Evaluation of Lamivudine Resistance Mutations in HBV/HIV Co-infected Patients

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ABSTRACT

Background and Aim: The drug resistance mutations are key elements in the failure of long-term treatment of Hepatitis B virus (HBV) and human immunodeficiency virus (HIV) infections. The mutation in the YMDD motif in the P gene of HBV is the most critical factor in antiviral drug (especially lamivudine) resistance. This study aimed to assess the YMDD motif and other polymerase gene mutations in individuals with HBV/HIV co-infection.

Materials and Methods: All enrolled patients were under lamivudine treatment. Blood samples were collected from 37 HBV/HIV-positive patients, and DNA was extracted. The P gene was amplified by the PCR method with appropriate primers. The PCR products for detecting mutations in the P gene were sent to the Macrogen. To investigate the P gene mutations, the obtained sequences were compared with the polymerase gene of the HBV standard sequence in the GeneBank ( accession number AB033559).

Results: The mean age of the patients was 34.1±5.7 years, of which 59.5% were male, and 40.5% were female. Of all patients, 56% were drug abusers, 35% had risky sexual behavior, 56% had prison history, and 33% had addicted wives. The 37 extracted samples were sequenced successfully. Among the studied samples (n =37), 28 patients had simultaneous mutations of YIDD and FLMAQ, 1 patient had YIDD and FSLAQ and 1 patient had YIDD and FSLAQ.

Conclusion: In summary, drug-resistant variants were detectable in most coinfected patients with chronic Hepatitis B (CHB) and HIV. As a result of mutations, therapeutic strategies sometimes are not effective. Therefore, recognition and monitoring of drug resistance mutations are critical.

Keywords: Coinfection, HBV, HIV, Lamivudine, Mutation, P gene

1. Introduction

Hepatitis B virus (HBV) is known as a blood-borne virus and a member of the Hepadnaviridae family, which with approximately 400 million carriers, it is one of the major concerns for public health (1). HBV and human immunodeficiency virus (HIV) have a common transmission route and therefore, coinfection is common in both infections. Almost 10% of patients worldwide infected with HIV are chronically coinfected with HBV (2). Some studies have reported that HBV/HIV coinfected in the course of HBV infection can increase HBV DNA levels in patients and eventually can lead to cirrhosis and Hepatocellular carcinoma (1, 2). After HBV infection in HIV-infected people, the development of chronic hepatitis is 6
times more common than HIV-negative people (3). This is more common in men with HIV infection and individuals with decreased CD4. In addition, in people with HIV infection development, protective anti-HBs responses may be defective (4). The genetic characteristics of the HBV are very effective in the progression of the disease and the development of cirrhosis and even Hepatocellular carcinoma (5).

By harboring four overlapping open reading frames (ORFs), including S, C, P, and X, HBV has a unique genomic structure (6). The HBV P ORF is the longest ORF and encodes a 90-kDa protein with polymerase activity (7). The YMDD motif in the catalytic domain of the reverse transcriptase region of the HBV P gene consists of tyrosine, methionine, and aspartate (8, 9). It is also a common motif in RNA-dependent DNA polymerase in retroviruses, hepatitis viruses, retrotransposons, group II intron, and the catalytic subunit of telomerase and is involved in nucleotide binding in the polymerase (10, 11). The mutation in the YMDD motif is the most important factor in antiviral drug (especially lamivudine (LAM)) resistance (12). These mutations may reduce the efficiency of the drug against mutated strains (13). In addition, the FLLAQ motif (glutamine, alanine, leucine, and phenylalanine) is also located in the reverse transcriptase region of the polymerase gene and, together with YMDD, can cause resistance to lamivudine (14, 15).

For this reason, the management and treatment of HBV/HIV coinfection is a significant concern. Although anti-HIV and anti-HBV treatments can relieve symptoms and possibly minimize the level of HIV RNA and HBV DNA, Long-term LAM therapy may induce YMDD mutations and drug resistance (16). Therefore, the study of the YMDD motif in patients with hepatitis B can provide beneficial information for physicians to adopt appropriate therapies. In this regard, the present research was undertaken to evaluate the HBV polymerase gene mutations in individuals with HBV/HIV coinfection under lamivudine therapy and determine other mutations associated with antiviral drugs.

2. Materials and Methods

Sample Collection

In this study, 37 HBV/HIV coinfected patients under lamivudine therapy were selected. Blood samples from patients were collected and separated plasma stored in -70°C. All patients were tested for the HBV serological markers (HbsAg and HBeAg) and HIV antibodies using an ELISA kit (Acontech, California, USA). Patients vaccinated for HBV, individuals with a history of immunoglobulin therapy, and antibodies against hepatitis C and D viruses were excluded. The study was approved by the Ethics Committee of Golestan University of Medical Sciences with the code number 773A2032980.

DNA Extraction and PCR Assay

The extraction of the viral genome from plasma was performed using the High Pure Viral Nucleic Acid Kit (Roche, Hamburg, Germany). The extraction of HBV DNA was performed according to the manufacturer’s protocol, and then the extracted DNA was stored at -20°C for PCR assay. Totally, the DNA extraction was successful in 37 samples. The P gene of HBV was amplified by PCR method with appropriate primers. The length of the DNA sequence was 645 bp, and PCRs were performed with the sets of forward primer (5’ GAT GTG TCT GCC GCG TTT TA 3’) and reverse primer (5’ CAG CAA AGC CCA AAA GAC CCA C 3’), corresponding to the nucleotide positions 376-395 and 1021-1000 for forward and reverse primers, respectively (15). For PCR, 5 μL of extracted DNA was added to an amplification mixture containing 1 × PCR buffer, 2.5 mM MgCl₂, 0.2 mM/L dNTP mixture, 2.5U of Taq DNA polymerase (QIAGEN, Hamburg, Germany) and 1 pmol of each primer in a total volume of 50 μL. The amplification thermal conditions were as: an initial incubation at 94°C for 3 min and 30 cycles comprised of denaturation for 30 sec at 94°C, annealing for 30 sec at 55.9°C and extension for 50 sec at 72°C, with a final extension at 72°C for 2 min. The PCR products were electrophoresed, as shown in Figure 1. The PCR products for detecting mutations in P gene were sent to the Macrogen (South Korea).

DNA Sequencing and Mutation Analysis

To identify the location of the mutation, we compared our sequences with the reference sequence attained from GeneBank (accession number AB033559). In addition, the sequenced P gene was compared with the HBV genome sequence in Iran, which was registered in the GeneBank by Tehran University of Medical Sciences and Digestive Disease Research Institute. To determine the mutations, the resultant sequences were blasted with the reference sequences for HBV genotypes. Gene Runner software was also utilized to determine mutations in amino acid levels.

Statistical Analysis

Statistical analysis was performed using the SPSS version 22 (SPSS Inc., Chicago, IL, USA). Descriptive data were presented as mean± SD. T-Test was used to compare means. P-values less than 0.05 were considered statistically significant.
3. Results

Mutations in Nucleotide Sequences

In this study, the mean age of patients was 34.1±5.7 years, of which 59.5% were male and 40.5% were female. All HIV-positive patients were HBsAg positive, and 6 (10%) of patients were HBeAg positive. Of all patients, 56% were drug abusers, 35% had risky sexual behavior, 56% had prison history, and 33% had addicted wives. Demographic characteristics of enrolled patients are listed in Table 1.

Table 1. Demographic and other characteristics of enrolled patients

| Patient No. | Gender | Age | Intravenous drug abuse | Risky Sexual Behavior | Prison | Wife addicted | YMDD Mutation | FLLAQ Mutation |
|-------------|--------|-----|-------------------------|-----------------------|--------|---------------|----------------|----------------|
| 1           | MALE   | 48  | yes                     | yes                   | no     | no            | Negative       | Negative       |
| 2           | FEMALE | 39  | no                      | no                    | no     | yes           | Negative       | Negative       |
| 3           | FEMALE | 25  | no                      | no                    | no     | yes           | Negative       | Negative       |
| 4           | FEMALE | 54  | no                      | no                    | no     | yes           | Negative       | Negative       |
| 5           | MALE   | 35  | yes                     | yes                   | yes    | no            | Negative       | Negative       |
| 6           | FEMALE | 17  | no                      | no                    | no     | yes           | positive       | positive       |
| 7           | MALE   | 54  | yes                     | no                    | yes    | no            | positive       | positive       |
| 8           | FEMALE | 35  | no                      | no                    | no     | yes           | positive       | positive       |
| 9           | FEMALE | 28  | no                      | no                    | yes    | no            | positive       | positive       |
| 10          | FEMALE | 32  | yes                     | yes                   | yes    | no            | positive       | positive       |
| 11          | FEMALE | 26  | no                      | no                    | no     | yes           | positive       | positive       |
| 12          | MALE   | 45  | yes                     | yes                   | yes    | no            | positive       | positive       |
| 13          | MALE   | 24  | yes                     | no                    | yes    | no            | positive       | positive       |
| 14          | MALE   | 25  | yes                     | no                    | no     | yes           | positive       | positive       |
| 15          | FEMALE | 30  | no                      | yes                   | no     | yes           | positive       | positive       |
| 16          | MALE   | 30  | yes                     | no                    | yes    | no            | positive       | positive       |
| 17          | MALE   | 26  | yes                     | yes                   | yes    | no            | positive       | positive       |
| 18          | FEMALE | 17  | no                      | yes                   | no     | yes           | positive       | positive       |
| 19          | FEMALE | 45  | no                      | no                    | no     | yes           | positive       | positive       |
After sequencing, all sequences were aligned. According to our previous study, all isolates corresponded to Genotype D (16). Generally, 7 deletions at position 419 and 13 deletions at position 1009 were found. In addition, 5 mutations at positions 420-421 and 20 mutations at positions 1013-1014 were insertion mutations, and the other mutations were translocation as listed in Table 2.

Table 2. Frequency of mutations in nucleic acid level

| Position | Type     | Nucleotide | Frequency | Frequency |
|----------|----------|------------|-----------|-----------|
| 419      | Deletion | C          | 7         |           |
| 420-421  | Insertion| G          | 5         |           |
| 420      | translocation | T-G  | 8         |           |
| 427      | translocation | C-G  | 7         |           |
| 472      | translocation | T-G  | 34        |           |
| 493      | translocation | A-T  | 35        |           |
| 514      | translocation | C-A  | 36        |           |
| 533      | translocation | C-A  | 36        |           |
| 561      | translocation | C-A  | 36        |           |
| 574      | translocation | A-C  | 36        |           |
| 592      | translocation | C-T  | 34        |           |
| 667      | translocation | T-C  | 7         |           |
Investigation Mutations in Amino Acid Level

Gene runner software was used to investigate mutations at amino acid levels. Also, these sequences were double-checked with ClustalW. In the term of YMDD motif, 29 cases showed substitution of methionine to isoleucine (YIDD), and in one patient, aspartic was also converted to asparagine (YINN). In terms of the FLLAQ motif, 28 patients showed the conversion of leucine to methionine (FLMAQ). In a patient, leucine was converted to serine (FSLAQ). Also, in a patient, leucine was converted to isoleucine, alanine was converted to proline, and glutamine was converted to histidine (FLIPH). Among the studied samples (n=37), 28 patients had simultaneous mutations of YIDD and FLMAQ, 1 patient had YINN and FLIPH and 1 patient had YIDD and FSLAQ. However, seven patients indicated no mutation in YMDD and FLLAQ motifs. The frequency of mutations based on positions is listed in Table 3.

Table 3. Frequency of mutations in amino acid level

| Frequency | Mutation type | Position |
|-----------|--------------|----------|
| 5         | A to V, C, S, G | 97       |
| 5         | H to D        | 100      |
| 30        | L to V, F     | 115      |
| 32        | I to F        | 122      |
| 32        | L to M, I     | 129      |
| 32        | S to Y        | 135      |
### Frequence

| Frequency | Mutation type | Position |
|-----------|---------------|----------|
| 32        | K to Q        | 149      |
| 29        | L to M        | 180      |
| 30        | M to I        | 204      |
| 37        | N to H        | 248      |
| 37        | W to Y        | 257      |
| 36        | N to D        | 263      |
| 36        | H to Q        | 267      |
| 36        | H to N        | 279      |
| 4         | L to F, R to L| 293      |
| 13        | L to W, G     | 294      |
| 23        | G to A, P, L-V to L | 295 |
| 12        | A to C, S     | 297      |
| 16        | A to G, W     | 298      |
| 10        | P to N, K, F-L to F | 299 |

### Drug Resistance Mutations

The data from Table 4 depicts that mutation at amino acid position 204 in the region C of the sequenced P gene caused the substitution of the methionine with isoleucine and caused resistance to lamivudine and telbivudine. Also, mutation at amino acid position 180 in region B changed the leucine to methionine and resulted in resistance to lamivudine. The L180M mutation alone had little effect on lamivudine resistance, while its combination with M204V and sometimes M204I increased resistance to both emtricitabine and lamivudine compared to M204V and M204I mutations individually.

#### Table 4. Drug resistance mutations

| Agent         | Mutations | Patients No. | Cross-resistant to                             | Sensitive to               |
|---------------|-----------|--------------|-----------------------------------------------|---------------------------|
| Lamivudine    | M204I     | 30           | Other nucleoside Analogs (Telbivudine, Adefovir) | Adefovir                  |
|               | L180A     | 28           |                                               | Tenofovir                 |
| Telbivudine   | M204I     | 30           |                                               | Entecavir±MPA             |
| Emtericitabine| L180A±M204I| 28           |                                               | Lamivudine                |

### Discussion

The HBV polymerase is the main target of therapeutic agents. Long-term LAM therapy may induce YMDD mutations and drug resistance, which would limit the effects of LAM treatment (15). LAM therapy in HBV/HIV co-infection inhibits both HIV and HBV reverse transcriptase and may result in undetectable HBV DNA levels, relieving liver damages and causing HBeAg seroconversion (17). In previous studies, the rate of YMDD mutations after one year of LAM therapy was 14–70% (8, 11). In contrast, in the present study, the rate of YMDD mutation was 81% among patients with HBV. The YMDD-specific cytotoxic T lymphocytes may have an incomplete cross-reactivity with the YIDD and YVDD motifs, which often results in drug resistance (18, 19).

One of the factors related to a mutation in lamivudine resistance is the simultaneous presence of two mutations, YIDD and YVDD (20, 21). Our findings demonstrated the simultaneous mutation of YIDD and FLMAQ in 28 patients, YINN and FLIPH in one patient, and YIDD and FSLAQ in another patient. Kobayashi et al.'s study has reported that the liver's defense mechanism occurs in association with a single mutation, and none of the patients had two concurrent mutations in YIDD and YVDD, which is inconsistent with our findings (22). Another factor related to lamivudine resistance is mutations in YMDD and FLLAQ motifs (23). This observation is in agreement with a previous study conducted at Golestan Province of Iran (24).
A survey on HBsAg-positive patients has reported that all patients with lamivudine resistance have the YMDD mutation; however, no FLLAQ mutation was observed in any of the patients (22). Mutations affect the YMDD motif in the reverse transcriptase catalytic domain of the HBV polymerase gene, rendering the conversion of methionine to valine or isoleucine in codon 741 (19). Mutation in the amino acid position 204 in the C region of the P gene has been shown to change the methionine to valine, leucine, and serine. In addition, a mutation in the amino acid position 180 (in the B region) changes the leucine to methionine (25). Inoue et al. (2011) detected the mutation of codon 552 from methionine to valine (rtM204V) and methionine to isoleucine (rtM204I) in 42.9% and 28.6% of the patients, respectively. They also found a mutation in codon 528 from leucine to methionine (rtL180M) in 28.6% of the patients (26). The YSDD mutation, which is a substitution of methionine to serine in codon 204 in the C-terminal polymerase region, was first reported by Bozdayi et al. in 2001 (27).

In the present study, amino acid variations (change in methionine to isoleucine amino acids or change in leucine to methionine amino acids) resulted in resistance to lamivudine, adefovir, tenofovir, and entecavir. The viral resistance to adefovir is lower than lamivudine, tenofovir, and entecavir. In most cases, the rtM204I mutation in the YMDD motif disappears after treatment with adefovir. Delaney et al.’s (2011) study on lamivudine-resistant and adefovir-treated patients showed a link between the rtV173L mutation and both M204 and L180 mutations (28). In addition, the rtV173L mutation is involved in resistance to lamivudine and famciclovir (29). Co-occurrence of HBV/HIV infection and combination therapy with lamivudine and adefovir can be the reasons for this mutation (28). Long-term treatment with nucleoside/nucleotide analogs can cause HBV drug resistance in patients with chronic hepatitis B. Arrese et al. have shown limited responses in patients with monotherapy and stronger responses with combination therapy (30). Unlike their study, in which they detected rtM204I and rtM204V + rtL180M mutations, we did not find such mutations. Lacking observation of the other mutations in our study could be due to the research methods used above (31).

The rate of YMDD mutations varies among different populations, and this variation may be related to the genotype of the infected population (24). Relation between the types of mutations and the virus genotype in the population is one of the main factors in lamivudine resistance mutations (32). Wang et al.’s study in Southern China disclosed that although genotype B is predominant in the region, the highest drug resistance was among individuals with genotype C (33). Horgan et al. reported a connection between the genotype C and YMDD mutation and drug resistance (34). Zhang et al. (2003) reported that 55.9% and 44.1% of patients are infected with genotypes B and C, respectively. They also recorded 22 mutations in 54.7% (238/435) of patients, in which the frequency of drug resistance mutations in genotype C was much higher than B (63.0% vs. 48.1%, P=0.003). The positions 180, 181, 191, 200, 202, 221, 229, and 224 were common mutation sites in the genotype C, and mutation in position 236 was more common in genotype B (18). In patients with virological breakthrough, 11 (169, 202, 250, 173, 180, 200, 207, 214, 237, 242, and 245) and 9 (191, 207, 213, 218, 221, 224, 229, 238 and 242) mutations sites coexisted with M204I or M204V. In our study, all the infected individuals had a D genotype, and the YMDD mutation was found in this genotype. It must be mentioned that one of the genotypes that have YMDD mutation is genotype D.

The present survey detected M204I and L180M mutations in 30 and 28 patients, respectively; however, both M204V + L180M and M204I + M204V mutations were observed in all the patients. In this regard, Liaw et al.’s study, which was performed on patients with HBV, reported 12 patients with M204V + L180M mutation and 20 patients with M204I mutation (11). Our results showed that the L180M mutation alone provides little resistance to lamivudine. Still, its combination with M204V and M204I gives rise to resistance due to the increased reverse transcriptase and transcription activity. In contrast, previous investigations in HBV/HIV coinfected patients have displayed that these mutations alone can induce resistance to lamivudine, though the M204I mutation was more resistant to this agent (35, 36).

4. Conclusion

In summary, drug-resistant variants were detectable in the majority of coinfected patients with chronic Hepatitis B and HIV. Today, different drug agents are active toward both HIV and HBV and different treatment algorithms. Therefore, we suggest that physicians draw a separate algorithm for HIV and HBV before starting treatment, and additionally, drug resistance mutations in both HBV and HIV are examined.

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Author Contribution
Conceive and design of the experiments: A.M.; data analysis: A.M, E.B; writing of the paper: A.M, E.B, M.N, I.S; performance of the experiments: F.S.

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Conflict of Interest
None declared.

References
1. Ribeiro CRA, de Almeida NAA, Martinelli KG, et al. Cytokine profile during occult hepatitis B virus infection in chronic hepatitis C patients. Virol J. 2021;18(1):15. Published 2021 Jan 12. [DOI:10.1186/s12985-021-01487-2] [PMID] [PMCID]
2. Ireland G, Simmons R, Balogun K, et al. HIV coinfection among persons diagnosed with hepatitis B in England in 2008-2014. HIV Med. 2019;20(4):255-263. [DOI:10.1111/hiv.12707] [PMID] [PMCID]
3. Soriano V, Puoti M, Bonacini M, Brook G, Cargnel A, Rockstroh J, Thio C, Benhamou Y. Care of patients with chronic hepatitis B and HIV coinfection: recommendations from an HIV-HBV International Panel. Aids. 2005; 18;19(3):221-40. [DOI:10.1097/01.aids.0000163948.62176.e7]
4. Rajbhandari R, Jun T, Khalili H, Chung RT, Ananthakrishnan AN. HBV/HIV coinfection is associated with poorer outcomes in hospitalized patients with HBV or HIV. J viral hepatitis. 2016; 23(10):820-9. [DOI:10.1111/jvhe.12555] [PMID] [PMCID]
5. Hanazaki K. Antiviral therapy for chronic hepatitis B: a review; JCDT-I, Allergy. 2004; 3(1):63-70. [DOI:10.2174/1568010043483908] [PMID]
6. Lavanchy D, Kane M. Global Epidemiology of Hepatitis B Virus Infection. In: Liaw Y-F, Zoulim F, editors. Hepatitis B Virus in Human Diseases. Cham: Springer International Publishing; 2016. p. 187-203. [DOI:10.1007/978-3-319-22330-8_9]
7. Maucort-Boulch D, de Martel C, Franceschi S, Plummer M. Ratio and incidence of liver cancer attributable to hepatitis B and C viruses worldwide. J Cancer. 2018;142(12):2471-7. [DOI:10.1002/jic.31280] [PMID]
8. Lai CL, Dienstag J, Schiff E, Leung NW, Atkins M, Hunt C, Brown N, Woessner M, Boehme R, Condrea L. Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. Clin Infect Dis. 2003; 15;36(6):687-96. [DOI:10.1086/368083] [PMID]
9. Alter H, Block T, Brown N, Brownstein A, Brosgart C, Chang KM, et al. A research agenda for curing chronic hepatitis B virus infection. Hepatology. 2018; 67(3):1127-31. [DOI:10.1002/hep.29509] [PMID] [PMCID]
10. Pappoe, F., Hagan, C.K.O., Obiri-Yeboah, D. Seroprevalence of hepatitis B and C viral infections in Ghanaian HIV-positive cohort: a consideration for their health care. BMC Infect. Dis 2019; 19:380. [DOI:10.1186/s12879-019-4027-y] [PMID] [PMCID]
11. Liaw Y-F, Sung JJ, Chow WC, Farrell G, Lee C-Z, Yuen H, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med. 2004; 351(15):1521-31. [DOI:10.1056/NEJMoa033364] [PMID]
12. Feng B, Wei L, Chen M, Wang LM. Dynamic changes of hepatitis B virus polymerase gene including YMDD motif in lamivudine-treated patients with chronic hepatitis B. Microbiol Res. 2018; 163(4):487-92. [DOI:10.1016/j.micres.2016.11.004] [PMID]
13. Liu KZ, Hou W, Zumbika E, Ni Q. Clinical features of chronic hepatitis B patients with YMDD mutation after lamivudine therapy. J Zhejiang Uni SCI. 2005; 6(12):1182-7. [DOI:10.1631/jzus.2005.81182] [PMID] [PMCID]
14. Bennet JE, Blaser MJ, Dolin R. Principles and practice of infectious diseases: Elsevier Saunders 2015.
15. Lee CZ, Lee HS, Huang GT, Yang PM, Sheu JC. Detection of YMDD mutation using mutantspecific primers in chronic hepatitis B patients before and after lamivudine treatment. World J Gastroenterol. 2006;12:5301-5. [DOI:10.3748/wjg.v12.i33.5301] [PMID] [PMCID]
16. Karami C, H Adli A, Zhänd S, Tabarraei A, Talei R, Saedt M, Moradi A. Study of Genotype, Subtype and Mutation in the S Gene in Hepatitis B Patients Co-infected with HIV in Iran. Jundishapur J Microbiol. 2016; 9(12):e34009. [DOI:10.5812/jjm.34009]
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17. Kobayashi S, Ide T, Sata MJJoH. Detection of YMDD motif mutations in some lamivudine-unintreated asymptomatic hepatitis B virus carriers. J Hepatol. 2011; 34(4):584-6. [DOI:10.1016/j.jhep.2010.12.001] [PMID:20987235] [PMCID:PMC2980808]

18. Zhang X, Zhang Y, Sun L, Wen Q, Zhou L, Fan G, et al. Study of gene chips in the detection of YMDD mutations in the region of HBV polymeras. Zhonghua yi xue za zhi. 2003; 83(6):459-62.

19. Yan M, Zhang C, Ling Q, Zhou RF. Detection of YMDD motif mutations in lamivudine-unintreated patients with chronic hepatitis B. Zhonghua gan zang bing za zhi. 2013;11(7):430-1.

20. Cuestas ML, Mathet VL, Oubiña JR, Sosnik AJPr. Drug delivery systems and liver targeting for the improved pharmacotherapy of the hepatitis B virus (HBV) infection. Pharm Res. 2010; 27(7): 1184-202. [DOI:10.1007/s11095-010-0112-z] [PMID:20934254]

21. Lin C, Tsai S, Lee T-H, Chien R, Liao S, Liaw YJG. High frequency of functional anti-YMDD and mutant cytotoxic T lymphocytes after in vitro expansion correlates with successful response to lamivudine therapy for chronic hepatitis B. Gut. 2015; 54(1):152-61. [DOI:10.1136/gut.2003.032920] [PMID:01129135] [PMCID:PMCID5803061]

22. Kobayashi M, Suzuki F, Akuta N, Yatsuji H, Hosaka T, Sezaki H, et al. Correlation of YMDD mutation and breakthrough hepatitis with hepatitis B virus DNA and serum ALT during lamivudine treatment. Hepatology Res. 2010; 40(2):125-34. [DOI:10.1111/j.1872-034X.2009.00565.x] [PMID:20231363]

23. Zoulim F, Poynard T, Degos F, Slama A, El Hassnaoui A, Blin P, et al. A prospective study of the evolution of the YMDD resistance mutations in patients with chronic hepatitis B treated with lamivudine. J Viral Hepat. 2006; 13(4):278-88. [DOI:10.1111/j.1365-2893.2005.00712.x] [PMID:16789482] [PMCID:PMCID16789482]

24. Gholamreza R, Shahryar S, Abbasali K, Hamidreza J, Abdolvahab M, Khodaberdi K, et al. Seroprevalence of hepatitis B virus and its coinfection with hepatitis D virus and hepatitis C virus in Iranian adult population. Indian J Med Sci. 2017; 61(5):263-8. [DOI:10.4103/0019-5359.32092] [PMID:28911284]

25. Lo CM, Liu CL, Lau GK, Chan SC, Ng IO, Fan ST. Liver transplantation for chronic hepatitis B with lamivudine-resistant YMDD mutant using add-on adefovir dipivoxil plus lamivudine. Liver Transplant. 2015;11(7):807-13. [DOI:10.1002/lt.20416] [PMID:26528970] [PMCID:PMCID4452392]

26. Inoue J, Ueno Y, Wakui Y, Niitsuma H, Fukushima K, Yamagiwa Y, et al. Four-year study of lamivudine and adefovir combination therapy in lamivudine-resistant hepatitis B patients: influence of hepatitis B virus genotype and resistance mutation pattern. J Viral Hepatitis. 2011;18(3):206-15. [DOI:10.1111/j.1365-2893.2010.01301.x] [PMID:20710874]

27. Bozdayi A, Uzunalımoğlu Ö, Türkyılmaz A, CinAR K, Sezgin 0, Bozkaya H, et al. A new mutation pattern (YMDD→YSDD) in YMDD motif of HBV DNA polymerase gene in chronic B hepatitis infection resistant to lamivudine treatment. J Hepatology. 2001; 34:162. [DOI:10.1016/S0168-8278(01)80594-8]

28. Delaney IV WE, Locarnini S, Shaw TJAC, Chemotherapy. Resistance of hepatitis B virus to antiviral drugs: current aspects and directions for future investigation. Antivir Chem Chemother. 2011; 12(1):1-35. [DOI:10.1177/095632020101200101] [PMID:11221633]

29. Yildiz O, Aygen B, Demirtürk N, Demirdal T, Inan D, Yildirmak T, et al. Lamivudine resistance mutations in patients infected with hepatitis B virus genotype D. World J Gastroenterol. 2011; 17(45):4987. [DOI:10.3748/wjg.v17.i45.4987] [PMID:21852133] [PMCID:PMCID3650002]

30. Arrese E, Basarab M, Blanco S, Ruiz P, Cisterna R. Evolution of hepatitis B virus during long-term therapy in patients with chronic hepatitis B Ann Hepatol. 2011;10(4), 434-440. [DOI:10.1016/S0915-6086(10)90010-5] [PMID:21666897] [PMCID:PMCID21666897]

31. Kim J-K, Lee H-J, Lee Y-J, Chun J-Y, Lee I-K, Lim Y-S, et al. Direct detection of lamivudine-resistant hepatitis B virus mutants by a multiplex PCR using dual-priming oligonucleotide primers. J Virol Methods. 2018;149(1):76-84. [DOI:10.1016/j.jviromet.2008.01.003] [PMID:28661713]

32. Tamandjou Tchuem CR, Brandt L, Nel EDIR, Cotton MF, Matthews P, Kaindjee Tamandjou Tchuem CR, Brandt L, Nel EDlR, et al. Hepatitis B virus drug resistance mutations in HIV/HBV coinfected children in Windhoek, Namibia. PLoS ONE. 2020; 15(9): e0238839. [DOI:10.1371/journal.pone.0238839] [PMID:32898202] [PMCID:PMCID6276293]

33. Wang C, Yu S, Zhang Y, Zhang M, Lv L, Huang C, et al. Viral quasispecies of hepatitis B virus in patients with YMDD mutation and lamivudine resistance may not predict the efficacy of lamivudine/adeovir rescue therapy. Exp Ther Med. 2019;17(4):2473-84. [DOI:10.3892/ettm.2019.7255] [PMID:31764300] [PMCID:PMCID6512995]
34. Horgan M, Brannigan E, Crowley B, Levis J, Fanning LJ. Hepatitis B genotype and YMDD profiles in an untreated Irish population. J Clin Virol. 2016; 35(2):203. [DOI:10.1016/j.jcv.2005.08.004] [PMID]

35. Thio CL, Locarnini SJA. Treatment of HIV/HBV coinfection: clinical and virologic issues. AIDS Rev. 2017; 9(1):40-53.

36. Mirandola S, Campagnolo D, Bortoletto G, Franceschini L, Marcolongo M, Alberti A. Large-scale survey of naturally occurring HBV polymerase mutations associated with anti-HBV drug resistance in untreated patients with chronic hepatitis B. J Viral Hepatitis. 2011;18 (7): e212-e6. [PMID] [DOI:10.1111/j.1365-2893.2011.01435.x]