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BRIEF REPORT

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Prevalence of restriction fragment length polymorphism patterns of hepatitis B virus compatible with genotype D in Lebanon

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In a national study, 167 Lebanese patients with various manifestations of hepatitis B virus (HBV) infection were investigated to determine the prevalence of HBV genotypes in Lebanon. The patients were seen at nine medical centers throughout the country from June 2002 to August 2003. Serum HBV DNA was detected in all cases, and the overwhelming majority of the patients (97%) were anti-HBeAg-positive. Genotyping revealed 166 samples as genotype D, while only one sample was non-typeable. Thus, genotype D seems to be the predominant HBV genotype in Lebanon and possibly in the region.

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Despite significant progress in vaccine development, infection with HBV remains a major health problem leading to 1–2 million deaths annually worldwide [1]. A wide range of manifestations has been established for chronic HBV infection ranging from asymptomatic carriage to cirrhosis and hepatocellular carcinoma [2]. Currently, HBV can be classified into seven genotypes, A through G, based on an intergroup divergence of 8% or more in the nucleotide sequence, and recently an eighth genotype, H, has been described [3]. Various HBV genotypes are found in different geographic areas [4, 5].

Recent studies have demonstrated that individual HBV genotypes may be related to severity of liver disease and response to antiviral treatment [6]. However, the results of studies on the clinical significance of HBV genotypes have not been consistent. For example, one study suggested that genotype B is associated with an increased risk of hepatocellular carcinoma while other studies have refuted this finding [7]. Moreover, almost all Asian studies on HBV genotypes and liver disease were restricted to comparing genotypes B and C, while in Europe, most patients with genotype A have chronic hepatitis while most patients with genotype D are reported to have acute hepatitis [8].

Lebanon is considered to be moderately endemic for hepatitis B with an overall HBsAg carrier rate of 2.2% [9]. To date, there are no data on the prevalence and clinical significance of HBV genotypes in Lebanon. Thus, the aim of this national study was to determine the prevalence of HBV genotypes and to study the association between these genotypes and the clinical status of HBV-infected patients in the Lebanese population.

One hundred sixty-seven individuals (123 male, 64 female) with HBV infection were seen at nine medical centers in Lebanon and included in our cross-sectional national study between June 2002 and August 2003. The

mean age of the patients was 42±12 years. A questionnaire designed to gather demographic, clinical and laboratory data was completed for each individual. The demographic data included sex, age, place of birth and travel history. The clinical information included mode of presentation (asymptomatic, symptoms of chronic liver disease), presumed source of infection (sexual, parenteral, other), liver histology and treatment. The manifestations of HBV infection in the 167 individuals were as follows: 46 of the patients were asymptomatic carriers in whom infection was discovered following blood donation, 82 had symptomatic chronic hepatitis, 24 had cirrhosis, and 15 had hepatocellular carcinoma.

All patients were HBsAg-positive. HBeAg and antibody to HBeAg were tested at the Molecular Virology Laboratory, Faculty of Health Sciences, American University of Beirut using a commercially available enzyme-linked immunosorbent assay (VIDAS HBe/Anti-HBe; bioMérieux, Boxtel, The Netherlands). DNA extraction was performed using the QIAmp DNA Blood Mini Kit (Qiagen, Chatsworth, CA, USA) according to the manufacturer's instructions. HBV genotyping was performed using PCR amplification and restriction fragment length polymorphism analysis of the fragment of the HBV genome between nucleotide positions 256 and 796 in the S region of the HBV genome, as described by Lindh et al. [15], with minor modifications. In brief, 10 μl from the extracted DNA was used for the PCR reaction using primers P7 (sense primer: 5′-GTG GTG GAC TTC TCT CAA TTT TC) and P8 [antisense primer: 5′-CGG TA (A/T) AAA GGG ACT CA (A/C) GAT]. Amplified DNA was digested using *Tsp509I* (New England Biolabs, Beverly, MA, USA) and *HinfI* (Amersham Biosciences, Uppsala, Sweden). Resulting DNA fragments were separated by electrophoresis in 3% agarose gel, stained by ethidium bromide, and the restriction pattern was read according to the description of Lindh et al. [10]. Three restriction patterns representing genotype D were obtained. The fragments created by *Tsp509I* and *HinfI* were typical of D_1, D_2 and D_6. Genotypes B and C were included in all runs as controls. All genotype B strains have a unique *Tsp509I* site yielding a characteristic 316 bp fragment. As for genotype C strains, they have a *HinfI* site at nt 633 producing a unique pattern.

Serum HBV DNA was detected in all cases and a 541 bp fragment was obtained using primers P7 and P8. Genotyping revealed 166 samples as genotype D and only one sample was non-typeable (Fig. 1). The results were confirmed by a second expert PCR technologist who tested 25 randomly chosen samples. Nucleotide sequencing was performed using the Applied Biosystems DNA sequencer 3100 (Perkin Elmer Corp., Foster City, CA, USA) on 5% of our samples, which were randomly selected. Sequencing results were comparable with published sequences of HBV genotype D. Five (3%) of our patients were positive for HBeAg and 162 (97%) were positive for anti-HBeAg. Of the five HBeAg-positive patients, three were relatively young (30–34 years old) blood donors, and all had a history of traveling abroad within a year preceding blood donation; however, none reported any known risk behavior leading to HBV infection. The other two HBeAg-positive patients had liver cirrhosis.

Host-related factors, such as age and time of infection, environmental factors, such as alcohol abuse, and the emergence of HBV mutants have all been identified as factors that may influence the long-term outcome of chronic HBV infection [1]. Recently, studies from different parts of the world have demonstrated the increasing clinical relevance of HBV genotypes. For example, in cross-sectional, case-controlled and prospective studies convincing lines of evidence have appeared indicating that genotype C is significantly more closely associated with severe liver disease than genotype B. In patients in Western countries, where genotypes A and D are frequent, the long-term outcome of chronic HBV infection is different than in patients infected with genotypes A, D or F. Clearance of HBV DNA has been noted to occur at a higher rate in patients infected with genotype A than in patients with genotype D or F, and death related to liver disease is more frequent in patients with genotype F than with the other genotypes [8].

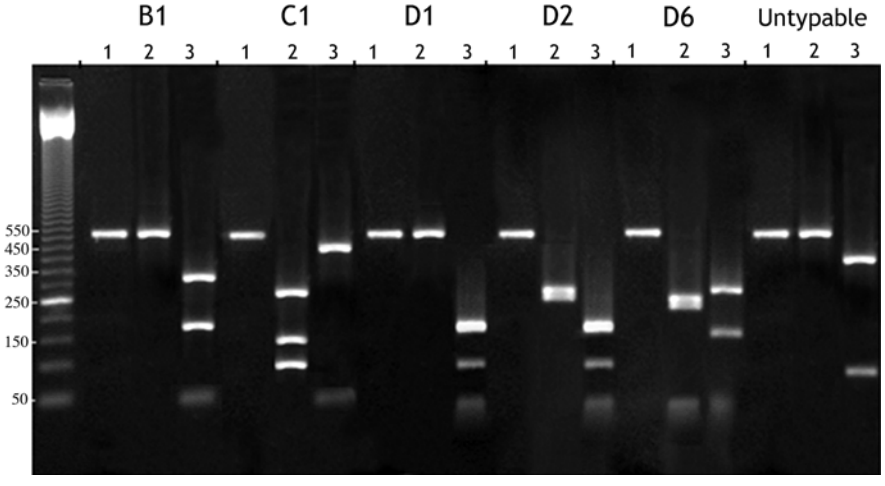

Fig. 1 Restriction fragment length polymorphism patterns of hepatitis B virus representing genotypes B and C as controls and genotype D and the untypeable pattern from positive chronic carriers. Each sample was run in three lanes: *lane 1*, untreated PCR product; *lane 2*, incubated with HifI; *lane 3*, incubated with *Tsp509I*

To the best of our knowledge, our national study is the first of its kind to be conducted in Lebanon or the surrounding region, and the results clearly show a predominance of HBV genotype D. Only 1 of our 167 patients was infected with a HBV genotype that could not be confirmed as D, but since this finding did not affect the results, further classification of this case was not attempted. Similar to our results, HBV genotype D was also the sole genotype found in studies conducted in Egypt [11] and Turkey [12]. Therefore, genotype D seems to be the predominant HBV genotype in Lebanon and the Middle East. The overwhelming majority of our patients (>97%) were anti-HBe-positive, which reflects the findings of other studies in which patients infected with HBV genotype D had high anti-HBe positivity [7]. Seroconversion from HBeAg to anti-HBe is triggered, most commonly, by the stop codon mutation in the precore region (G 1896A). HBV genomes of genotypes A, C and F possess cytosine at position 1858 that makes a Watson–Crick pair with G at position 1896. Hence, seroconversion to antibody to HBeAg is delayed or hindered in individuals who carry C 1858 and G 1896. This is in contrast to strains of genotype B, D or E, in which C 1858 is rarely, if ever, present.

The homogeneity of the HBV genotype in our population indicates that other HBV genotypes have not been introduced to Lebanon despite the country's long history of immigration. Because of this homogeneity, we were unable to study the influence of various genotypes on the severity of liver disease in our patients. Moreover, in most of our cases, the route of HBV transmission as well as the time of first exposure to the virus could not be identified, and there were no reliable data on the duration of infection before enrolment in the study. These factors, which are of relevance in elucidating the national history of chronic HBV infection, could not, unfortunately, be evaluated in this study.

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