REFERENCES

1. Girones, R. and Miller, R. H. " Mutation rate of the hepadnavirus genome" , Virology, 1989; 170: 595-7.

2. Blum, H. E. , Moradpour, D. , von Weizsacker, F. , Wieland, S. , Peters, T. , and Rasenack, J. W. " [Hepatitis B virus mutants--clinical significance] " , Z Gastroenterol, 1997; 35: 347-55.

3. Thomas, H.C. "The hepatitis B virus and the host response", J Hepatol, 1990; 11 Suppl 1: S83-9.

4. Benhamou, Y., Bochet, M., Thibault, V., Di Martino, V., Caumes, E., Bricaire, F., *et al.* "Long-term incidence of hepatitis B virus resistance to lamivudine in human immunodeficiency virus-infected patients", Hepatology, 1999; 30: 1302-6.

5. Pessoa, M. G. , Gazzard, B. , Huang, A. K. , Brandao-Mello, C. E. , Cassetti, I. , Mendes-Correa, M.C., *et al.* "Efficacy and safety of entecavir for chronic HBV in HIV/ HBV coinfected patients receiving lamivudine as part of antiretroviral therapy", AIDS, 2008; 22: 1779-87.

6. Locarnini, S. and Zoulim, F. " Molecular genetics of HBV infection" , Antivir Ther, 2010; 15 Suppl 3: 3-14.

7. Takahashi, K. , Ohta, Y. , Kanai, K. , Akahane, Y. , Iwasa, Y. , Hino, K. , *et al.* "Clinical implications of mutations C-to-T1653 and T-to-C/A/G1753 of hepatitis B virus genotype C genome in chronic liver disease" , Arch Virol, 1999; 144: 1299-308.

8. Chu, C.M., Lin, C.C., Chen, Y.C., Jeng, W.J., Lin, S.M., and Liaw, Y.F. "Basal core promoter mutation is associated with progression to cirrhosis rather than hepatocellular carcinoma in chronic hepatitis B virus infection" , Br J Cancer, 2012; 107: 2010-5.

9. Carman, W.F., Jacyna, M.R., Hadziyannis, S., Karayiannis, P., McGarvey, M.J., Makris, A., *et al.* "Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection", Lancet, 1989; 2: 588-91.

10. Buckwold, V.E., Xu, Z., Chen, M., Yen, T.S., and Ou, J.H. "Effects of a naturally occurring mutation in the hepatitis B virus basal core promoter on precore gene expression and viral replication", J Virol, 1996; 70: 5845-51.

11. Baptista, M., Kramvis, A., and Kew, M.C. "High prevalence of 1762(T) 1764(A) mutations in the basic core promoter of hepatitis B virus isolated from black Africans with hepatocellular carcinoma compared with asymptomatic carriers", Hepatology, 1999; 29: 946-53.

12. Wai, C.T. and Fontana, R.J. "Clinical significance of hepatitis B virus genotypes, variants, and mutants", Clin Liver Dis, 2004; 8: 321-52.

13. Bahn, A., Hilbert, K., Martine, U., Westedt, J., von Weizsacker, F., and Wirth, S. "Selection of a precore mutant after vertical transmission of different hepatitis B virus variants is correlated with fulminant hepatitis in infants", J Med Virol, 1995; 47: 336-41.

14. Cheng, H., Su, H., Wang, S., Shao, Z., Men, K., Li, M., *et al.* "Association between genomic heterogeneity of hepatitis B virus and intrauterine infection", Virology, 2009; 387: 168-75.

15. Jamieson, D.J., Theiler, R.N., and Rasmussen, S.A. "Emerging infections and pregnancy", Emerg Infect Dis, 2006; 12: 1638-43.

16. Okoko, B.J., Enwere, G., and Ota, M.O. "The epidemiology and consequences of maternal malaria: a review of immunological basis", Acta Trop, 2003; 87: 193-205.

17. Paryani, S.G. and Arvin, A.M. "Intrauterine infection with varicella-zoster virus after maternal varicella", N Engl J Med, 1986; 314: 1542-6.

18. Rogerson, S.J., Hviid, L., Duffy, P.E., Leke, R.F., and Taylor, D.W. "Malaria in pregnancy: pathogenesis and immunity", Lancet Infect Dis, 2007; 7: 105-17.

19. ter Borg, M.J., Leemans, W.F., de Man, R.A., and Janssen, H.L. "Exacerbation of chronic hepatitis B infection after delivery", J Viral Hepat, 2008; 15: 37-41.

20. Soderstrom, A., Norkrans, G., and Lindh, M. "Hepatitis B virus DNA during pregnancy and post partum: aspects on vertical transmission", Scand J Infect Dis, 2003; 35: 814-9.

21. Almog, Y., Klein, A., Adler, R., Laub, O., and Tur-Kaspa, R. "Estrogen suppresses hepatitis B virus expression in male athymic mice transplanted with HBV transfected Hep G-2 cells", Antiviral Res, 1992; 19: 285-93.

22. Ngui, S.L., Andrews, N.J., Underhill, G.S., Heptonstall, J., and Teo, C.G. "Failed postnatal immunoprophylaxis for hepatitis B: characteristics of maternal hepatitis B virus as risk factors", Clin Infect Dis, 1998; 27: 100-6.

23. Beasley, R.P., Trepo, C., Stevens, C.E., and Szmuness, W. "The e antigen and vertical transmission of hepatitis B surface antigen", Am J Epidemiol, 1977; 105: 94-8.

24. Khamduang, W., Gaudy-Graffin, C., Ngo-Giang-Huong, N., Jourdain, G., Moreau, A., Borkird, T., *et al.* "Analysis of residual perinatal transmission of hepatitis B virus (HBV) and of genetic variants in human immunodeficiency virus and HBV co-infected women and their offspring", J Clin Virol, 2013; 58: 415-21.

25. Hawkins, A.E., Gilson, R.J., Beath, S.V., Boxall, E.H., Kelly, D.A., Tedder, R.S., *et al.* "Novel application of a point mutation assay: evidence for transmission of hepatitis B viruses with precore mutations and their detection in infants with fulminant hepatitis B", J Med Virol, 1994; 44: 13-21.

26. Colin, J.F., Cazals-Hatem, D., Loriot, M.A., Martinot-Peignoux, M., Pham, B.N., Auperin, A., *et al.* "Influence of human immunodeficiency virus infection on chronic hepatitis B in homosexual men", Hepatology, 1999; 29: 1306-10.

27. Falade-Nwulia, O., Seaberg, E.C., Rinaldo, C.R., Badri, S., Witt, M., and Thio, C.L. "Comparative risk of liver-related mortality from chronic hepatitis B versus chronic hepatitis C virus infection", Clin Infect Dis, 2012; 55: 507-13.

28. Thio, C.L., Seaberg, E.C., Skolasky, R., Jr., Phair, J., Visscher, B., Munoz, A., *et al.* "HIV-1, hepatitis B virus, and risk of liver-related mortality in the Multicenter Cohort Study (MACS)", Lancet, 2002; 360: 1921-6.

29. Audsley, J., Littlejohn, M., Yuen, L., Sasadeusz, J., Ayres, A., Desmond, C., *et al.* " HBV mutations in untreated HIV-HBV co-infection using genomic length sequencing", Virology, 2010; 405: 539-47.

30. Cassino, L., Torres, C., Mbayed, V., Laufer, N., Campos, R.H., and Quarleri, J. " Comparative analysis of hepatitis B virus genotype a molecular evolution in patients infected with HBV and in patients co-infected with HBV and HIV" , J Med Virol, 2012; 84: 562-9.

31. Svicher, V., Gori, C., and Trignetti, M. *HIV-driven Immunosuppression Regulates HBV Evolution in HBV/ HIV-co-infected Patients*. in *6th Conference on Retroviruses and Opportunistic Infections (CROI 2009)*. 2009. Montreal, Canada.

32. Tangkijvanich, P. , Sa-Nguanmoo, P. , Avihingsanon, A. , Ruxrungtham, K. , Poovorawan, K. , and Poovorawan, Y. " Characterization of hepatitis B virus mutations in untreated patients co-infected with HIV and HBV based on complete genome sequencing", J Med Virol, 2013; 85: 16-25.

33. Alter, M.J. "Epidemiology of hepatitis B in Europe and worldwide", J Hepatol, 2003; 39 Suppl 1: S64-9.

34. Prince, A.M. "Relation of Australia and SH antigens", Lancet, 1968; 2: 462-3.

35. Nielsen, J.O., Dietrichson, O., Elling, P., and Christoffersen, P. "Incidence and meaning of persistence of Australia antigen in patients with acute viral hepatitis: development of chronic hepatitis", N Engl J Med, 1971; 285: 1157-60.

36. Wright, R., McCollum, R.W., and Klatskin, G. "Australia antigen in acute and chronic liver disease", Lancet, 1969; 2: 117-21.

37. London, W.T., Sutnick, A.I., and Blumberg, B.S. "Australia antigen and acute viral hepatitis", Ann Intern Med, 1969; 70: 55-9.

38. Seeger, C. and Mason, W.S. "Hepatitis B virus biology", Microbiol Mol Biol Rev, 2000; 64: 51-68.

39. Sprengel, R., Kaleta, E.F., and Will, H. "Isolation and characterization of a hepatitis B virus endemic in herons", J Virol, 1988; 62: 3832-9.

40. Ganem, D. and Varmus, H.E. "The molecular biology of the hepatitis B viruses", Annu Rev Biochem, 1987; 56: 651-93.

41. Chai, N., Chang, H.E., Nicolas, E., Han, Z., Jarnik, M., and Taylor, J. "Properties of subviral particles of hepatitis B virus", J Virol, 2008; 82: 7812-7.

42. Block, T.M., Guo, H., and Guo, J.T. "Molecular virology of hepatitis B virus for clinicians", Clin Liver Dis, 2007; 11: 685-706, vii.

43. Locarnini, S., McMillan, J., and Bartholomeusz, A. "The hepatitis B virus and common mutants", Semin Liver Dis, 2003; 23: 5-20.

44. Ngui, S.L., Hallet, R., and Teo, C.G. "Natural and iatrogenic variation in hepatitis B virus", Rev Med Virol, 1999; 9: 183-209.

45. Zhang, Q. and Cao, G. "Genotypes, mutations, and viral load of hepatitis B virus and the risk of hepatocellular carcinoma: HBV properties and hepatocarcinogenesis", Hepat Mon, 2011; 11: 86-91.

46. Wang, Y.X., Luo, C., Zhao, D., Beck, J., and Nassal, M. "Extensive mutagenesis of the conserved box E motif in duck hepatitis B virus P protein reveals multiple functions in replication and a common structure with the primer grip in HIV-1 reverse transcriptase", J Virol, 2012; 86: 6394-407.

47. Stuyver, L.J., Locarnini, S.A., Lok, A., Richman, D.D., Carman, W.F., Dienstag, J.L., *et al.* "Nomenclature for antiviral-resistant human hepatitis B virus mutations in the polymerase region", Hepatology, 2001; 33: 751-7.

48. Oberhaus, S.M. and Newbold, J.E. "Detection of an RNase H activity associated with hepadnaviruses", J Virol, 1995; 69: 5697-704.

49. Kramvis, A. and Kew, M.C. "The core promoter of hepatitis B virus", J Viral Hepat, 1999; 6: 415-27.

50. Ganem, D. and Schneider, R.J., *Hepadnaviridae: the viruses and their replication*. 4th ed. 2001, Lippincott Williams: Wilkins, Philadelphia, USA.

51. Magnius, L.O. and Norder, H. "Subtypes, genotypes and molecular epidemiology of the hepatitis B virus as reflected by sequence variability of the S-gene", Intervirology, 1995; 38: 24-34.

52. Yano, Y., Azuma, T., and Hayashi, Y. "Variations and mutations in the hepatitis B virus genome and their associations with clinical characteristics", World J Hepatol, 2015; 7: 583-92.

53. Chen, H.S., Kaneko, S., Girones, R., Anderson, R.W., Hornbuckle, W.E., Tennant, B.C., *et al.* "The woodchuck hepatitis virus X gene is important for establishment of virus infection in woodchucks", J Virol, 1993; 67: 1218-26.

54. Zoulim, F., Saputelli, J., and Seeger, C. "Woodchuck hepatitis virus X protein is required for viral infection in vivo", J Virol, 1994; 68: 2026-30.

55. Birrer, R.B., Birrer, D., and Klavins, J.V. "Hepatocellular carcinoma and hepatitis virus", Ann Clin Lab Sci, 2003; 33: 39-54.

56. Sung, W.K., Lu, Y., Lee, C.W., Zhang, D., Ronaghi, M., and Lee, C.G. "Deregulated direct targets of the hepatitis B virus (HBV) protein, HBx, identified through chromatin immunoprecipitation and expression microarray profiling", J Biol Chem, 2009; 284: 21941-54.

57. Klingmuller, U. and Schaller, H. "Hepadnavirus infection requires interaction between the viral pre-S domain and a specific hepatocellular receptor", J Virol, 1993; 67: 7414-22.

58. Yan, H., Zhong, G., Xu, G., He, W., Jing, Z., Gao, Z., *et al.* "Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus", Elife, 2012; 1: e00049.

59. Zhang, Y.Y., Zhang, B.H., Theele, D., Litwin, S., Toll, E., and Summers, J. "Single-cell analysis of covalently closed circular DNA copy numbers in a hepadnavirus-infected liver", Proc Natl Acad Sci U S A, 2003; 100: 12372-7.

60. Bruss, V. "Hepatitis B virus morphogenesis", World J Gastroenterol, 2007; 13: 65-73.

61. Germer, J.J., Qutub, M.O., Mandrekar, J.N., Mitchell, P.S., and Yao, J.D. "Quantification of hepatitis B virus (HBV) DNA with a TaqMan HBV analyte-specific reagent following sample processing with the MagNA pure LC instrument", J Clin Microbiol, 2006; 44: 1490-4.

62. McMahon, B.J., Alward, W.L., Hall, D.B., Heyward, W.L., Bender, T.R., Francis, D.P., *et al.* "Acute hepatitis B virus infection: relation of age to the clinical expression of disease and subsequent development of the carrier state", J Infect Dis, 1985; 151: 599-603.

63. Hyams, K.C. "Risks of chronicity following acute hepatitis B virus infection: a review", Clin Infect Dis, 1995; 20: 992-1000.

64. Shepard, C.W., Simard, E.P., Finelli, L., Fiore, A.E., and Bell, B.P. "Hepatitis B virus infection: epidemiology and vaccination", Epidemiol Rev, 2006; 28: 112-25.

65. Lok, A.S. and McMahon, B.J. "Chronic hepatitis B: update 2009", Hepatology, 2009; 50: 661-2.

66. Liang, T.J. "Hepatitis B: the virus and disease", Hepatology, 2009; 49: S13-21.

67. Gitlin, N. "Hepatitis B: diagnosis, prevention, and treatment", Clin Chem, 1997; 43: 1500-6.

68. Kwon, H. and Lok, A.S. "Hepatitis B therapy", Nat Rev Gastroenterol Hepatol, 2011; 8: 275-84.

69. Ribeiro, R.M., Lo, A., and Perelson, A.S. "Dynamics of hepatitis B virus infection", Microbes Infect, 2002; 4: 829-35.

70. McMahon, B.J. "The natural history of chronic hepatitis B virus infection", Hepatology, 2009; 49: S45-55.

71. Manno, M., Camma, C., Schepis, F., Bassi, F., Gelmini, R., Giannini, F., *et al.* "Natural history of chronic HBV carriers in northern Italy: morbidity and mortality after 30 years", Gastroenterology, 2004; 127: 756-63.

72. McMahon, B.J., Holck, P., Bulkow, L., and Snowball, M. "Serologic and clinical outcomes of 1536 Alaska Natives chronically infected with hepatitis B virus", Ann Intern Med, 2001; 135: 759-68.

73. Hoofnagle, J.H. "Reactivation of hepatitis B", Hepatology, 2009; 49: S156-65.

74. Funk, M.L., Rosenberg, D.M., and Lok, A.S. "World-wide epidemiology of HBeAg-negative chronic hepatitis B and associated precore and core promoter variants", J Viral Hepat, 2002; 9: 52-61.

75. Guidotti, L.G. and Chisari, F.V. "Immunobiology and pathogenesis of viral hepatitis", Annu Rev Pathol, 2006; 1: 23-61.

76. Milich, D.R. and McLachlan, A. "The nucleocapsid of hepatitis B virus is both a T-cell-independent and a T-cell-dependent antigen", Science, 1986; 234: 1398-401.

77. Bond, W.W., Favero, M.S., Petersen, N.J., Gravelle, C.R., Ebert, J.W., and Maynard, J.E. "Survival of hepatitis B virus after drying and storage for one week", Lancet, 1981; 1: 550-1.

78. Beeson, P.B. "Jaundice occurring one to four months after transfusion of blood or plasma: Report of seven cases", Journal of the American Medical Association, 1943; 121: 1332-1334.

79. Roth, W.K., Weber, M., Petersen, D., Drosten, C., Buhr, S., Sireis, W., *et al.* "NAT for HBV and anti-HBc testing increase blood safety", Transfusion, 2002; 42: 869-75.

80. Allain, J.P. and Candotti, D. "Diagnostic algorithm for HBV safe transfusion", Blood Transfus, 2009; 7: 174-82.

81. Seo, D.H., Whang, D.H., Song, E.Y., and Han, K.S. "Occult hepatitis B virus infection and blood transfusion", World J Hepatol, 2015; 7: 600-6.

82. Lettau, L.A., McCarthy, J.G., Smith, M.H., Hadler, S.C., Morse, L.J., Ukena, T., *et al.* "Outbreak of severe hepatitis due to delta and hepatitis B viruses in parenteral drug abusers and their contacts", N Engl J Med, 1987; 317: 1256-62.

83. Polish, L.B., Shapiro, C.N., Bauer, F., Klotz, P., Ginier, P., Roberto, R.R., *et al.* "Nosocomial transmission of hepatitis B virus associated with the use of a spring-loaded finger-stick device", N Engl J Med, 1992; 326: 721-5.

84. Alter, M.J., Ahtone, J., Weisfuse, I., Starko, K., Vacalis, T.D., and Maynard, J.E. "Hepatitis B virus transmission between heterosexuals", JAMA, 1986; 256: 1307-10.

85. Borgia, G., Carleo, M.A., Gaeta, G.B., and Gentile, I. "Hepatitis B in pregnancy", World J Gastroenterol, 2012; 18: 4677-83.

86. Li, X.M., Yang, Y.B., Hou, H.Y., Shi, Z.J., Shen, H.M., Teng, B.Q., *et al.* "Interruption of HBV intrauterine transmission: a clinical study", World J Gastroenterol, 2003; 9: 1501-3.

87. Bai, H., Zhang, L., Ma, L., Dou, X.G., Feng, G.H., and Zhao, G.Z. "Relationship of hepatitis B virus infection of placental barrier and hepatitis B virus intra-uterine transmission mechanism", World J Gastroenterol, 2007; 13: 3625-30.

88. Gentile, I. and Borgia, G. "Vertical transmission of hepatitis B virus: challenges and solutions", Int J Womens Health, 2014; 6: 605-11.

89. Zhang, S.L., Yue, Y.F., Bai, G.Q., Shi, L., and Jiang, H. "Mechanism of intrauterine infection of hepatitis B virus", World J Gastroenterol, 2004; 10: 437-8.

90. Pan, C.Q., Zou, H.B., Chen, Y., Zhang, X., Zhang, H., Li, J., *et al.* "Cesarean section reduces perinatal transmission of hepatitis B virus infection from hepatitis B surface antigen-positive women to their infants", Clin Gastroenterol Hepatol, 2013; 11: 1349-55.

91. de Oliveira, P.R., Yamamoto, A.Y., de Souza, C.B., de Araujo, N.M., de Andrade Gomes, S., Heck, A.R., *et al.* "Hepatitis B viral markers in banked human milk before and after Holder pasteurization", J Clin Virol, 2009; 45: 281-4.

92. Zheng, Y., Lu, Y., Ye, Q., Xia, Y., Zhou, Y., Yao, Q., *et al.* "Should chronic hepatitis B mothers breastfeed? a meta analysis", BMC Public Health, 2011; 11: 502.

93. Lee, C., Gong, Y., Brok, J., Boxall, E.H., and Gluud, C. "Effect of hepatitis B immunisation in newborn infants of mothers positive for hepatitis B surface antigen: systematic review and meta-analysis", BMJ, 2006; 332: 328-36.

94. Hino, K., Okuda, M., Hashimoto, O., Ishiko, H., Okazaki, M., Fujii, K., *et al.* "Glycine-to-arginine substitution at codon 145 of HBsAg in two infants born to hepatitis B e antigen-positive carrier", Dig Dis Sci, 1995; 40: 566-70.

95. Schweitzer, A., Horn, J., Mikolajczyk, R.T., Krause, G., and Ott, J.J. "Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013", Lancet, 2015; 386: 1546-55.

96. Lavanchy, D. and Kane, M., *Global Epidemiology of Hepatitis B Virus Infection*, in *Hepatitis B Virus in Human Diseases*, Y.-F. Liaw and F. Zoulim, Editors. 2016, Springer International Publishing: Cham. p. 187-203.

97. Jutavijittum, P., Yousukh, A., Jiviriyawat, Y., Kunachiwa, W., and Toriyama, K. " Genotypes of hepatitis B virus among children in Chiang Mai, Thailand" , Southeast Asian J Trop Med Public Health, 2008; 39: 394-7.

98. Chongsrisawat, V., Yoocharoen, P., Theamboonlers, A., Tharmaphornpilas, P., Warinsathien, P., Sinlaparatsamee, S., *et al.* " Hepatitis B seroprevalence in Thailand: 12 years after hepatitis B vaccine integration into the national expanded programme on immunization", Trop Med Int Health, 2006; 11: 1496-502.

99. Chimparlee, N., Oota, S., Phikulsod, S., Tangkijvanich, P., and Poovorawan, Y. "Hepatitis B and hepatitis C virus in Thai blood donors", Southeast Asian J Trop Med Public Health, 2011; 42: 609-15.

100. Sungkanuparph, S., Vibhagool, A., Manosuthi, W., Kiertiburanakul, S., Atamasirikul, K., Aumkhyan, A., *et al.* " Prevalence of hepatitis B virus and hepatitis C virus co-infection with human immunodeficiency virus in Thai patients: a tertiary-care-based study", J Med Assoc Thai, 2004; 87: 1349-54.

101. Tsuchiya, N., Pathipvanich, P., Rojanawiwat, A., Wichukchinda, N., Koga, I., Koga, M., *et al.* " Chronic hepatitis B and C co-infection increased all-cause mortality in HAART-naive HIV patients in Northern Thailand", Epidemiol Infect, 2013; 141: 1840-8.

102. Aurpibul, L., Lumbiganon, P., Kolasaraksa, P., Hansudewechakul, R., Sa-Nguanmoo, P., Taeprasert, P., *et al.* " HIV and Hepatitis B coinfection among perinatally HIV-infected Thai adolescents", Pediatr Infect Dis J, 2012; 31: 943-7.

103. Zhang, Z.H., Wu, C.C., Chen, X.W., Li, X., Li, J., and Lu, M.J. "Genetic variation of hepatitis B virus and its significance for pathogenesis", World J Gastroenterol, 2016; 22: 126-44.

104. Kay, A. and Zoulim, F. " Hepatitis B virus genetic variability and evolution" , Virus Res, 2007; 127: 164-76.

105. Okamoto, H. , Tsuda, F. , Sakugawa, H. , Sastrosoewignjo, R. I. , Imai, M. , Miyakawa, Y. , *et al.* " Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes" , J Gen Virol, 1988; 69 (Pt 10): 2575-83.

106. Stuyver, L., De Gendt, S., Van Geyt, C., Zoulim, F., Fried, M., Schinazi, R.F., *et al.* " A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness", J Gen Virol, 2000; 81: 67-74.

107. Arauz-Ruiz, P., Norder, H., Robertson, B.H., and Magnius, L.O. "Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America" , J Gen Virol, 2002; 83: 2059-73.

108. Norder, H. , Courouce, A. M. , and Magnius, L. O. " Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes", Virology, 1994; 198: 489-503.

109. Tran, T. T. , Trinh, T. N. , and Abe, K. " New complex recombinant genotype of hepatitis B virus identified in Vietnam", J Virol, 2008; 82: 5657-63.

110. Tatematsu, K., Tanaka, Y., Kurbanov, F., Sugauchi, F., Mano, S., Maeshiro, T., *et al.* "A genetic variant of hepatitis B virus divergent from known human and ape genotypes isolated from a Japanese patient and provisionally assigned to new genotype J", J Virol, 2009; 83: 10538-47.

111. Jutavijittum, P., Jiviriyawat, Y., Yousukh, A., Kunachiwa, W., and Toriyama, K. " Genotypes of hepatitis B virus among voluntary blood donors in northern Thailand", Hepatol Res, 2006; 35: 263-6.

112. Louisirirotchanakul, S., Olinger, C.M., Arunkaewchaemsri, P., Poovorawan, Y., Kanoksinsombat, C., Thongme, C., *et al.* "The distribution of hepatitis B virus genotypes in Thailand", J Med Virol, 2012; 84: 1541-7.

113. Sunbul, M. " Hepatitis B virus genotypes: global distribution and clinical importance", World J Gastroenterol, 2014; 20: 5427-34.

114. Chen, X., Dai, B., Liu, Z., Gao, J., Ji, Z., Guo, J., *et al.* "A novel B/C inter-genotype recombinant of hepatitis B virus identified in north-west China", J Gen Virol, 2014; 95: 153-5.

115. Suwannakarn, K., Tangkijvanich, P., Theamboonlers, A., Abe, K., and Poovorawan, Y. "A novel recombinant of Hepatitis B virus genotypes G and C isolated from a Thai patient with hepatocellular carcinoma", J Gen Virol, 2005; 86: 3027-30.

116. Baumert, T.F., Barth, H., and Blum, H.E. "Genetic variants of hepatitis B virus and their clinical relevance", Minerva Gastroenterol Dietol, 2005; 51: 95-108.

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

118. Pawlotsky, J.M. "The concept of hepatitis B virus mutant escape", J Clin Virol, 2005; 34 Suppl 1: S125-9.

119. Chang, J.J. and Lewin, S.R. "Immunopathogenesis of hepatitis B virus infection", Immunol Cell Biol, 2007; 85: 16-23.

120. Akahane, Y., Yamanaka, T., Suzuki, H., Sugai, Y., Tsuda, F., Yotsumoto, S., *et al.* "Chronic active hepatitis with hepatitis B virus DNA and antibody against e antigen in the serum. Disturbed synthesis and secretion of e antigen from hepatocytes due to a point mutation in the precore region", Gastroenterology, 1990; 99: 1113-9.

121. Tong, S., Diot, C., Gripon, P., Li, J., Vitvitski, L., Trépo, C., *et al.* "In vitro replication competence of a cloned hepatitis B virus variant with a nonsense mutation in the distal pre-C region", Virology, 1991; 181: 733-737.

122. Raimondo, G., Campo, S., Smedile, V., Rodino, G., Sardo, M.A., Brancatelli, S., *et al.* "Hepatitis B virus variant, with a deletion in the preS2 and two translational stop codons in the precore regions, in a patient with hepatocellular carcinoma", Journal of Hepatology, 1991; 13, Supplement 4: S74-S77.

123. Okamoto, H., Yotsumoto, S., Akahane, Y., Yamanaka, T., Miyazaki, Y., Sugai, Y., *et al.* "Hepatitis B viruses with precore region defects prevail in persistently infected hosts along with seroconversion to the antibody against e antigen" , J Virol, 1990; 64: 1298-303.

124. Baumert, T.F., Rogers, S.A., Hasegawa, K., and Liang, T.J. "Two core promotor mutations identified in a hepatitis B virus strain associated with fulminant hepatitis result in enhanced viral replication", J Clin Invest, 1996; 98: 2268-76.

125. Parekh, S., Zoulim, F., Ahn, S.H., Tsai, A., Li, J., Kawai, S., *et al.* "Genome replication, virion secretion, and e antigen expression of naturally occurring hepatitis B virus core promoter mutants", J Virol, 2003; 77: 6601-12.

126. Jammeh, S., Tavner, F., Watson, R., Thomas, H.C., and Karayiannis, P. "Effect of basal core promoter and pre-core mutations on hepatitis B virus replication" , J Gen Virol, 2008; 89: 901-9.

127. Matthews, G.V., Bartholomeusz, A., Locarnini, S., Ayres, A., Sasaduesz, J., Seaberg, E., *et al.* " Characteristics of drug resistant HBV in an international collaborative study of HIV-HBV-infected individuals on extended lamivudine therapy", AIDS, 2006; 20: 863-70.

128. Tenney, D.J., Levine, S.M., Rose, R.E., Walsh, A.W., Weinheimer, S.P., Discotto, L., *et al.* "Clinical Emergence of Entecavir-Resistant Hepatitis B Virus Requires Additional Substitutions in Virus Already Resistant to Lamivudine" , Antimicrobial Agents and Chemotherapy, 2004; 48: 3498-3507.

129. Protzer-Knolle, U., Naumann, U., Bartenschlager, R., Berg, T., Hopf, U., Meyer zum Buschenfelde, K.H., *et al.* " Hepatitis B virus with antigenically altered hepatitis B surface antigen is selected by high-dose hepatitis B immune globulin after liver transplantation", Hepatology, 1998; 27: 254-63.

130. Lukhwareni, A., Burnett, R.J., Selabe, S.G., Mzileni, M.O., and Mphahlele, M.J. "Increased detection of HBV DNA in HBsAg-positive and HBsAg-negative South African HIV/AIDS patients enrolling for highly active antiretroviral therapy at a Tertiary Hospital", J Med Virol, 2009; 81: 406-12.

131. Carman, W.F., Zanetti, A.R., Karayiannis, P., Waters, J., Manzillo, G., Tanzi, E., *et al.* "Vaccine-induced escape mutant of hepatitis B virus", Lancet, 1990; 336: 325-9.

132. Torresi, J. " The virological and clinical significance of mutations in the overlapping envelope and polymerase genes of hepatitis B virus", J Clin Virol, 2002; 25: 97-106.

133. Kamili, S., Sozzi, V., Thompson, G., Campbell, K., Walker, C.M., Locarnini, S., *et al.* "Efficacy of hepatitis B vaccine against antiviral drug-resistant hepatitis B virus mutants in the chimpanzee model", Hepatology, 2009; 49: 1483-91.

134. Leon, B., Taylor, L., Vargas, M., Luftig, R.B., Albertazzi, F., Herrero, L., *et al.* "HBx M130K and V131I (T-A) mutations in HBV genotype F during a follow-up study in chronic carriers", Virol J, 2005; 2: 60.

135. Chen, G. G., Li, M. Y., Ho, R. L., Chak, E. C., Lau, W. Y., and Lai, P. B. "Identification of hepatitis B virus X gene mutation in Hong Kong patients with hepatocellular carcinoma", J Clin Virol, 2005; 34: 7-12.

136. Tu, H., Bonura, C., Giannini, C., Mouly, H., Soussan, P., Kew, M., *et al.* " Biological impact of natural COOH-terminal deletions of hepatitis B virus X protein in hepatocellular carcinoma tissues", Cancer Res, 2001; 61: 7803-10.

137. Gilson, R.J., Hawkins, A.E., Beecham, M.R., Ross, E., Waite, J., Briggs, M., *et al.* "Interactions between HIV and hepatitis B virus in homosexual men: effects on the natural history of infection", AIDS, 1997; 11: 597-606.

138. Puoti, M., Airoldi, M., Bruno, R., Zanini, B., Spinetti, A., Pezzoli, C., *et al.* " Hepatitis B virus co-infection in human immunodeficiency virus-infected subjects", AIDS Rev, 2002; 4: 27-35.

139. Ockenga, J., Tillmann, H.L., Trautwein, C., Stoll, M., Manns, M.P., and Schmidt, R.E. "Hepatitis B and C in HIV-infected patients. Prevalence and prognostic value", J Hepatol, 1997; 27: 18-24.

140. Nguyen, G., Garcia, R.T., Nguyen, N., Trinh, H., Keeffe, E.B., and Nguyen, M.H. "Clinical course of hepatitis B virus infection during pregnancy", Aliment Pharmacol Ther, 2009; 29: 755-64.

141. Wong, S., Chan, L.Y., Yu, V., and Ho, L. "Hepatitis B carrier and perinatal outcome in singleton pregnancy", Am J Perinatol, 1999; 16: 485-8.

142. Tse, K.Y., Ho, L.F., and Lao, T. "The impact of maternal HBsAg carrier status on pregnancy outcomes: a case-control study", J Hepatol, 2005; 43: 771-5.

143. Kock, K., Kock, F., Klein, K., Bancher-Todesca, D., and Helmer, H. "Diabetes mellitus and the risk of preterm birth with regard to the risk of spontaneous preterm birth", J Matern Fetal Neonatal Med, 2010; 23: 1004-8.

144. Leung, T.Y., Chan, L.W., Tam, W.H., Leung, T.N., and Lau, T.K. "Risk and prediction of preterm delivery in pregnancies complicated by antepartum hemorrhage of unknown origin before 34 weeks", Gynecol Obstet Invest, 2001; 52: 227-31.

145. Wang, Z., Zhang, J., Yang, H., Li, X., Wen, S., Guo, Y., *et al.* "Quantitative analysis of HBV DNA level and HBeAg titer in hepatitis B surface antigen positive mothers and their babies: HBeAg passage through the placenta and the rate of decay in babies", J Med Virol, 2003; 71: 360-6.

146. Wong, V.C., Ip, H.M., Reesink, H.W., Lelie, P.N., Reerink-Brongers, E.E., Yeung, C.Y., *et al.* "Prevention of the HBsAg carrier state in newborn infants of mothers who are chronic carriers of HBsAg and HBeAg by administration of hepatitis-B vaccine and hepatitis-B immunoglobulin. Double-blind randomised placebo-controlled study", Lancet, 1984; 1: 921-6.

147. Okada, K., Kamiyama, I., Inomata, M., Imai, M., and Miyakawa, Y. "e antigen and anti-e in the serum of asymptomatic carrier mothers as indicators of positive and negative transmission of hepatitis B virus to their infants", N Engl J Med, 1976; 294: 746-9.

148. Lallemant, M., Jourdain, G., Le Coeur, S., Kim, S., Koetsawang, S., Comeau, A.M., *et al.* "A trial of shortened zidovudine regimens to prevent mother-to-child transmission of human immunodeficiency virus type 1. Perinatal HIV Prevention Trial (Thailand) Investigators", N Engl J Med, 2000; 343: 982-91.

149. Lallemant, M., Jourdain, G., Le Coeur, S., Mary, J.Y., Ngo-Giang-Huong, N., Koetsawang, S., *et al.* "Single-dose perinatal nevirapine plus standard zidovudine to prevent mother-to-child transmission of HIV-1 in Thailand", N Engl J Med, 2004; 351: 217-28.

150. Gunther, S., Li, B.C., Miska, S., Kruger, D.H., Meisel, H., and Will, H. "A novel method for efficient amplification of whole hepatitis B virus genomes permits rapid functional analysis and reveals deletion mutants in immunosuppressed patients", J Virol, 1995; 69: 5437-44.

151. Gunther, S., Sommer, G., Von Breunig, F., Iwanska, A., Kalinina, T., Sterneck, M., *et al.* "Amplification of full-length hepatitis B virus genomes from samples from patients with low levels of viremia: frequency and functional consequences of PCR-introduced mutations", J Clin Microbiol, 1998; 36: 531-8.

152. Kimura, M. " A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences", J Mol Evol, 1980; 16: 111-20.

153. Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. "MEGA6: Molecular Evolutionary Genetics Analysis version 6.0", Mol Biol Evol, 2013; 30: 2725-9.

154. Tamura, K. and Nei, M. "Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees", Mol Biol Evol, 1993; 10: 512-26.

155. Su, H.X., Xu, D.Z., Li, D., Zhang, J.X., Lu, J., Choi, B.C., *et al.* "Heterogeneity analysis of the hepatitis B virus genome in intrauterine infection", J Med Virol, 2005; 77: 180-7.

156. Pollicino, T., Cacciola, I., Saffioti, F., and Raimondo, G. "Hepatitis B virus PreS/S gene variants: pathobiology and clinical implications", J Hepatol, 2014; 61: 408-17.

157. Wu, Q., Xu, C., Li, J., Li, L., Yan, G., Yue, L., *et al.* "Evolution and mutations of hepatitis B virus quasispecies in genotype B and C during vertical transmission", J Med Virol, 2016; 88: 1018-26.

158. Livingston, S.E., Simonetti, J.P., Bulkow, L.R., Homan, C.E., Snowball, M.M., Cagle, H.H., *et al.* "Clearance of hepatitis B e antigen in patients with chronic hepatitis B and genotypes A, B, C, D, and F", Gastroenterology, 2007; 133: 1452-7.

159. Chu, C.J., Keeffe, E.B., Han, S.H., Perrillo, R.P., Min, A.D., Soldevila-Pico, C., *et al.* "Hepatitis B virus genotypes in the United States: results of a nationwide study", Gastroenterology, 2003; 125: 444-51.

160. Petit, M.A., Dubanchet, S., Capel, F., Voet, P., Dauguet, C., and Hauser, P. "HepG2 cell binding activities of different hepatitis B virus isolates: inhibitory effect of anti-HBs and anti-preS1(21-47)", Virology, 1991; 180: 483-91.

161. Maeng, C.Y., Oh, M.S., Park, I.H., and Hong, H.J. "Purification and structural analysis of the hepatitis B virus preS1 expressed from Escherichia coli", Biochem Biophys Res Commun, 2001; 282: 787-92.

162. Okamoto, H., Imai, M., Kametani, M., Nakamura, T., and 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: 231-6.

163. Osiowy, C., Giles, E., Tanaka, Y., Mizokami, M., and Minuk, G.Y. "Molecular evolution of hepatitis B virus over 25 years", J Virol, 2006; 80: 10307-14.

164. Lim, S.G., Cheng, Y., Guindon, S., Seet, B.L., Lee, L.Y., Hu, P., *et al.* "Viral quasi-species evolution during hepatitis Be antigen seroconversion" , Gastroenterology, 2007; 133: 951-8.

165. Blackberg, J. and Kidd-Ljunggren, K. "Occult hepatitis B virus after acute self-limited infection persisting for 30 years without sequence variation" , J Hepatol, 2000; 33: 992-7.

166. Bozkaya, H., Akarca, U.S., Ayola, B., and Lok, A.S. "High degree of conservation in the hepatitis B virus core gene during the immune tolerant phase in perinatally acquired chronic hepatitis B virus infection", J Hepatol, 1997; 26: 508-16.

167. Harrison, A., Lemey, P., Hurles, M., Moyes, C., Horn, S., Pryor, J., *et al.* " Genomic analysis of hepatitis B virus reveals antigen state and genotype as sources of evolutionary rate variation", Viruses, 2011; 3: 83-101.

168. Chu, C.J., Hussain, M., and Lok, A.S. "Hepatitis B virus genotype B is associated with earlier HBeAg seroconversion compared with hepatitis B virus genotype C", Gastroenterology, 2002; 122: 1756-62.

169. Ren, X., Xu, Z., Liu, Y., Li, X., Bai, S., Ding, N., *et al.* "Hepatitis B virus genotype and basal core promoter/precore mutations are associated with hepatitis B-related acute-on-chronic liver failure without pre-existing liver cirrhosis" , J Viral Hepat, 2010; 17: 887-95.

LIST OF PUBLICATION

1. Whole genome sequence analysis of Hepatitis B virus (HBV) strains recovered from HBV/HIV-1 co-infected pregnant women. International Graduate Research Conference, 2015. Chiang Mai, Thailand. December 11, 2015. *(Proceedings)*

Whole genome sequence analysis of hepatitis B virus (HBV) strains recovered from HBV/HIV-1 co-infected pregnant women

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ABSTRACT

Background & Aims: HBV genetic diversity has been reported to have an impact on diagnostic assays, failure to hepatitis B vaccination, response to antiviral therapy, liver disease progression and long-term clinical outcomes. HBV, as well as HIV, infections are major public health concerns in Southeast Asia. To date, data on HBV genetic diversity among HBV/HIV co-infected pregnant women is limited. This study aimed to analyze whole genome HBV sequences of HBV/HIV co-infected pregnant women.

Methods: Whole genome of HBV from HBV/HIV co-infected pregnant women was directly sequenced using BigDye Terminator cycle sequence. Genotyping and specific mutations were analyzed using a web-based analysis tools (HIV-grade: HBV-resistance interpretation tool). HBV genotype was also confirmed by phylogenetic analysis of the whole genome sequences together with HBV reference sequences using MEGA version 6.0.

Results: Two complete HBV genomes, 3,215 base pairs in length, were successfully amplified. Phylogenetic analysis showed that the two women were infected with HBV genotype C1. Analysis of *surface* gene showed the presence of a mutation K141N. Although this mutation has been described as an immune escape mutation, the woman infected with this virus did not transmit HBV to her baby. No mutation related to drug resistant within the *polymerase* gene was observed. The mutation in *precore/core* gene was not found in both samples even one sample showed HBeAg-negative phenotype.

Conclusion: Whole genome sequencing provides complete information that may be useful for clinical outcomes and epidemiological perspectives.

KEYWORDS

Hepatitis B virus (HBV), Genetic diversity, HBV/HIV co-infection, Whole genome (Full-genome) sequencing, pregnant woman

INTRODUCTION

Hepatitis B virus (HBV) belongs to the *Hepadnaviridae* family. It is a small DNA virus with approximately 3.2 kb partially double stranded circular DNA genome. This compact genome consists of four overlapping open reading frames (ORFs) (1) including *polymerase* (*P*), *preS/S*, *precore/core* (*preC/C*) and *X* region. HBV replicates by reverse transcriptase, a viral enzyme which lacks proofreading activity; hence, incorrect bases incorporation are more occur than other DNA viruses. HBV has a high viral production, which is up to 10^{11} virions per day and high mutation rate with an estimated of 1.4-3.2 x 10^{-5} nucleotides substitutions per site per year (2). This unique life cycle leads to a heterogeneous viral population. HBV variants which can survive under endogenous pressure (host immune system) and exogenous pressure (anti-viral treatment and vaccination) are the predominant. Thus, HBV variants are related to immune response and response to treatment. HBV can be divided into 10 genotypes (3-8) based on a divergence of ≥ 8% in the complete genomic sequence. HBV genotypes have a distinct geographical distribution worldwide. In Thailand, HBV genotype C and B are the 2

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predominant genotypes, accounting for 54-91% and 7-24%, respectively (9-12).

Impact of HBV genetic diversity, i.e. genotype, subtype and mutations, has been related to diagnostic assay detection problems (13, 14), failure to hepatitis B vaccination (15, 16), response to antiviral therapy (17), disease progression and long-term clinical outcomes (18-20). Several studies suggested the impact of specific mutations of the 4 ORFs related to clinical outcome. For example, in *core* region, substitution of thymine to cytosine at nucleotide 1753 (T1753C) and double mutation A1762T/G1764A have been associated with a decrease of HBeAg expression (21-23) and lead to a high risk of cirrhosis (24). In *surface* region, glycine at position 145 was substituted by arginine or lysine or alanine (sG145R/K/A) has been widely reported as vaccine escape mutant, which can infect vaccinated individuals (15, 16). Amino acid substitution in the reverse transcriptase region of the polymerase gene, rtM204I/V/S, is known to be associated with lamivudine resistance (25). Due to the overlapping between polymerase and surface genes, mutations in polymerase will effect to surface gene encoding. The mutation rtA181T in the polymerase gene results in a stop codon at amino acid position 172 in the overlapping *S* region (sW172*). This mutation was reportedly associated with an oncogenic role leading to hepatocellular carcinoma (HCC) (26).

Hepatitis B virus and human immunodeficiency virus (HIV-1) infections are the major public health concerns, particularly in Southeast Asia (27). Co-infection of HBV and HIV has been common due to shared routes of transmission, e.g. perinatal transmission, contact with infectious fluid, and sexual exposure (28). HIV infection has a significant impact on HBV infection in terms of natural progression (29) and genetic diversity (30). In some particular condition, pregnancy may have an impact on HBV replication due to hormones produced and immune changes. However, there has been some controversial information of pregnancy on HBV (31, 32). To date, data on HBV genetic diversity among HBV/HIV co-infected pregnant women is limited. This study aimed to demonstrate the possibility of performing a whole genome HBV sequences among HBV/HIV co-infected pregnant women. These preliminary data provide a better knowledge of HBV whole genome sequence that circulates in this population.

METHODOLOGY/EXPERIMENTAL DESIGN

Sample

Two plasma samples of HIV/HBV co-infected pregnant women from the perinatal HIV prevention trial (PHPT) were used (39). HBV DNA levels were previously quantified by the COBAS Amplicor HBV monitor test (Roche Diagnostics) or Abbott real-time HBV DNA™ assay (Abbott laboratories) (33).

HBV DNA amplification and sequencing

HBV DNA was extracted from 200 µL of plasma samples using Abbott Molecular's m2000sp sample extraction automation (Abbott Molecular Inc., IL, USA). Full-length HBV genome was amplified by PCR using primers P1-F and P2-R, as shown in Figure 1 and Table 1 (34). For each reaction, the total volume was 50 µL, containing 10 µL DNA template and Platinum Taq DNA polymerase high fidelity reaction mix (Invitrogen). Amplification conditions were as follows: 94°C for 2 min, followed by 40 cycles consists of denaturation at 94°C for 40 sec, annealing at 60°C for 90 sec and extension at 68°C for 3 min with an increment of 12 second per PCR cycle. All PCR products were purified using E.Z.N.A. Cycle Pure Kit (Omega Bio-Tek, Norcross, GA). Purified PCR products were applied to sequence with the set of HBV specific primers, as shown in Table 1 (34, 35) and BigDye Terminator Mix V. 3.1 reagents (Applied Biosystems, Foster

city, CA) using the ABI 3100 genetic analyzer (Applied Biosystems, Foster city, CA).

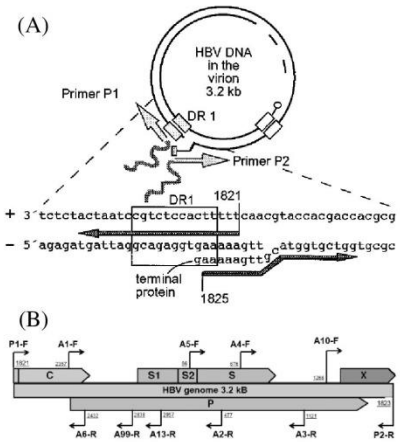

Figure 1 (A) Primers used for whole HBV genome amplification and sequencing, (B) primer binding sites of each specific primer used in HBV genome sequencing.

Sequence Analysis

Complete reference genome sequences were collected from GenBank database (National Center for Biotechnology Information, Bethesda, MD) and HBVdb: Hepatitis B Virus Database (https://hbvdb.ibcp.fr) and aligned using the ClustalW method in BioEdit software version 7.2.5. The phylogenetic analysis was performed using whole genome sequences of the analyzed samples and HBV reference sequences. Phylogenetic tree was constructed using neighbor-joining tree method in Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0 (The Biodesign Institute, Tempe, AZ). HBV genotyping and mutations in polymerase and surface regions were identified using a web-based analysis tools, HIV-grade: HBV-resistance interpretation tool (www.hiv-grade.de).

Table 1 The set of HBV specific primers

Primer name	Nucleotide position	Nucleotide sequence	Ref.
P1-F	1821 - 1841	CCG GAA AGC TTG AGC TCT TCT TTT TCA CCT CTG CCT AAT CA	(34)
P1-R	1823 -1806	CCG GAA AGC TTG AGC TCT TCA AAA AGT TGC ATG GTG CTG G	(34)
A1-F	2357 – 2380	GGC AGG TCC CCT AGA AGA AGA ACT	(34)
A2-R	477 – 455	GGA CAA ACG GGC AAC ATA CCT TG	(35)
A3-R	1121 – 1100	AGA AAG GCC TTG TAA GTT GGC G	(35)
A4-F	676 – 699	TTT ACT AGT GCC ATT TGT TCA GTG	(35)
A5-F	66 – 90	GCT CCA GTT CAG GAA CAG TAA ACC C	(35)
A6-R	2432 – 2408	ATT GAG ATC TTC TGC GAC GCG GCG A	(35)
A10-F	1266 – 1286	CCA TAC TGC GGA ACT CCT AGC	(34)
A13-R	2957 – 2935	TTG GGA TTG AAG TCC CAA TCT GG	(34)
A99-R*	2838 – 2814	CTT GTT CCC AAG AAT ATG GTG ACC C	-

*Unpublished primers were designed to sequence *pol* gene using primer design software

RESULTS AND DISCUSSION

Two HBV/HIV co-infected pregnant women were initially selected in this preliminary study. First woman (0496) was 22 years old and another (0675) was 27 years old at enrollment. CD4+ T-cell count were 500 and 250 cells/mm^3 and HBV DNA levels were 5.68 $\log_{10}$ and 7.84 $\log_{10}$ IU/ml in woman 0496 and woman 0675, respectively. The woman 0496 was negative for HBeAg while woman 0675 was positive. HIV viral load were 4.49 $\log_{10}$ and 5.01 $\log_{10}$ copies/mL in woman 0496 and woman 0675, respectively.

The two complete HBV genomes, 3,215 basepairs in length, were successfully amplified and analyzed (Fig.2). Using a web-based analysis tool,

the viruses from both women were identified as genotype C, which is a genotype predominantly found in Thailand. The previous study, in 1998 by Theamboonlers, A. *et. al.* reported that genotype C (54.4%) was dominated over B (23.5%) by nested PCR and sequencing of the *"a"* determinant in surface gene in wide-range HBV-infected population (12). In addition, the study among voluntary blood donors in 2006 found that genotype C was highly predominant (89.3%) and genotype B was only 7.4% (9). Also, in the recent study of Louisirirotchanakul, S. *et. al.* in 2012 found that 87.5% of genotype C and 10.5% of genotype B based on surface gene sequences (11). Our result was confirmed by phylogenetic analysis, the two genomes were clustered together with genotype C sequences collected from Thailand and Myanmar and HBV subgenotype C1 (Fig. 3), suggesting that these two women were infected with HBV genotype C1.

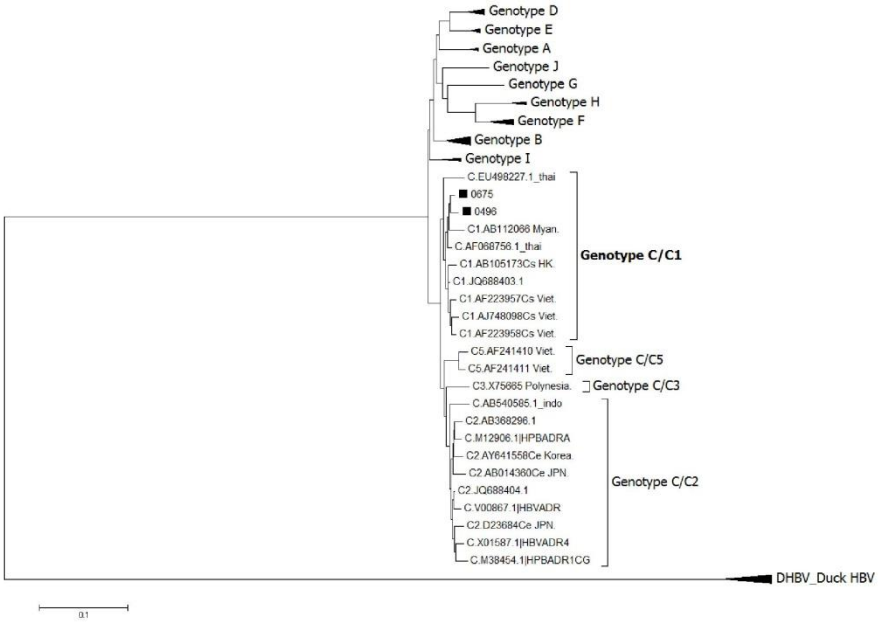

Figure 3 Phylogenetic analysis of the HBV strains isolated compared with 46 reference sequences on the basis of full genome sequence

Hepatitis B surface antigen (HBsAg) contains the major epitopes that localized in the region known as the *"a"* determinant, which is the target for neutralizing antibody produced during natural infection or following vaccination. Mutations of HBsAg have been associated to clinical aspects of HBV infection. We observed that the woman 0496 had only one substitution within the *"a"* determinant, from lysine to asparagine at the position 141 (sK141N), as shown in Figure 4. In the previous study, this mutant was also found in HBV infected children despite vaccinated with hepatitis B vaccine suggesting that this mutation may play an important role in immune escape (36). However, in this study, these two women did not transmit

Figure 2 Whole genome HBV sequences (3,215 base pairs in length)

either HBV or HIV to their baby although one woman was infected with sK141N HBV mutant.

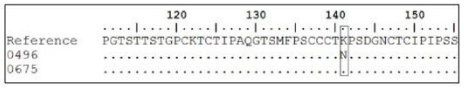

Figure 4 Amino acid sequence of the "a" determinant region of HBsAg

Selection of antiviral therapy is critical issue in management of HIV/HBV co-infection. Several available anti-HIV drugs are also active against HBV and can lead to drug-resistant HBV. Lamivudine (3TC) widely uses as an oral therapy for chronic HBV infection. Drug resistance mutations are usual with prolonged 3TC therapy (37). Recent study showed that the prevalence of 3TC mutants were 5.5% in naïve HBV/HIV individuals who has never experienced to any antiviral drugs (38). This present data, amino acid substitution in the polymerase gene which related to antiviral resistance was not detected, indicating that these two women were not infected with drug-resistant HBV.

Furthermore, this study investigated the presence of mutation associated with HBeAg serological status. The A1762T and G1764A mutations in the basal core promoter (BCP) region and the G1896A mutation in the precore (PC) region are found commonly in HBeAg-negative patients (21-23). The mutation in BCP/PC region was not found in both two samples even the 0496 sample showed HBeAg negative phenotype.

The limitation of this study was a small number of samples analyzed. Further study with a larger sample sizes is needed to confirm the finding.

CONCLUSION

Current molecular methods allow us to better understand the genetic diversity of HBV that are associated with clinical outcomes and epidemiological perspectives. Identification of HBV genotype and specific mutations are useful in guiding treatment of chronic infection. In this study, whole genome HBV sequences of two HBV/HIV co-infected pregnant women were successfully sequenced in our setting. Therefore, further investigations are possible and necessary to find out the patterns of HBV mutation in our country and Southeast Asia, particularly in HBV/HIV co-infected pregnant population.

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REFERENCES

1. Kramvis A. Genotypes and genetic variability of hepatitis B virus. Intervirology. 2014; 57:141-50.
2. Orito E, Mizokami M, Ina Y, *et al.* Host-independent evolution and a genetic classification of the hepadnavirus family based on nucleotide sequences. Proc Natl Acad Sci U S A. 1989; 86:7059-62.
3. Okamoto H, Tsuda F, Sakugawa H, *et al.* Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. J Gen Virol. 1988; 69 (Pt 10):2575-83.
4. Norder H, Courouce AM, Magnius LO. Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. Virology. 1994; 198:489-503.
5. Stuyver L, De Gendt S, Van Geyt C, *et al.* A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. J Gen Virol. 2000; 81:67-74.
6. Arauz-Ruiz P, Norder H, Robertson BH, *et al.* Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. J Gen Virol. 2002; 83:2059-73.
7. Tran TT, Trinh TN, Abe K. New complex recombinant genotype of hepatitis B virus identified in Vietnam. J Virol. 2008; 82:5657-63.

8. Tatematsu K, Tanaka Y, Kurbanov F, *et al.* A genetic variant of hepatitis B virus divergent from known human and ape genotypes isolated from a Japanese patient and provisionally assigned to new genotype J. J Virol. 2009; 83:10538-47.
9. Jutavijittum P, Jiviriyawat Y, Yousukh A, *et al.* Genotypes of hepatitis B virus among voluntary blood donors in northern Thailand. Hepatol Res. 2006; 35:263-6.
10. Jutavijittum P, Yousukh A, Jiviriyawat Y, *et al.* Genotypes of hepatitis B virus among children in Chiang Mai, Thailand. Southeast Asian J Trop Med Public Health. 2008; 39:394-7.
11. Louisirirotchanakul S, Olinger CM, Arunkaewchaemsri P, *et al.* The distribution of hepatitis B virus genotypes in Thailand. J Med Virol. 2012; 84:1541-7.
12. Theamboonlers A, Tangkijvanich P, Pramoolsinsap C, *et al.* Genotypes and subtypes of hepatitis B virus in Thailand. Southeast Asian J Trop Med Public Health. 1998; 29:786-91.
13. Coleman PF, Chen YC, Mushahwar IK. Immunoassay detection of hepatitis B surface antigen mutants. J Med Virol. 1999; 59:19-24.
14. Louisirirotchanakul S, Kanoksinsombat C, Theamboonlert A, *et al.* Mutation of the *"a"* determinant of HBsAg with discordant HBsAg diagnostic kits. Viral Immunol. 2004; 17:440-4.
15. Hsu HY, Chang MH, Ni YH, *et al.* Surface gene mutants of hepatitis B virus in infants who develop acute or chronic infections despite immunoprophylaxis. Hepatology. 1997; 26:786-91.
16. Luongo M, Critelli R, Grottola A, *et al.* Acute hepatitis B caused by a vaccine-escape HBV strain in vaccinated subject: sequence analysis and therapeutic strategy. J Clin Virol. 2015; 62:89-91.
17. Zoulim F, Locarnini S. Hepatitis B virus resistance to nucleos(t)ide analogues. Gastroenterology. 2009; 137:1593-608 e1-2.
18. Liu S, Zhang H, Gu C, *et al.* Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: a meta-analysis. J Natl Cancer Inst. 2009;101:1066-82.
19. Ito K, Yotsuyanagi H, Yatsuhashi H, *et al.* Risk factors for long-term persistence of serum hepatitis B surface antigen following acute hepatitis B virus infection in Japanese adults. Hepatology. 2014; 59:89-97.
20. Wai CT, Fontana RJ, Polson J, *et al.* Clinical outcome and virological characteristics of hepatitis B-related acute liver failure in the United States. J Viral Hepat. 2005;12:192-8.
21. Baumert TF, Rogers SA, Hasegawa K, *et al.* Two core promotor mutations identified in a hepatitis B virus strain associated with fulminant hepatitis result in enhanced viral replication. J Clin Invest. 1996; 98:2268-76.
22. Parekh S, Zoulim F, Ahn SH, *et al.* Genome replication, virion secretion, and e antigen expression of naturally occurring hepatitis B virus core promoter mutants. J Virol. 2003; 77:6601-12.
23. Jammeh S, Tavner F, Watson R, *et al.* Effect of basal core promoter and pre-core mutations on hepatitis B virus replication. J Gen Virol. 2008; 89:901-9.
24. Thomas HC. The hepatitis B virus and the host response. J Hepatol. 1990;11 Suppl 1:S83-9.
25. Damerow H, Yuen L, Wiegand J, *et al.* Mutation pattern of lamivudine resistance in relation to hepatitis B genotypes: hepatitis B genotypes differ in their lamivudine resistance associated mutation pattern. J Med Virol. 2010; 82:1850-8.
26. Lai MW, Yeh CT. The oncogenic potential of hepatitis B virus rtA181T/ surface truncation mutant. Antivir Ther. 2008; 13:875-9.
27. Hoffmann CJ, Thio CL. Clinical implications of HIV and hepatitis B co-infection in Asia and Africa. Lancet Infect Dis. 2007;7:402-9.
28. Alter MJ. Epidemiology of viral hepatitis and HIV co-infection. J Hepatol. 2006; 44:S6-9.
29. Thio CL, Seaberg EC, Skolasky R, Jr., *et al.* HIV-1, hepatitis B virus, and risk of liver-related mortality in the Multicenter Cohort Study (MACS). Lancet. 2002; 360:1921-6.
30. Audsley J, Littlejohn M, Yuen L, *et al.* HBV mutations in untreated HIV-HBV co-infection using genomic length sequencing. Virology. 2010; 405:539-47.

31. Almog Y, Klein A, Adler R, *et al.* Estrogen suppresses hepatitis B virus expression in male athymic mice transplanted with HBV transfected Hep G-2 cells. Antiviral Res. 1992; 19:285-93.
32. Soderstrom A, Norkrans G, Lindh M. Hepatitis B virus DNA during pregnancy and post partum: aspects on vertical transmission. Scand J Infect Dis. 2003; 35:814-9.
33. Khamduang W, Gaudy-Graffin C, Ngo-Giang-Huong N, *et al.* Analysis of residual perinatal transmission of hepatitis B virus (HBV) and of genetic variants in human immunodeficiency virus and HBV co-infected women and their offspring. J Clin Virol. 2013; 58:415-21.
34. Gunther S, Li BC, Miska S, *et al.* A novel method for efficient amplification of whole hepatitis B virus genomes permits rapid functional analysis and reveals deletion mutants in immunosuppressed patients. J Virol. 1995; 69:5437-44.
35. Gunther S, Sommer G, Von Breunig F, *et al.* Amplification of full-length hepatitis B virus genomes from samples from patients with low levels of viremia: frequency and functional consequences of PCR-introduced mutations. J Clin Microbiol. 1998; 36:531-8.
36. Karthigesu VD, Allison LM, Fortuin M, *et al.* A novel hepatitis B virus variant in the sera of immunized children. J Gen Virol. 1994; 75 (Pt 2):443-8.
37. Matthews GV, Bartholomeusz A, Locarnini S, *et al.* Characteristics of drug resistant HBV in an international collaborative study of HIV-HBV-infected individuals on extended lamivudine therapy. AIDS. 2006; 20:863-70.
38. Tuma P, Pineda JA, Labarga P, *et al.* HBV primary drug resistance in newly diagnosed HIV-HBV-coinfected individuals in Spain. Antivir Ther. 2011; 16:585-9.
39. Lallemant M, Jourdain G, Le Coeur S, Mary JY, Ngo-Giang-Huong N, Koetsawang S, *et al.* Single-dose perinatal nevirapine plus standard zidovudine to prevent mother-to-child transmission of HIV-1 in Thailand. N Engl J Med. 2004; 351(3):217-28.