

## Molecular Analysis of Hepatitis B Virus Isolates in Libya: Predominant Circulation of Hepatitis B Virus Genotype D

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### INTRODUCTION

Hepatitis B virus (HBV) infection is one of the most important infectious diseases worldwide and is a major global health problem. Approximately one million people die annually because of acute and chronic HBV infection despite the availability of effective vaccines and antiviral medications<sup>[1]</sup>. HBV is the prototype member of the Hepadnaviridae family; it is a circular, partially double-stranded DNA virus of approximately 3 200 nucleotides. This highly compact genome contains four open reading frames (ORF) encoding the envelope (PreS1, PreS2, S), core (core, precore), polymerase, and X proteins<sup>[2]</sup>. HBV has been classified into nine genotypes (A-I) according to the sequence divergence of the entire genome of >8%. These genotypes have distinct geographical distribution. In general, genotype A is pandemic and most prevalent in North West Europe, North America, Central Africa and India. Genotypes B and C are prevalent in Eastern Asia and the Far East origin. Genotype D is predominant in the Mediterranean area and the Middle East, Central Asia and South America<sup>[3]</sup>. Genotype E is restricted to West Africa<sup>[4]</sup> and genotype F is found in Central and South America<sup>[5]</sup>. Genotype G has been identified in France and North America<sup>[6]</sup> and genotype H has been described in Mexico and Central America<sup>[7]</sup>, while genotype I has been recently identified in Vietnam and Laos<sup>[8]</sup>. There have been growing lines indicate clinical relevance of HBV genotypes. HBV genotype C is more closely associated with severe liver disease. Genotype D is a significant association with a high rate of developing hepatocellular carcinoma, genotype A is associated with early seroconversion to anti-HBe. A Swiss study demonstrated that progression from acute to chronic hepatitis is more likely with genotype A than with D. Another report from Japan suggested that genotype A more often induced chronic hepatitis than genotypes B and C. Genotypes B and D are associated with lower prevalence of HBeAg than genotype A. In HBeAg-negative patients, HBV DNA level is higher in patients with genotype D. The response to interferon alpha

### ABSTRACT

**AIM:** To determine prevalence of hepatitis B virus (HBV) genotypes and HBV variants among Libyan patients.

**METHODS:** HBV DNA levels were determined using RoboGene® HBV Quantification kit (AJ Roboscreen GmbH, Germany) in a total of 121 HBV isolates. HBV isolates with higher DNA levels were amplified by PCR, but only 60 isolates tested for genotypes by line probe assay (INNO-LiPA HBV Genotyping; INNOGENETICS NV, Ghent, Belgium). Precore and core promoter variants in 39 of the 60 HBV isolates genotyped were also performed by line probe assay (INNO-LiPA HBV PreCore; INNOGENETICS NV, Ghent, Belgium).

**RESULTS:** Overall, HBV genotype D was found in 54 (90%) out of the 60 isolates studied. HBV genotypes A, and E were found in 1 (1.7%) isolate for each. In the remaining 4 isolates the results were suggestive of mixed or recombinants of HBV genotypes D and E. In the precore region, all of the 39 isolates investigated were genotype D and showed mixed wild type (G1896) and precore mutant (G1896A). The combination of wild type (A1762) and (G1764) of the core promoter was found in 30/39 (77%) of the isolates. The variant profiles (A1762T) and (G1764A) or (A1762) and (A1764) were detected in 6 (15%) and 3 (8%) of the isolates, respectively.

**CONCLUSION:** HBV genotype D was the most prevalent HBV genotype in Libya. High circulation of mixed wild type and precore mutant are common in HBV genotype D in Libya.

(IFN) is higher in patients with genotype A than genotypes D or E, likewise, is better in patients with genotype B than genotype C<sup>[9]</sup>. Mutations in the precore and core promoter regions of HBV have been also related with genotypes and may play an important role in the clinical outcome of HBV infection. Precore and core promoter mutations have been observed mainly in HBV genotypes D and B, as well as A and C<sup>[10]</sup>, respectively.

Libya has an intermediate prevalence of the HBV infection, the HBsAg carriers rate reaching around 2.2%<sup>[11]</sup>. Because studies on the molecular aspects of HBV infection in the country remain very limited, this study investigated for HBV genotypes and precore and basal core promoter mutations in Libyan patients with chronic HBV infection.

## MATERIALS AND METHODS

### HBV isolates and DNA detection

The study was approved by the biomedical ethics committee of Saint James Hospital and informed consent was received from patients before plasma samples were obtained from 121 HBV infected Libyan patients, 79 males and 42 females, aged 15-66 years. HBV DNA levels were determined using RoboGene® HBV Quantification kit (AJ Roboscreen GmbH, Germany), then isolates with higher DNA levels were extracted using Wizard® SV genomic DNA purification system, Promega, USA, as recommended by the manufacturer.

### HBV genotyping

The extracted DNA was amplified with genotyping primers by nested PCR according to the instructions of the manufacturer (INNOGENETICS N. V., Ghent, Belgium) for amplification of the HBsAg region to provide a biotinylated product. The HBV genomic region amplified extends from nucleotides 415 to 824 for the outer primers and nucleotides 456 to 798 for the nested inner primers (the numbering is based on the sequence with GenBank accession number AY128092). These procedures have been described previously<sup>[10]</sup>. Each stage of amplification was monitored by visualization of the PCR products on a 2% agarose gel. If the first-stage PCR products were visible, they were used for hybridization to the test strips for genotyping; otherwise, the second-stage products were used.

Genotype detection was performed on 60 isolates by (INNO-LiPA HBV Genotyping; INNOGENETICS N. V., Ghent, Belgium) according to the instructions of the manufacturer.

### HBV precore and core promoter mutation characterization

The extracted DNA was additionally amplified with basal core promoter and precore primers by nested PCR as recommended by the manufacturer (INNOGENETICS N. V., Ghent, Belgium) for amplification of parts of basal core promoter and precore regions to provide a biotinylated product.

Characterization of precore mutations was performed on 39 of 60 genotyped isolates by (INNO-LiPA HBV PreCore; INNOGENETICS N.V., Ghent, Belgium) according to the manufacturer's instructions<sup>[12]</sup>.

## RESULTS

### HBV DNA quantity

The HBV-DNA quantity in 85 of 121 isolate were higher exceeding  $5 \times 10^3$  copies/ml of viral DNA.

### HBV genotyping by INNO-LiPA

INNO- LiPA HBV genotyping assay was able to provide HBV

genotypes results for 60/121 HBV isolates with higher DNA levels (Table 1).

HBV genotype D was predominate genotype in this study, HBV genotypes A and E were found in 1 isolates for each, while 4 isolates were demonstrated a mixed pattern possibly indicating infection with both genotypes D and E.

**Table 1** Distribution of HBV genotypes in the populations studied *n* (%).

Genotypes <i>n</i> (%)			
A	D	E	D/E
1(1.70)	54(90)	1(1.70)	4(6.70)

### HBV precore mutations

All of 39 isolates investigated were genotype D. In precore region mixed wild type (G1896) and precore mutant (G1896A) were observed in all isolates (Table 2).

In the core promoter region, 30/39 (77%) isolates had wild type sequence (A1762) and (G1764), 6/39 (15%) isolates showed the classical dual variant (A1762T) and (G1764A), and 3/39 (8%) had an indeterminate sequence (A1762) and (G1764A).

**Table 2** Prevalence of precore and core promoter variants in Libyan HBV genotype D isolates.

Region and variant	<i>n</i> (%)
Precore (nt 1896)	
Wild type+variant (G+A)	39(100)
Core promoter (nt 1762 and 1764)	
Wild type (AG)	30(77)
Dual variant	6(15)
Indeterminate	3(8)

## DISCUSSION

Previous studies suggested that genotype D has a worldwide distribution but prevails in the Mediterranean area and the Middle East. However, although many reports documenting predominance of genotype D in countries from the north side of the Mediterranean Sea<sup>[8]</sup> were published, information on those of the south side is limited or based on very small numbers of patients studied specially in Libya. To our knowledge, this study reporting consistent data on circulating HBV genotypes in a country from North Africa. Furthermore, the genotype distribution that was found in Libya in this study differs from what was previously reported in European Mediterranean countries, genotype D counted for 90% of the patients studied. Beyond the HBV genotype, several studies reported that a precore stop codon mutation at nucleotide 1896, which is also prevalent in Mediterranean region most frequently in association with genotype D<sup>[13]</sup>. More recently, other type of HBV mutants were identified such as mutants in the basal core promoter which remain less frequently studied. The present study describes mixed wild type (G1896) and precore mutant (G1896A) in 39/39 (100%) isolates which are genotype D. While concerning mutations in the core promoter region, it is remarkable that the wild type (A1762, G1764), classical dual variant (A1762T, G1764A), and indeterminate (A1762, G1764A) sequences were observed.

The INNO-LiPA HBV genotyping and precore assays have proved to be sensitive and specific diagnostic method, fast time to result and clear identification of mixed genotypes infections. In addition, HBV genotyping has shown its utility not only in epidemiology studies but also in predicting prognosis and therapeutic response<sup>[14]</sup>.

In conclusion, HBV genotype D is highly predominant in Libyan HBV isolates and genotypes A and E and mixed genotypes D/E also

observed. The frequency of mixed wild type and precore mutant in Libyan HBV isolates is high and associated only with HBV genotype D. Core promoter mutations seem to be low in HBV isolates of Libya.

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