

Hepatitis B virus genotypes and lamivudine resistance mutations in Jordan

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Abstract

AIM: To investigate and identify prevalent hepatitis B virus (HBV) genotypes and to explore lamivudine-resistant mutations among treated and untreated patients in Jordan.

METHODS: A total of 107 cases with chronic hepatitis B were recruited from different medical centers in Jordan. Serological tests were performed for all cases using a microparticle enzyme immunoassay. HBV Genotyping was performed for 70 cases using Line probe genotyping assay. The YMDD mutations were explored for 20 cases (4 were lamivudine naive) using the INNO-LiPA HBV DR assay.

RESULTS: Genotype D was the only detected genotype. A total of 6 YMDD mutations were detected in 5 treated patients (31%) while one mutation was detected in the naive patients. Seventeen percent of cases were positive for HBeAg and had statistically significant higher levels of serum aminotransferases.

CONCLUSION: HBV genotype D appears to be the only circulating type in Jordanian patients. The YMDD mutations were detected in 31% of lamivudine-treated cases with similar patterns to those found in the literature. We also found a relatively low prevalence of HBeAg expression among examined cases (17%). Awareness of these serologic, genotypic and resistance patterns might help in the formulation of management

plans and for predicting clinical outcomes. Further larger scale studies are needed to confirm our results and to examine possible associations among clinical, serologic, and genetic patterns of HBV infections in Jordan.

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Key words: Hepatitis B virus; Genotypes; Lamivudine; YMDD mutation; Jordan

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INTRODUCTION

Infections with hepatitis B virus (HBV) continue to be a worldwide problem^[1] and a considerable proportion of these infections usually progress to chronic infection and hepatocellular carcinoma^[2]. In view of this significant diseases burden, vaccination against hepatitis B emerged as the most cost-effective prevention method^[3]. The available treatments of HBV infections include interferon-alpha (IFN- α) and nucleoside analogue agents (lamivudine, adefovir, entecavir, telbivudine, and others)^[4]. However, responses to these treatment regimens are variable and are still a long way from being perfect. Besides, treatment protocols are associated with considerable risks of evolving resistant mutants^[5]. These hepatitis B mutants can appear in patients as a consequence of the constant selection pressure from either the immune response or treatment choice. In the meantime, classification of HBV has changed from the serologic subtype classification to a more precise genotype genetic classifi-

cation. Hepatitis B virus has been classified into the eight genotypes (A-H) on the basis of nucleotide sequence differences, and these genotypes have typical geographic distributions and may have different pathogenicity and epidemiology^[6]. These genotypes are generated during replication of HBV DNA through an RNA reverse transcriptase intermediary step that lacks proofreading functions^[7,8]. Lamivudine, as a potent nucleoside analogue, has been used for chronic HBV infections therapy. However, the need for long-term courses is associated with emergence of lamivudine-resistant mutations. The most common of these mutations affect HBV polymerase-reverse transcriptase. The most commonly reported mutations are the substitution of either valine (M204V) or isoleucine (M204I) for methionine in the tyr-met-asp (YMDD) motif located in the polymerase active site (domain C), and substitution of methionine for leucine (L180M) in the active site (domain B)^[9,10]. The present study aimed to identify prevalent hepatitis B genotypes and lamivudine-resistant mutations among treated and untreated HBV patients in Jordan.

MATERIALS AND METHODS

Patients and setting

In this cross-sectional study, we recruited 107 patients with chronic hepatitis B from different departments of King Abdullah University Hospital, Jordan University Hospital, and Princess Bada'eh Hospital. All were positive for HBsAg. Genotyping and lamivudine-resistance mutation analysis were performed for 70, and 20 patients, respectively (budgetary restrictions meant we couldn't genotype all the patients). There was no recruitment discrimination made in respect to active or previous lamivudine treatment.

HBV serological markers testing

Twenty ml of peripheral blood were taken and serum samples were aliquoted and stored at -70°C until used. Sera were tested for HBsAg, anti-HBc, anti-HBs, HBeAg, and anti-HBe using the microparticle enzyme immunoassay (Abbott diagnostic laboratories/AXSYM system, v2, 200, Ireland).

HBV DNA extraction

HBV DNA was extracted from the serum using a DNA-sorb-B kit (Sacace Sr biotechnologies company, Italy). DNA was re-suspended in a final volume of 30 µL of sterile, nuclease-free water and then stored at -20°C till used.

HBV genotypes detection

HBV Genotyping was performed using the Line probe genotyping assay (INNO-LiPA HBV Genotyping assay; Innogenetics, Ghent, Belgium). It is a line probe assay designed to identify hepatitis B genotypes A to G by detection of type-specific sequences (328-619 nucleotides) in the HBV polymerase gene (domains B to C), which are overlapped with specific sequences in the HBV surface gene. In summary, the biotinylated PCR

product (after amplification) was denatured by adding denaturation solution at room temperature. It was then hybridized with specific oligonucleotides probes immobilized as parallel lines on membrane based-strips in hybridization solution at 50°C using a water bath. Stringency solution was then added to strengthen the binding between the probe and single stranded denatured DNA. Unhybridized DNA was washed from the strips by addition of rinse solution. Conjugate solution was then added to the strips at room temperature. Finally, a chromogenic substrate was added to the strips. A purple precipitate was observed in the lines, identifying stable hybrids formed between the DNA and the probes.

Amplification of HBV DNA and YMDD mutations detection

Amplification of HBV DNA was as described previously^[9]. YMDD mutations were detected using a lamivudine resistance assay (INNO-LiPA HBV DR; Innogenetics, Ghent, Belgium). The amplified region of the HBV genome is common between the HBsAg gene and the polymerase gene, therefore the same protocol was followed for both amplification and hybridization processes. This assay detects mutations or polymorphisms at codons 180, 204 and 207 of the HBV polymerase gene, in addition to HBsAg-specific codons, due to the overlapping reading frame.

Statistical analysis

Data were processed using the statistical package for statistical science software (SPSS, version 10, Chicago, Inc). Statistical analysis was carried out using Fisher's exact test, Chi-square test, and *t*-test wherever appropriate. A *P* value of < 0.05 was considered significant.

RESULTS

Patients' demographics

We recruited 107 patients (31 female and 76 male). Mean age of cases was 34.1 years (SD = 13). Chronicity was confirmed in all cases with positive results for HBsAg, anti-HBcAg-IgG and negative results for anti-HBsAg-IgM. Sixteen patients had a history of receiving lamivudine (Twelve were actively receiving it, while 4 were off treatment at time of enrolment). None of HBeAg negative cases had hepatitis-related symptoms (jaundice, liver enlargement, and Ascites), while 33% of the HBeAg positive cases had such symptoms (Table 1).

Serological patterns

Most patients did not express HBeAg (83%). However, the mean levels of alanine aminotransferase (ALT), and aspartate aminotransferases (AST) were significantly higher in the HBeAg positive group (121 *vs* 38 and 143 *vs* 37, respectively) (Table 1) and these ALT and AST values were statistically significantly abnormal.

HBV genotyping

Surprisingly and interestingly, the 70 selected samples (positive-HBsAg) were all positive for genotype D. We

Table 1 Clinical and serological patterns of all 107 HBsAg positive patients *n* (%)

Criteria	HBsAg positive	HBsAg negative	<i>P</i>
Number of cases	18 (17)	89 (83)	< 0.0001
Hepatitis-related symptoms	6 (330)	0 (0)	< 0.0001
Positive HBeAb	0 (0)	89 (100)	< 0.0001
Abnormal ALT	18 (100)	35 (39)	< 0.0001
Abnormal AST	16 (89)	12 (13)	< 0.0001
Abnormal ALP	4 (22)	18 (20)	0.85

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase.

did not detect any other genotypes. Detecting no other genotypes in our cohort made it impossible to compare between HBV genotypes and their effects on clinical course of disease or on response to antiviral drugs.

Lamivudine resistance mutations

Lamivudine resistant mutations were tested for 20 cases (4 were lamivudine naive and 16 with a history of lamivudine treatment) (Table 2). Six mutations were detected in 5 different patients with a history of lamivudine treatment (31%). Two mutations (M204Ile and L180M) were detected in one patient. On the other hand, one mutation only (M204V) was detected in a lamivudine naive patient. The mutation M204Ile was the most prevalent and durations of lamivudine treatment ranged from 1 to 13 years.

DISCUSSION

This is the first report from Jordan that investigates HBV genotypes and lamivudine resistance mutations. Our results showed that genotype D is at least the most (if not the only) prevalent genotype in Jordan. This genotype is also the most prevalent genotype worldwide, with more concentration in the Middle East and the regions around as Turkey, Egypt, and Gulf region. Recent studies have found that genotype D accounts for 81%-85% of all genotypes in Saudi Arabia and almost all genotypes in Egypt^[11-13]. This finding might have potential impact on selection of antiviral drugs, prediction of disease courses and clinical responses. There is accumulating evidence that patients with genotype D might achieve higher sustained viral response rate than patients with genotype A^[14], despite being less responsive to interferon treatment when compared to genotypes A and B^[5]. It is also known that that genotype D has a higher likelihood of developing advanced cirrhosis compared to genotype A^[15]. Furthermore, there is evidence that the rate of resistance to lamivudine is lower in patients infected with genotype D than in patients with genotype A^[5]. In our study, the prevalence of lamivudine resistant mutations was 31%. It is difficult to compare this prevalence to other studies because our cases received variable durations of lamivudine treatment. However, this prevalence seems broadly similar to those in other reports showing that there is a 20% yearly chance for resistance

Table 2 Profile of detected YMDD mutations among tested cases

Mutation	Patients with history of lamivudine treatment	lamivudine naive patients
Patients tested	16	4
HBeAg Positive	3	1
M204Ile	4	0
M204V	1	1
L180M	1	0
Total	6	1

to emerge in lamivudine-treated patients. The mutation M204Ile was the most prevalent, followed by the M204V mutation, which is similar to cases reported previously^[16]. We also detected a mutation in an apparently lamivudine-naive case. This might be due to transmission of the virus from a lamivudine-treated index case or, less likely, to a spontaneous mutation. In general, HBV mutations conferring drug resistance to lamivudine are rare in lamivudine naive patients but do exist^[17]. In one of our patients, the M204V mutation was accompanied by the mutation L180M. This combination is also common and has been described before^[18]. Our study has also found a relatively low rate of HBeAg positive patients (17%). This might be explained by the rapid clearance of HBeAg among patients with genotype D demonstrated in previous reports^[12].

In conclusion, HBV genotype D appears to be the only circulating type in Jordanian patients. The YMDD mutations were detected in 31% of lamivudine-treated cases with similar patterns to those in the literature. We also found a relatively low prevalence of HBeAg expression among examined cases (17%). Awareness of these serologic, genotypic and resistance patterns might help in the formulation of management plans and in predicting clinical outcomes. Further larger scale studies are needed to confirm our results and to examine possible associations among clinical, serologic, and genetic patterns of HBV infections in Jordan.

COMMENTS

Background

Hepatitis B virus (HBV) infections continue to impose huge medical, social, and economic burdens on patients and countries all over the world. Chronic liver disease, cirrhosis, and hepatocellular carcinoma are serious consequences of such infections. These infections are possibly transmitted through transfusion of blood products, unprotected sex, vertical transmission from mothers, or other different risky behaviours. As of today, and despite tremendous medical advances, there is permanent cure for these infections.

Research frontiers

Individualizing the management of patients with hepatitis B infections is based on understanding the local prevalence of different genotypes of this virus. It is also affected by the patterns of resistance manifested by local strains. We addressed these issues in this article. We studied the genotype profiles and resistance patterns of hepatitis B strains among Jordanian patients.

Innovations and breakthroughs

Even though we expected genotype D to be the most prevalent one among Jordanian patients, it was surprising that no other genotypes were detected. With a small number of strains tested for YMDD resistance mutations, it was apparent that our YMDD lamivudine resistance profiles were similar to those encountered

in other surrounding countries and other different parts of the world. During the twentieth century, huge advancements in understanding the basics of this disease have been achieved. Researchers have focused on different frontiers including pathogenesis, diagnostic approaches, genetic profiling, antiviral therapy, resistance patterns, and vaccines development.

Applications

This study may give certain insights to Jordanian physicians managing hepatitis B patients. Genotypic profiles and resistance patterns might help in decisions regarding selection of antiviral therapy, duration of treatments, and expected responses to treatment. Knowing the genetic profiling of hepatitis B strains might reduce the cost of diagnostic testing.

Terminology

Lamivudine is a well-known antiviral drug used for treatment of hepatitis B and other viral infections. YMDD is a motif that is commonly mutated in hepatitis B virus lamivudine resistant mutants.

Peer review

In this article, the authors have described HBV molecular epidemiology in Jordan. The objectives were clearly stated and a respectable sample size (107 subjects) was studied. All subjects were HBsAg positive. Only 17% of cases were positive for HBeAg. 100% of cases were genotyped as HBV genotype D. YMDD mutations were observed in 31% of cases analyzed. Overall, the manuscript is well written and results are clearly presented. Interpretation of the findings is appropriate.

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