Phylogenetic Analysis Related to Hepatitis B Virus based on Region Surfaces in Tuban, East Java Province, Indonesia

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Abstract

Hepatitis B virus (HBV) is a virus cause liver disease. This virus could be transmitted through blood product, and it becomes the major problems in developing countries. Genotype distribution of HBV depends on geographical characteristics. Here, we reported HBV genotyping analysis of blood donors with positive Hepatitis Surface Antigen (HbsAg) at Indonesian Red Cross Foundation in Tuban, East Java. Total 150 sera were collected from blood donors at Indonesian Red Cross Foundation in Tuban, East Java. Enzyme-Linked Immunosorbent Assay (ELISA) was conducted by PRISM®HBsAg kit (ABBOTT) according to the manufacture's instruction, to determine sample that has positive HbsAg. The genotyping analysis was done using polymerase chain reaction and sequencing method from 12 samples. Genetyx ver.10 software was used for identification of HBV type. Analysis of HBV genotype revealed three different types of HBV. HBV type B was the major HBV (50%). Interestingly, there was one unidentified HBV type suggested as a novel subtype of HBV. Further genotyping analysis is needed to confirm novelty of the unidentified HBV type. Analysis of HBV genotype revealed three different types of HBV. HBV type B was the major HBV (50%). Interestingly, there was one unidentified HBV type suggested as a novel subtype of HBV. Further genotyping analysis is needed to confirm novelty of the unidentified HBV type. This study was the first evidence of blood donor's genotyping analysis in Tuban.

Keywords: *blood donors, genotyping analysis, hepatitis B virus, region surface, Tuban.*

Introduction

Hepatitis B virus (HBV) is a major virus causing both acute and chronic liver diseases that are spread widely in Indonesia. Chronic infection of HBV has a risk to develop become to carcinoma and liver cirrhosis[1]. Indonesia is included in group of countries that have high prevalence of HBV with blood Hepatitis B surface antigen (HbsAg-emia) approximately 3-20%. Therefore, HBV infection still becomes the main health problem[2].

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To date, there are 10 HBV genotypes already identified all over the world coded from A to J[3,4]. These types are classified based on genomic properties of HBV. Classification is mainly observed from the genomic divergence, divergence with percentage >8% in nucleotide sequences from HBV whole genomes or >4% from region surface (S) genes[5,6].

Several regions in Indonesia have different prevalences of HBV infection. Lombok Island is a hyper endemic region in which about 10.6%-20.3% individuals in suburban area become a carrier of this infection[2]. Infection transmitted through blood product remains high. It is reported that the highest evidence of blood donors with positive HBV is found in Papua province, about 10.5%. In addition, it also explained the evidence

of blood donors with positive HBV at 11 big cities in Indonesia ranging from 2.1% to 9.5%[4].

Tuban is one of the districts in East Java, Indonesia. This district is located near Central Java boundaries; therefore, it becomes one of the regions that connects East Java and Central Java. Based on data from Indonesian Ministry of Health in East Java region in 2007, Tuban has relatively high prevalence of hepatitis with frequency of 0.24% and ranked seventh among all 38 districts and cities in East Java province. That frequency might increase because from the recent data, the frequency of hepatitis prevalence increased significantly from 2007 to 2013 in East Java province[2,3,4]. Besides that, it has been suggested that the geographical position of Tuban could support the increased frequency of hepatitis prevalence. Here, we reported the first evidence of HBV genotyping analyses based on region surface gene from blood donors with positive HbsAg at Indonesian Red Cross Foundation in Tuban, East Java, Indonesia.

Materials and Methods

Sample preparation

Sample used in this study was 150 sera from blood donors at Indonesian Red Cross Foundation in Tuban, East Java. Enzyme-Linked Immunosorbent Assay (ELISA) was conducted by PRISM®HBsAg kit (ABBOTT) according to the manufacture's instruction, to determine sample that has positive HbsAg. Genomic DNA was extracted from positive samples of human blood by Gentra Puregene Blood kit (QIAGEN) according to the manufacturer's instructions prior to genotyping analysis.

Genotyping Analysis

Samples with positive HbsAg were continued for genotyping analysis. HBV genotyping analysis was performed using nested polymerase chain reaction (PCR) and DNA sequencing. Nested PCR was done using two different primers, external and internal primers. Sequences of external primers used in the first step are 5'- GTG GTG GAC TTC TCT CAA TTT TC – 3' (P7) and 5'-CGG TAW[A/T] AAA GGG ACT CAM[A/C]GAT-3' (P8)[13]. And sequences of internal primers used in the second step are 5'-CAA GGT ATG TTG CCC GTT TG-3' (HBS1) and 5'-AAA GCC CTG CGA ACC ACT GA-3' (HBS2)[14].

DNA fragment resulted from PCR was used for sequencing after purification step. Purification was performed by QIAquick Gel Extraction kit (QIAGEN) according to the manufacturer's instruction. Purified DNA was then mixed with one of the primers and Big Dye Terminator kit reagent. Sequencing was conducted using ABI PRISM-310 Sequencer Machine (Applied Biosystems Inc.).

Identification of HBV genotype

The identification of HBV genotype was analysed by multiple alignments between sequencing results from sample and gen bank (NCBI) were performed using software Genetyx-Win Ver.10. Phylogenetic tree was made to determine the HBV genotype.

Results and Discussion

ELISA analyses from 150 samples of sera resulted in 13 samples that have positive HbsAg. DNA Sequence analysis was performed only in 12 samples. One sample failed to produce a good sequencing result. We used primers that amplified region surface gene in our study for genotyping analysis. The genotyping analysis showed that 12 samples could be included in two major groups. HBV type B was the be included in all groups of HBV type already identified.

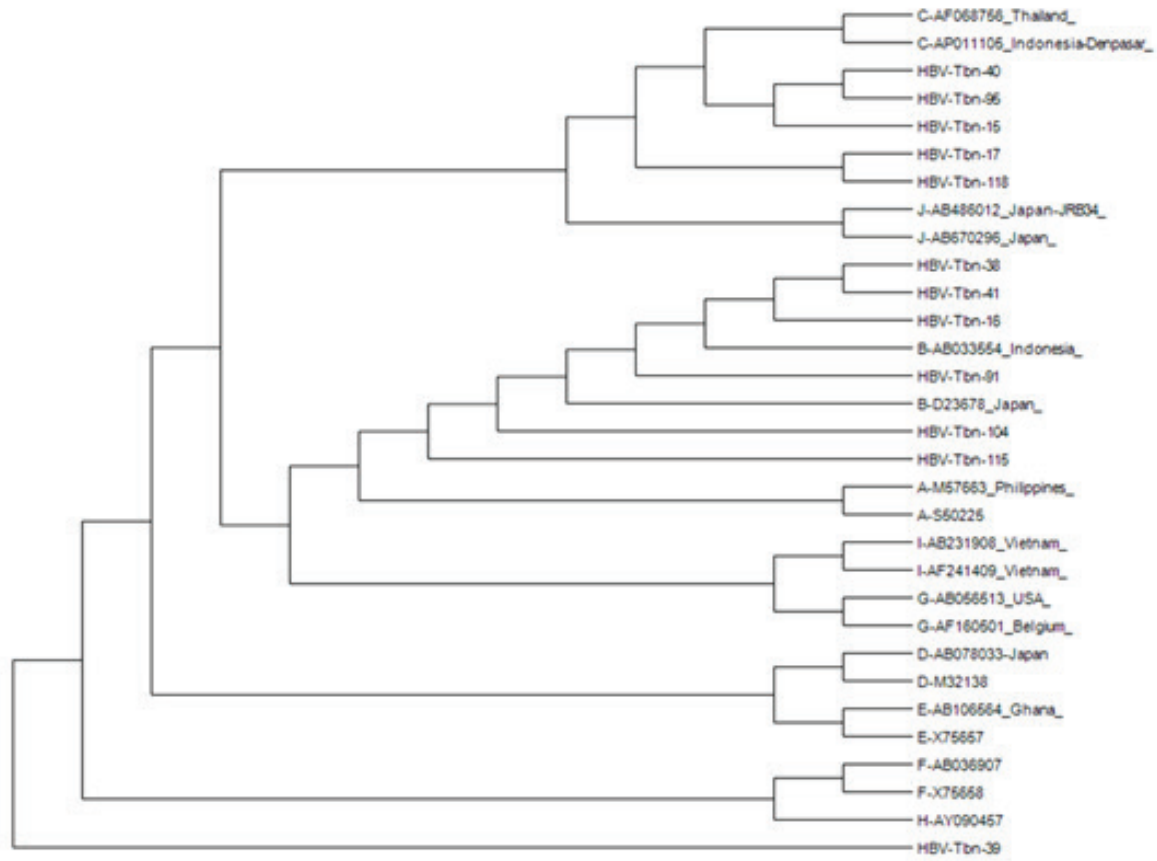

Figure 1. Phylogenetic tree was generated to show the genotype HBV and evolutionary relationship between the HBV in Tuban with the other several Asian countries.

Study conducted by Telenta *et al.* showed the usage of primers targeting the region of surface gene could showed genotype analysis results and analysis using whole genome of HBV[7]. Multiple alignments were conducted between sequencing results from 12 samples and sequence from previous studies already described[5,8,9]. Furthermore, phlyogenetic tree was made from the alignment analysis. The genotyping analysis showed that 12 samples could be included in two major groups. HBV type B was the dominant type indentified in six samples, thus frequency was 50%. This was followed by HBV type C identified in five samples, thus frequency was 41.7%. One sample with code VHB-Tbn-39 (frequency 8.3%) could not be included in all groups of HBV type already identified. HBV type C was considered prior to hepatocellular carcinomas and higher frequency of mutation at HBV core promoter region that related to susceptibility to chemical drugs, compared to HBV type B.

In general, our results showed a similarity with previous study conduct at other areas in Indonesia, whereas HBV types B and C are more dominant than other types[10,11]. Although Lombok and Papua are well known as regions with the highest prevalence of Hepatitis, HBV type B and C are also dominant in both regions[12,13]. But in Tuban, both types are almost equal in frequencies. Several Asian countries have similarities in dominant types of HBV such as China and Japan[14,15]. Compared with other regions such as America and Africa, there were predominant other types of HBV such as types A, D, and E[16,17].

Several factors were related to distribution of HBV such as: 1) the effects of mutation prior to drug resistances and vaccination to patient with HBV; 2) individual sex behaviour in those regions[18]; and 3) poor infection control at dental services[19]. Basically, social life was closely related to the HBV evidence. Therefore, although there was similarity in predominant HBV types among Indonesian regions but it might be possible that the clinical characteristics among patients in different geographic regions were also different, as suggested in previous study by Orito *et al.*[20]. Thus, it is possible that the distributions of HBV are different worldwide.

Conclusion

In summary, since there is no clinical study related to genotyping analysis in Tuban, at least in part, the data resulted from this study seemed to be an important preliminary data. In our case, one sample was found to be significantly different from any HBV type already known. Further research with large numbers of sample, clinical feature observation, randomized, placebo-controlled study may be needed to confirm the predominant HBV type in Tuban, East Java, Indonesia. In addition, more sensitive genotype analysis should be carried out to reveal one sample that could not be identified.

Conflict of Interest : The authors declare that they have no conflict of interest.

Source of Funding : The source of funding in this study from DP2M DIKTI for research grant (Fundamental Research Grant No: 0072.SP2H/PP/K7/ II/2012).

Acknowledgements : We thank Rasyadan Taufiq Probojati and Arif Nur Muhammad Ansori for editing the manuscript. We additionally to thank for all laboratory members in University of PGRI Ronggolawe, Tuban and University of Airlangga, Surabaya for technical support.

Ethical Approval: This study had been approved by local ethics committee Medical Faculty, University of Airlangga, Surabaya, East Java, Indonesia.

References

1. Ie SI, Turyadi, Sidarta E, *et al.* High prevalence of hepatitis b virus infection in young adults in Ternate, Eastern Indonesia. Am J Trop Med Hyg. 2015, 93(6): 1349–1355.
2. Ruff TA, Gertig DM, Otto BF, *et al*. Lombok Hepatitis B Model Immunization Project: Toward universal infant hepatitis B immunization in Indonesia. J Infect Dis. 1995, 171(2): 290-296.
3. Lusida MI, Victor EN, Soetjipto, RH, *et al*. Novel Subgenotypes of Hepatitis B Virus Genotypes C and D in Papua, Indonesia. J Clin Microbiol. 2008, 46(7):2160–2166.
4. Yano Y, Utsumi T, Lusida MI, *et al*. Hepatitis B virus infection in Indonesia. World J Gastroenterol. 2015, 21(38):10714-20.
5. Tatematsu K, Tanaka Yang, Kurbanov F, *et al*. A Genetic variant of hepatitis B virus divergent from known human and genotypes isolated from a Japanese patients and provisionally assigned to new genotype. J Virol. 2009, 83(20):10538-10547.
6. Arankalle VA, Gandhe SS, Borkakoty BJ, *et al*. A novel recombinant (genotype I) similar to Vietnam/ Laos in a primitive tribe in Eastern India. *J Viral Hepatol.* 2010, 17(7): 501-10.
7. Telenta PFS, Poggio GP, Lopez JL, *et al*. Increased prevalence of genotype F Hepatitis B virus isolates in Buenos Aires, Argentina. *J Clin Microbiol.* 1997, 35(7): 1873-1875.
8. Handajani R, Soetjipto MI, Lusida MI. Molecular analysis of various region of hepatitis B virus in chronic hepatitis B patients with and without Lamivudine therapy. 2nd year Competitive Res. Grant XI. Hepatitis Group-TDC UNAIR, Biomedical Research Unit – Mataram Hospital. 2004.
9. Lusida MI, Surayah MI, Sakugawa H, *et al*. Genotype and subtype analyses of hepatitis B virus (HBV) and possible co-infection of HBV and hepatitis C virus (HCV) or hepatitis D virus (HDV) in blood donors, patients with chronic liver disease and patients on hemodialysis in Surabaya, Indonesia. Microbiol Immunol. 2003, 47(12): 969–975.
10. Utsumi T, Lusida MI, Yano Yang, *et al*. Complete genome sequence and phylogenetic relatedness of hepatitis B virus isolates in Papua, Indonesia. J Clin Microbiol. 2009, 47(6): 1842–1847.

11. Utama A, Octavia TI, Dhenni R, *et al*. Hepatitis B virus genotypes/subgenotypes in voluntary blood donors in Makassar, South Sulawesi, Indonesia. Virology J. 2009, 6:128.
12. Mulyanto M, Depamede SN, Arif W, *et al*. Analysis of the Full-Length Genomes of Novel Hepatitis B Virus Subgenotypes C11 and C12 in Papua, Indonesia. J Med Virol. 2001, 1(83): 54–64.
13. Juniastuti J, Utsumi T, Nugrahaputra VE, *et al*. Another novel subgenotype of hepatitis B virus genotype C from Papuans of Highland origin. J Med Virol. 2011, 83(2): 225-234.
14. Zhu CT, Dong CL. Characteristics of general distribution of hepatitis B virus genotypes in China. Hepatobiliary Pancreat Dis Int. 2009, 8:397-401.
15. Matsuura K, Tanaka Y, Hige S, *et al*. Distribution of hepatitis B virus genotypes among patients with chronic infection in Japan shifting toward an increase of genotype A. J Clin Microbiol. 2009, 47(5):1476–1483.
16. Palumbo E, Scotto G, Faleo G, *et al*. Prevalence of HBV genotypes in South American immigrants affected by HBV-related chronic active hepatitis. The Brazilian J Infect Dis. 2007, 11(3):311-313.
17. Al Baqlani SA, TienSy B, Ratsch BA, *et al*. Molecular epidemiology and genotyping of Hepatitis B virus of HbsAg-positive patients in Oman. PlosOne. 2014, 9(5):1-10.
18. Abdo AA, Al-Jarallah BM, Sanai FM, *et al*. Hepatitis B genotypes: relation to clinical outcome in patients with chronic hepatitis B in Saudi Arabia. World J Gastroenterol. 2006, 12(43):7019-7024.
19. Mc Carthy GM. Risk of transmission of viruses in the dental office. J Can Dent Assoc. 2000, 66:554-557.
20. Orito E, Ichida T, Sakugawa H, *et al*. Geographic distribution of Hepatitis B Virus (HBV) genotype in patients with chronic HBV infection in Japan. Hepatology. 2001, 34(3):590-594.