specific HBV strain genotypes or even additional new sub-genotypes that are circulating in Saudi Arabia.

1. Introduction

Many nations have a heavy financial load from the high percentage of both individuals with chronic hepatitis B virus (HBV) infection and the approximately 400 million HBV carriers worldwide. HBV infection leads to the development of severe liver diseases including cirrhosis and hepatocellular carcinoma (HCC) (Norder et al., 1992, 1994). Human HBV is the prototype member of the family *Hepadnaviridae* that contains a circular, partially double-stranded DNA genome of about 3200 bp. HBV DNA contains four overlapping open reading frames encoding the pre S1/pre S2/S, pre C/C, pol, and X viral proteins (Fig. 1) (Magnius and Norder, 1995).

Compared to most DNA viruses, HBV has a high rate of nucleotide substitution although this is lower than the mutation rate of RNA viruses (Okamoto et al., 1987). Previously, HBV genomes were classified into four genotypes, but recently have been categorized into eight genotypes designated as A-H. This categorization was based on inter-genotype divergence of at least 8% of the complete nucleotide sequence or on more than 4% divergence in the *HBsAg* gene (Arauz-Ruiz et al., 1997; Okamoto et al., 1987). These genotypes possess different geographical distributions as illustrated in Fig. 2 (Arauz-Ruiz et al., 1997; Chu et al., 2003; Miyakawa and Mizokami, 2003). Recently, a complex recombinant of genotypes A, C, and G has been referred to as representing a new genotype (I), which was described and sequenced in northwestern China, Vietnam, and Brazil (Santos et al., 2010).

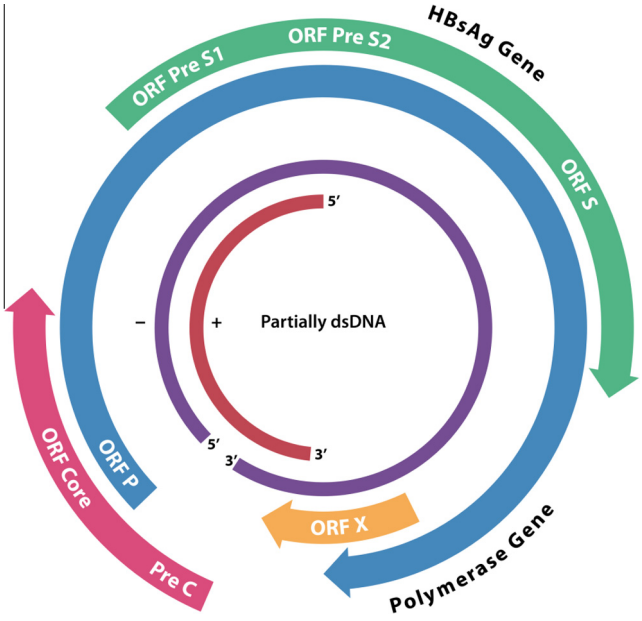

Figure 1 Genome organization of HBV. The colored arrows indicate overlapping genes: (ORF P) polymerase gene; (ORF C/pre C) core/pre core gene; (ORF X) X gene and (ORF Pre S1/ORF Pre S2/ORF S) *HBsAg* gene (Jalali and Alavian, 2006).

In addition, sub-genotypes have been described in certain HBV genotypes; these are A1-A6 in genotype A (HBV/A), and B1-B8, C1-C16, and D1-D8 in HBV/B, C, and D, respectively (Abdou Chekaraou et al., 2010; Mulyanto et al., 2009).

HBV genotypes show a direct correlation with the severity of liver disease. HBV/C, HVB/D, and HBV/B are associated with severe liver cirrhosis (Banerjee et al., 2006; Huy et al., 2006; Kramvis et al., 2008; Sakamoto et al., 2006) and appear to have a higher incidence of HCC (Chan and Sung, 2006; Liu et al., 2007; Liu et al., 2011; Sumi et al., 2003; Yuen et al., 2003, 2009). Additionally, patients with either HBV/C or HBV/D have a lower response rate to treatment with interferon (IFN)γ when compared with those carrying other HBV genotypes (Zöllner et al., 2001). The HBV genotype might also influence the emergence of lamivudine resistance mutations, which appear to be more strongly associated with HBV/A than HBV/D (Wen, 2004).

HBV surface antigen (HBsAg) can induce protection against HBV infection as it is related directly to B-cell epitopes and is considered as the major target of neutralizing antibodies; therefore, it is used an HBV vaccine (Kramvis et al., 2005). The complete *HBsAg* gene consists of three regions: *large S*, *Pre S2*, and *Pre S1*, which share their C-terminal 226 amino acid residues (Carman, 1997; Szmuness et al., 1981). Mutant *HBsAg* nucleotides might cause amino acid substitutions (EL Hadad et al., 2013), which could affect the binding of specific anti-HB antibodies and the detection by conventional diagnostic assays (Torresi, 2002). In addition, a relationship has been observed between low antigenicity of HBV (leading to HBV reinfection) and the increased incidence of HCC in Egyptian patients with chronic HBV (Tian et al., 2007). Furthermore, correlations between mutations in the *HBsAg* gene, particularly in the *Pre S* regions, and the development of HCC have been verified in patients with chronic HBV (Yang et al., 2010).

The prevalence of HBV infection is generally high in Asian and African countries (Lee, 1997). Saudi Arabia reported HBV as the most predominant type (53%) of hepatitis infection followed by HCV (30%) and HAV (17%) (Alshabanat et al., 2013). Furthermore, the Jeddah province of Saudi Arabia hosts large numbers of HBV infected individuals originating from countries with a high-HBV burden because Jeddah is a main entry point as well as being the largest commercial port in the country. For these reasons and because little is known regarding the *HBsAg* genotypes/sub-genotype sequences circulating in Saudi Arabia, and in particular in the Jeddah region (Al-Faleh et al., 1992; 1999), the present study was conducted to provide sequence data of the most common *HBsAg* genotypes/subgenotypes circulating in Jeddah for subsequent phylogenetic analysis.

2. Materials and methods

2.1. Patient samples

Serum samples were collected from 17 Saudi patients with chronic HBV (5 women and 12 men, mean age 32.7 years)