Gastroenterology and Hepatology From Bed to Bench. ***2010;3(3):108-114***

REVIEW ARTICLE

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

Zunaira Awan, Muhammad Idrees, Shazia Rafique, Irshad-ur Rehman, Haji Akbar, Sadia Butt, Sobia Manzoor, Liaqat Ali Khan, Saira Munir, Samia Afzal, Zareen Fatima, Bisma Rauff, Mahrukh Naudhani, Mohammad Ali, Sana saleem, Sadaf Badar

Division of Molecular Virology, National Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan

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.*
(Gastroenterology and Hepatology From Bed to Bench 2010;3(3):108-114).

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.

Received: 10 March 2010 *Accepted:* 3 May 2010
Reprint or Correspondence: Muhammad Idrees, PhD, Division of Molecular Virology, National Centre of Excellence in Molecular Biology, 87-West Canal Bank Road Thokar Niaz Baig Lahore-53700, University of the Punjab Lahore, Pakistan
E-mail: idreeskhan@cemb.edu.pk

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 frame-shift 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 Para-retroviruses, 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

1. Ayman M, Abdelhamed CM, Kelley TG, Miller PA, and Harriet CI. Rebound of Hepatitis B Virus Replication in HepG2 Cells after Cessation of Antiviral Treatment. J Virol 2002; 76: 8148-60.

2. Ganem D, Prince AM. Hepatitis B virus infection--natural history and clinical consequences. N Engl J Med 2004; 350: 1118-29.

3. Lee WM. Hepatitis B virus infection. N Engl J Med 1997; 337: 1733-45.

4. Gust I, and Crowe S. The global importance of viral hepatitis. Clin Trop Med Commun Dis 1986; 1: 281-301.

5. Hakim ST, Kazmi SU, and Bagasra O. Seroprevalence of Hepatitis B and C Genotypes among Young Apparently Healthy Females of Karachi-Pakistan. Libyan J Med 2007; 3: 66-70.

6. Dienstag JL, Schiff ER, Wright TL, Perrillo RP, Hann HW, and Goodman Z. Lamivudine as initial treatment for chronic hepatitis B in the United States. N Engl J Med 1999; 341: 1256-63.

7. Seeff LB, and Koff RS. Evolving concepts of the clinical and serologic consequences of hepatitis B virus infection. Semin Liver Dis 1986; 6: 11-22.

8. Margolis HS, Alter MJ, and Hadler SC. Hepatitis B: evolving epidemiology and implications for control. Semin Liver Dis 1991; 11: 84-92.

9. Beasley RP, and Hwang LY. Postnatal infectivity of hepatitis B surface antigen-carrier mothers. J Infect Dis 1983; 147: 185-90.

10. Seeff LB, Beebe GW, Hoofnagle JH. A serologic follow-up of the 1942 epidemic of post-vaccination hepatitis in the United States Army. N Engl J Med 1987; 316: 965-70.

11. Tassopoulos NC, Papaevangelou GJ, Sjogren MH, Roumeliotou-Karayannis A, Gerin JL, and Purcell RH. Natural history of acute hepatitis B surface antigen-positive hepatitis in Greek adults. Gastroenterology 1987; 92: 1844-50.

12. Dane DS, Cameron CH, and Briggs M. Virus-like particles in serum of patients with Australia-antigen-associated hepatitis. Lancet 1970; 1: 695–98.

13. Kann M, Bischof A, and Gerlich WH. In vitro model for the nuclear transport of the hepadnavirus genome. J Virol 1970; 71: 1310-16.

14. Ganem D, Schneider RJ. Hepadnaviridae: the viruses and their replication. In: Knipe DM, Howley PM, eds. Fields virology. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p.2923-69.

15. Tavis J. The replication strategy of the hepadanaviruses. Viral Hepatitis Rev 1996; 2: 205-18.

16. Bavand M, Feitelson M, Laub O. The hepatitis B virus-associated reverse transcriptase is encoded by viral pol gene. J Virol 1989; 63: 1019–21.

17. Radziwill G, Tucker W, Schaller H. Mutational analysis of the hepatitis virus P gene product: domain structure and RNase H activity. J Virol 1990; 64: 613-20.

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.

19. Wang GH, Seeger C. The reverse transcriptase of hepatitis B virus acts as a protein primer for viral DNA synthesis. EMBO J 1992; 71: 663–70.

20. Will H, Reiser W, Weimer T, Pfaff E, Buscher M, Sprengel R, et al. Replication strategy of human hepatitis B virus. J Virol 1987; 61: 904–11.

21. Okamoto H, Imai M, Kametani M, Nakamura T, Mayumi M. Genomic heterogeneity of hepatitis B virus in a 54 year old woman who contracted the infection through materno-fetal transmission. Jpn J Exp Med 1987; 57: 231-36.

22. Lok AS, Akarca U, Greene S. Mutations in the pre-core region of hepatitis B virus serve to enhance

the stability of the secondary structure of the pre-genome encapsidation signal. Proc Natl Acad Sci USA 1994; 91: 4077-81.

23. Revill P, Locarnini S. HBV variants. Clinical significance and public health implications 2005; 2: 74-92.

24. Hans LT. Antiviral therapy and resistance with hepatitis B virus Infection, Antiviral therapy and resistance with hepatitis B virus infection. World J Gastroenterol 2007; 13: 125-40.

25. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007; 45: 507–39.

26. Owens DK, Black M. Assessing the benefits and costs of new therapies for hepatitis B virus infection. Ann Intern Med 2005; 142: 863-64.

27. Zoulim F. Mechanism of viral persistence and resistance to nucleoside and nucleotide analogs in chronic hepatitis B virus infection. Antivir Res 2004; 64: 1-15.

28. Doo E, and Liang TJ. Molecular anatomy and pathophysiologic implications of drug resistance in hepatitis B virus infection. Gastroenterology 2001; 120: 1000-1008.

29. Locarnini S, Hatzakis A, Heathcote J. Management of antiviral resistance in patients with chronic hepatitis B. Antivir Ther 2004; 9: 679-93.

30. Zhou DY, Lin LY, Wang H, Huang JS. The relationship between HBV lamivudine resistance and HBV genotypes or basic core promoter mutations. Hepatobiliary Pancreat Dis Int 2003; 2: 85-89.

31. Carman WF. The clinical significance of surface antigen variants of hepatitis B virus. J Viral Hepat 1997; 4: 11-20.

32. Mesut A, Aylin S, Ethem T, Ayca AS, Omer T, Hale A, Yusuf H. (2006). YMDD Motif Variants in Inactive Hepatitis B Carriers detected by Inno-Lipa HBV DR Assay. J Gastroenterol Hepatol 2006; 21: 1783-88.

33. Xiao Z, Xiao J, Jiang J, Zhang, Yu M, Zhao J, Wei D, Cao H. A novel method based on ligase detection reaction for low abundant YIDD mutants detection in hepatitis B virus. Hepatology 2005; 34: 150-55.

34. Hiroyuki N, Masayuki K, Yasuhiro A, Yuko O, Ken U, Yuki N, et al. Polymerase domain B mutation is associated with hepatitis relapse during long-term lamivudine therapy for chronic hepatitis B. Intervirology 2005; 48: 381–88.

35. Marcelle B, Francisco JD, Souto K, Marcia A, Carlos E, Christian N, et al. Hepatitis B virus genotypes and resistance mutations in patients under long term lamivudine therapy: characterization of genotype G in Brazil. BMC Microbiol 2008; 8: 11.

36. Sun J, Wang Z, Ma S, Zeng G, Zhou Z, Luo K, et al. Clinical and virological characteristics of lamivudine resistance in chronic hepatitis B patients: a single center experience. J Med Virol 2005; 75: 391-98.

37. Lai CL, Dienstag J, Schiff E, Leung NW, Atkins M, Hunt C. Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. Clin Infect Dis 2003; 36: 687-96.

38. Delaney WE, Locarnini S, Shaw T. Resistance of hepatitis B virus to antiviral drugs: Current aspects and directions for future investigation. Antivir Chem Chemother 2001; 12: 1-35.

39. Yuen MF, Wong DK, Sablon E, Yuan HJ, Sum SM, Hui CK. Hepatitis B virus genotypes B and C do not affect the antiviral response to lamivudine. Antiviral Ther 2003; 8: 531-34.

40. Zollner B, Schafer P, Feucht H, Schroter M, Petersen J, Laufs R. Correlation of hepatitis B virus load with loss of e antigen and emerging drug-resistant variants during lamivudine therapy. J Med Virol 2001; 65: 659–63.

41. Ayres A, Bartholomeusz A, Lau G, Lam KC, Lee JY, Locarnini S. Lamivudine and famciclovir resistant hepatitis B virus associated with fatal hepatic failure. J Clin Virol 2003; 27: 111–16.

42. Wang JH, Lu SN, Lee CM, Lee JF, and Chou YP. Fatal hepatic failure after emergence of the hepatitis B virus mutant during lamivudine therapy in a patient with liver cirrhosis. Scand J Gastroenterol 2002; 37: 366–69.

43. Zhang JM, Wang XY, and Huang YX. Fatal liver failure with the emergence of hepatitis B surface antigen variants with multiple stop mutations after discontinuation of lamivudine therapy. J Med Virol. 2006; 78: 324–28.

44. Marcelle B, Nilo I, Christian N, Natalia MA, Kycia MR, Selma AG. Lamivudine resistance and other mutations in the polymerase and surface antigen genes of hepatitis B virus associated with a fatal hepatic failure case. J Gastroenterol Hepatol 2008; 23: 67–72.

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

after emergence of YMDD motif mutation during lamivudine therapy. Hepatology 1999; 30: 567–72.

46. Man F. Long-term follow-up study of Chinese patients with YMDD mutations: significance of hepatitis B virus genotypes and characteristics of biochemical flares. J Clin Microbiol 2004; 9: 3932-36.

47. Zollner B, Petersen J, Schro¨ter M, Laufs R, Volker S, Feucht H. 20-fold increase in risk of lamivudine resistance in hepatitis B virus subtype adw. Lancet 2001; 357: 934–35.

48. Kao JH, Liu CJ, Chen DS. Hepatitis B viral genotypes and lamivudine resistance. J Hepatol 2002; 36: 303-304.

49. Li D, Gu HX, Zhang SY, Zhong ZH, Zhuang M, Hattori T. YMDD mutations and genotypes of hepatitis B virus in northern China. Jpn J Infect Dis 2006; 59: 42–45.

50. Akuta N, Suzuki F, Kobayashi M. Virological and biochemical relapse after discontinuation of lamivudine monotherapy for chronic hepatitis B in Japan: comparison with breakthrough hepatitis during long-term treatment. Intervirology 2005; 48: 174–82.

51. Horgan M, Brannigan E, Crowley B, Levis J, Fanning L. Hepatitis B genotype and YMDD profile in an untreated Irish population. J Clin Virol 2006; 35: 203–204.

52. Jafri W, Jafri N, Yakoob J, Islam M, Tirmizi A, Jafar T, et al. Hepatitis B and C: prevalence and risk factors associated with seropositivity among children in Karachi, Pakistan. BMC Infect Dis 2006; 6: 101.

53. Qureshi H, Arif A, Ahmed W, Alam SE. Response to lamivudine treatment in core and pre core mutants of HBV. (Abstract) PSG 2006 Karachi.

54. Lai CL, Dienstag J, Schiff E, Leung NW, Atkins M, Hunt C. Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. Clin Infect Dis 2003; 36: 687-96.

55. Baig S, Siddiqui AA, Ahmed W, Qureshi H, Arif A. The association of complex liver disorders with HBV genotypes prevalent in Pakistan. Virol J 2007; 4: 128.

56. Alam MM, Zaidi SZ, Shaukat S, Naeem A, Sharif S, Angez M, et al. Molecular epidemiology of Hepatitis B virus genotypes in Pakistan. BMC Infect Dis 2007; 7: 115.

57. Idrees M, Khan S, and Riazuddin SM. Common genotypes of hepatitis B Virus. J Coll Physicians Surg Pak 2004; 14: 344-47.

58. Alam MM, Zaidi SZ, Shaukat S, Sharif S, Angez M, Naeem A, et al. Common genotypes of hepatitis B virus prevalent in injecting drug abusers (addicts) of North West frontier province of Pakistan. Virol J 2007; 4: 63.