Hepatitis B virus YMDD-motif mutations with emergence of lamivudine-resistant mutants: a threat to recovery


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ABSTRACT

Hepatitis B virus (HBV) is a crucial public health problem with approximately 350 million affected people and a death rate of 0.5 to 1.2 million per year worldwide. It proceeds to end-stage liver diseases including cirrhosis with hepatic decompensation and hepatocellular carcinoma (HCC). Pakistan lies in the endemic region with 3% HBV carrier rate in the country. The differences in disease outcome and antiviral treatment response among HBV infected patients are variable in different regions of the world due to marked differences in HBV genotypes. These variants are different in their serological reactivity patterns, virus replication, liver disease severity and antiviral treatment responsiveness. The highly mutating nature of HBV is the major cause of its ever increasing antiviral resistance. Importantly the tyrosine-methionine-aspartate-aspartate (YMDD)-motif mutants emerge during prolonged lamivudine treatments that elicit immune clearance. This review deals with the HBV nature, mutation appearance and particularly emphasizes on lamivudine induced mutations in a conserved region (YMDD motif) which is further worsening the antiviral therapies with passage of time.

Keywords: Hepatitis B virus, Mutants, Public problem, Drug resistance, Chronic carrier.

INTRODUCTION

Today, chronic hepatitis B (CHB) is a widespread problem specifically in Asia at a rate of 70% (1). Out of the 350 million affected people worldwide, 15 to 40% develop cirrhosis, liver failure, or HepatoCellular Carcinoma (HCC) (2). Pakistan is included in the endemic region where Hepatitis B virus (HBV) infected individuals are 3% of general population (3-5). Self limited HBV acute infections are common accompanied by virus clearance and immunity improvement. On the other hand, it is observed that chronic Hepatitis B virus infection is 5% to 10% in adults and 85% to 95% in children (4). Exposure rate of Hepatitis B virus (HBV) in Pakistan is not fully known, however part of data shows 35-38% prevalence with 4% carrier rate and 32% with anti-HBV surface antibodies by natural conversion (5). The natural history of HBV infection is quite different in Asian and Western patients. Asian patients are prenatal infected and rarely confirm acute hepatitis-like clinical illness. In addition they consistently remain chronically infected and are at substantial risk for cirrhosis and HCC.
Western adult patients are usually infected by percutaneous or sexual contact and present acute hepatitis-like clinical symptoms; however chronic infection is rare over there (6-11).

**HEPATITIS B VIRUS**

Human hepatitis B virus (HHBV) is a member of *Hepadnaviruses* family of small, enveloped DNA viruses, (12) which shows considerable liver tropism which results in acute and chronic liver infections in their hosts. HBV is a partially double-stranded virus with 3.2 kb long DNA genome arranged into 4 overlapping open-reading frames (ORF) in a frameshift manner. The viral polymerase (Pol) is encoded by the longest ORF, which overlaps the envelope ORF. After the infection of a susceptible cell and genomic DNA enters the cell nucleus (13), as a result of viral core disassembly, where the partially double-stranded genomic viral DNA is converted into an important and crucial intermediate covalently closed circular (ccc) DNA by the host cell in the HBV life cycle. Viral cccDNA after being chromatinised is converted into viral mini-chromosome by the action of cellular repair enzymes such as the topoisomerases: which then acts as the key transcriptional virus template. This mini-chromosome transcribes four sets of mRNAs using host cell machinery (RNA polymerase II) (14, 15) and their transportation is facilitated by cellular proteins to the cell cytoplasm, where viral proteins are translated which include hepatitis B core antigen (HBcAg, or nucleocapsid protein); the soluble and secreted hepatitis B e antigen (HBeAg); the Polymerase protein; the viral envelope proteins, which express HBsAg; and hepatitis B X protein.

**REVERSE TRANSCRIPTION**

In addition to DNA genome, HBV also replicates by a reverse transcription process, using its own coded polymerase (16). This polymerase consists of four domains (17) which are specified for different important functions. From the N terminus, first one is the terminal protein (TP), which initiates reverse transcription by covalently linking to negative-strand DNA. Second is the spacer, which is believed to be mutation tolerant with no other specific function. Then is the RNase H domain which digests the RNA in RNA-DNA hybrids. Next is the reverse transcriptase (RT), which bears YMDD consensus motif which is also present in human immunodeficiency virus type 1 (HIV-1) reverse transcriptase. Key processes like priming reaction, reverse transcription, complementary sequences translocation and DNA second-strand synthesis (18-20) makes HBV genome a complex process.

**HBV RECOMBINATION**

HBV is believed to be highly mutating virus with the occurrence of recombination at a frequency of approximately 1.4 to 3.2 x 10^-5 nucleotide substitutions per site per year (21), which is believed to be around 10-fold higher than that of other DNA viruses. This considerably high recombination rate is due to the inability of viral reverse transcriptase to proof read. Mutation generation process involves the magnitude as well as the rate of virus replication. Theoretically it is estimated that the mean half-life of the virus in serum is less than a day and the translating rate of *de novo* HBV production is above 10 virions per day (22). Mutation appearance and HBV quasispecies complexity is highly influenced by high viral loads and turnover rates coupled with poor replication fidelity. These mutation rates, however, are much lower than the other Pararetroviruses, primarily because of the overlapping reading frame pressure. However, most of the HBV mutants pre-exist in the quasispecies pool prior to the influence of the selection pressure (23).

**HBV TREATMENT AND MUTATIONS**

There are different mechanisms for HBV inhibition. Mechanisms involve an antiviral approach inhibiting the HBV polymerase with
substances like acyclovir, ganciclovir, lamivudine, adefovir, entecavir, telbivudine and immune modulating approach with substances like interferon (24). The treatment duration for the oral nucleos(t)ides is not well defined and depends on the achievement of intermediate endpoints that are considered to be durable after discontinuation of therapy (25). Although HBV therapy issues are still unsettled, but evidences still exist to validate antiviral therapy for an extensive proportion of chronically infected individuals (26). The level of viral suppression and the probability of emergence of HBV resistance are directly related parameters under antiviral therapy (27). A low probability of resistance is expected if the drug achieves poor or complete viral suppression (27-29). On the other hand, any antiviral medication with incomplete (moderate) suppression of HBV viral replication facilitates the selection of specific mutations that will confer resistance to treatment (28).

**LAMIVUDINE MUTATIONS**

HBV Polymerase gene is responsible for the appearance of quasispecies primarily because of the use of nucleoside and nucleotide analogues as treatment agents. Due to the overlapping nature of polymerase and envelope genes, these Polymerase gene mutations also affect both. A major neutralization HBV domain is 'a' determinant (between amino acids 99 and 169) which overlaps the polymerase protein catalytic regions (domain A and B) (29, 30). Sequence variations in and around this region may decrease the affinity of HBsAg to anti-HBs, thus causing diagnostic problems. It also ceases the infection prevention after vaccination or HB immunoglobulin injection. YMDD mutations are actually the amino acid substitutions in HBsAg at position numbers 195 and 196, downstream of the 'a' determinant (30).

Lamivudine is an oral nucleoside analogue which inhibits the HBV replication by acting as a chain terminator and by competing with dCTP (31, 32) along with the reduction in serum HBV DNA levels as well as normalization of the ALT levels (33). Long term treatment is suggested since short term therapy does not completely eliminate HBV in liver (34), as a result of which its efficiency is limited by YMDD (tyrosine, methionine, aspartate, aspartate) motif mutations in the catalytic domain (C domain) of the reverse transcriptase, where methionine is substituted either by valine (YVDD) or isoleucine (YIDD) associated with biochemical flares of liver diseases (35). Virological breakthrough and alanine transaminase (ALT) flares are observed within two to three months after the YMDD mutant emergence respectively (36). Continuous increase in resistance has been seen during the treatment at an annual rate of 20-25% which ultimately touches 70% after three years of treatment (37). The factors predictive of clinical outcome of a patient have not been defined yet, as they can be different because of wide variability in patients (34). Whereas some factors increase the risk of resistance like high serum HBV DNA and alanine transferase (ALT) levels before therapy and inadequate suppression of virus replication (37). As a result of these mutations, the *in vitro* sensitivity to the drug decreases more than 100-fold in IC$_{50}$ (38).

The duration of treatment depends on the attainment of intermediary endpoints which include HBeAg seroconversion and HBsAg seroconversion. HBsAg seroconversion rarely occurs, and HBeAg seroconversion occurs gradually within 3–5 years or more and is only possible for HBeAg-positive patients. It was observed in a study that HBV DNA levels remains the same after six months of lamivudine therapy and suggested that there is correlation between DNA levels and future incidence of viral resistance in Asian patients with HBeAg-positive CHB (39). Only 8.3% of patients who achieved complete viral suppression (<200 copies/ml) at 6 months developed viral resistance compared to
59.9%, suggesting that patients without complete viral suppression at 24 weeks with lamivudine may benefit from additional or alternative treatment regimen for the prevention of viral resistance development after long-term therapy. In a limited study of primarily non-Asian patients, those with HBV DNA <100 copies per milliliter after 24 weeks had a reduced rate of viral resistance and were the only patients to subsequently clear their HBeAg (40).

**TYPES OF LAMIVUDINE MUTATIONS**

Four common types of YMDD mutations have so far been investigated i.e rtL 180M/M204V mutation (most prominent), rtL 180M/M204I mutation, rtM2004I mutation, and rtL180M mutation. Moreover, some uncommon mutations associated with lamivudine resistance in fatal hepatic failure cases have also been observed. In a study, mutations rtA222T and rtL336V, associated with surface antigen I195M and M213I mutations, were reported in addition to the double rtL180M, rtM204V mutation (41). In another study, L426I mutation in combination with rtM204I was reported after 10 months of lamivudine treatment and patient died after next 3 months (42). Another study has described a patient with fatal liver failure, who was treated with lamivudine for four months but treatment was terminated without consulting. After restarting lamivudine, HBV-DNA reappeared with HBsAg negative and development of anti-HBs antibodies. After nucleotide sequencing classical G145R escape mutant were revealed along with several stop codons in the coding region of HBsAg (43). Furthermore, two rare polymerases (rtS117Y and rtV142A) and three HBsAg (L109I, F134L, and I208T) substitutions were also observed along with 180M and M204I mutations in a study (44). These observations show correlation between fatal liver failure and the occurrence of mutations other than those occurring at positions 204 and 180 of the HBV polymerase.

As mentioned in various long term follow-up studies within Asia (37, 45), there are 14% to 38% probability of YMDD mutations after one to two years while 57% to 67% after three to four years. Generally it is known that mutations of YMDD motif occur after lamivudine long term treatment, but now it has become increasingly evident that YMDD mutations may also occur naturally even without lamivudine intake. Unfortunately the pretreatment selection of YMDD mutations by lamivudine during the treatment is not known yet (46). The risk of hepatitis B relapse and HBV DNA reappearance occurs at cumulative ratio of 14-32% after one year and 38-49% by the second year and has become an important issue in patients undergoing long term treatment with lamivudine (37).

**Lamivudine Mutations and Genetic Variability**

A possible aspect still to be evaluated fully is the effect of HBV genotypic variability on the emergence of YMDD mutations. One study shows that genotype A is possibly associated with YMDD mutations than genotype D (47) but in another study it is observed that there is no chance difference in having YMDD mutations between HBV genotype B and C patients (48, 33). Another study reveals that YVDD mutation is more frequent in genotype B whereas YIDD being common in genotype C (49, 50). Among the untreated patients, Horgan and coworkers revealed that the D genotype of HBV had no mutation in the YMDD motif, whereas HBV genotype C had these mutations (51).

**CONCLUSION**

HBV has the marvelous property of mutating day by day which is alarming for drug
development and treatment success. These mutations are not the only cause of relapse but any HBV mutation occurrence increases the pressure of resistance outcome. Pakistan lies in the intermediate HBV prevalence area where HBV carrier rate is 3–4% (52). Here, lamivudine is the cheapest option, yet beyond the reach of most patients. Its seroconversion rate is around 50% and drug resistance is 6% at 3 years (53). This is contrary to other reports from the west where many show over 70% resistance at 3-4 years (54). The possible reason for this disparity is that genotype D is common in Pakistan and has the least drug resistance rate (55-58). There is a lot to discover yet about these mutations, their impact on treatment, cellular effect, disease severity and drug designing strategies.

REFERENCES

18. Miller RH, Marion PL, Robinson WS. Hepatitis B viral DNA-RNA hybrid molecules in particles from infected liver are converted to viral DNA molecules during an endogenous DNA polymerase reaction. Virology 1984; 139: 64–72.
22. Lok AS, Akarca U, Greene S. Mutations in the pre-core region of hepatitis B virus serve to enhance


45. Liaw YF, Chien RN, Yeh CT, Tsai SL, Chu CM. Acute exacerbation and hepatitis B virus clearance...


