

SHORT COMMUNICATION

Genotype of Hepatitis B Virus Coinfection in Typhoid Patients

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Typhoid fever can cause liver disorder and may result in complication. Studies revealed hepatic involvement in typhoid known as typhoid hepatitis. Our previous paper reported the existence of hepatitis B virus (HBV) coinfection in the serum of patients with abdominal typhoid using nested-polymerase chain reaction (PCR). Based on its genomic sequence divergence, HBV has been classified into 10 genotypes (A-J), which used in the prediction of therapeutic response and correlates with the clinical outcome of chronic HBV infection. This study was conducted to determine the genotypes of HBV in typhoid patients coinfecting with HBV in Tuban. Sera were obtained from 5 typhoid patients with positive HBV infection (proven by nested PCR). Study was performed by direct sequencing using BigDye V1.1 Terminator Cycle Sequencing kit and ABI Prism 310 Genetic Analyzer. Bioinformatic analysis had been done using the GenetixMac version 10 software to create the phylogenetic tree. Phylogenetic analysis showed 3 samples as genotype B and 2 two samples as genotype C.

Key words: coinfection, genotype, hepatitis B virus

Demam tifoid sering dikenal sebagai abdominalis tifus dapat menyebabkan gangguan hati dan penyakit komplikasi. Penyakit ini sering dikenal sebagai hepatitis tifoid. Dari penelitian sebelumnya, ditemukan adanya koinfeksi virus hepatitis B (VHB) pada pasien abdominalis tifus di kota Tuban dengan menggunakan teknik Polymerase Chain Reaction (PCR). Virus hepatitis B diklasifikasikan menjadi sepuluh genotipe (A-J) dan diketahui memiliki peran dalam keberhasilan terapi. Tujuan dari penelitian ini adalah untuk mengetahui genotip pada pasien tifoid yang koinfeksi dengan VHB di Tuban. Metode dalam penelitian ini adalah melakukan sekuensing langsung dari lima sampel pasien yang positif terinfeksi VHB. Analisis filogenetik dari hasil sekuensing langsung sampel berhasil mengidentifikasi tiga sampel dikategorikan sebagai VHB genotipe B dan dua sampel termasuk sebagai VHB genotipe C.

Kata kunci: genotip, koinfeksi, virus hepatitis B

Typhoid fever, also known as abdominal typhoid, is caused primarily by *Salmonella enterica*. Cases of hepatic involvement with typhoid fever have been reported by Khosla (1990), which known as typhoid hepatitis. Coinfection of typhoid fever with hepatitis A and E also have been reported in previous studies (Bhat *et al.* 2008; Zhang *et al.*, 2015; Tulara 2013). This coinfection makes the diagnosis and management a challenging task. Our previous paper reported that hepatitis B virus (HBV) was found in abdominal typhoid patients in Tuban. From 30 serum samples of abdominal typhoid patients, we found HBV DNA in 5 samples using nested Polymerase Chain Reaction technique (Nurtjahyani 2013). Hepatitis B virus (HBV) infection is commonly known worldwide to causes more than one million deaths every year (WHO 2015). This disease is also still a health problem in

Indonesia. The prevalence of HBV infection in Indonesia is around 9.4% (Riskedas 2007).

Hepatitis B virus is a small DNA virus belonging to the Hepa dna viridae family. The genome size of this virus is around 3.2 kilo bases (kb) with a diameter of 42-47 nm (Dane *et al.* 1970; Summers *et al.* 1975). The HBV virion (Dane particle) consists of an outer protein envelope and an inner protein core (Kott 2010). Hepatitis B Virus genome consisted of 4 ORFs (Open reading Frames) encoding for the Surface (S), Pre Core and Core (pre-C and C), X and Polymerase (P) proteins (Quarleri 2014). HBV has been classified into 10 genotypes: A, B, C, D, E, F, G, H, I, and J. The prevalence of HBV genotypes varies markedly between different regions. Genotype A is more abundantly found in the Northern and Middle Europe, compared to the North of America and Africa. Genotype B and C are found in Asia, genotype D is distributed and detected mostly in Mediterranean, whereas genotype E is found in the West Africa nations.

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sequencing PCR technique (Applied Biosystems, USA). Nucleotide sequences of HBV S gene from serum samples were compared with the nucleotide sequences of S gene from HBVs with various genotypes that had been published in the international DNA data bank (DDBJ/EMBL/GeneBank). The Unweighted Pair Group Method using Arithmetic averages (UPGMA) method using Genetix MAC program version 10 was used to reconstruct a phylogenetic tree.

The results, a phylogenetic tree was constructed based on the PCR amplification products using primers P1-P2 and HBS1-HBS2 (222 nucleotide), and the DNA from five samples as templates (Fig 1). The distribution of HBV genotypes in our five samples 60% (three samples) B and 40 % (two samples) C.

Molecular phylogenetic analysis classifies HBV into ten major genotypes, A-J (Cao 2009). Studies showed that HBV genotypes correlated with distinct geographical distributions and clinical characteristics. The genotypes also play roles in the outcome of the infection, and on the patient's response to antiviral therapy (Erhardt *et al.* 2005; Fung and Lok 2004). Therefore is necessary to determine the genotypes in order to better understand the HBV coinfection and also to provide effective management therapy.

Several cases of typhoid-hepatitis coinfection were reported in some countries (Ahmad *et al.* 2010; Bhat *et al.* 2009), however, our previous publication was the first to report the presence of HBV in typhoid patients (Nurtjahyani 2015). In our current report, genotyping of HBVs isolated from 5 patients with typhoid. HBV coinfection indicated that 3 samples belonged to HBV genotype B and 2 samples belonged to HBV genotype C. This is consistent with the previous indications that genotypes B and C highly prevalent in Southeast Asia (Mans *et al.* 2004) and that they are present in some regions and groups in Indonesia (Yano *et al.* 2015; Maria *et al.* 2003; Utsumi *et al.* 2014, Handajani *et al.* 2004).

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REFERENCES

- Ahmed F, Chowdhury K, Alam J, Arefeen S, Alam MM. 2010. Co-infection of typhoid fever with hepatitis A, hepatitis E and dengue fever: A challenge to the physicians. *DS (Child) HJ.* 26(2):122-124.
- Akuta N, Kumada H. 2005. Influence of hepatitis B virus genotypes on the response to antiviral therapies. *J Antimicrob Chemother.* 55(2): 139-142. doi:10.1093/jac/dkh533.
- Arankalle VA, Gandhe SS, Borkakoty BJ, Walimbe AM, Biswas D, Mahanta J. 2010. A novel recombinant (Genotype I) similar to Vietnam/Laos in a primitive tribe in Eastern India. *J Virol Hepatol.* 10:1365-2893. doi:10.1111/j.1365-2893.2009.01206.x.
- Arauz-Ruiz P, Norder H, Visona KA, Magnius LO .1997. Molecular epidemiology of hepatitis B virus in Central America reflected in the genetic variability of the small S gene. *J Infect Dis.* 176(4):851-858. doi: 10.1086/516507.
- Bhat D., Dhooria G.S, Bains H.S. 2009. Coinfection of hepatitis A and E with *Salmonella* infection; a case report. *Iran J Pediatr.* 19(1): 79-81.
- Cao GW. 2009. Clinical relevance and public health significance of hepatitis B virus genomic variations. *World J Gastroenterol.* 15(46):5761-5769. doi: 10.3748/wjg.15.5761.
- Erhardt A, Blondin D, Hauck K, Sagir A, Kohnle T, Heintges T, Haussinger D. 2005. Response to interferon alfa is hepatitis B virus genotype dependent: genotype A is more sensitive to interferon than genotype D. *Gut.* 54: 1009-1012. doi:10.1136/gut.2004.060327.
- Fung L. 2004. Hepatitis B virus genotypes: do they play a role in the outcome of HBV infection? *Hepatology* 40(4):790-792. doi:10.1002/hep.20455.
- Handajani R, Soetjipto MI, Lusida MI. 2004. 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.
- Khosla SN. 1990. Typhoid hepatitis. *Postgrad Med J.* 66(781):923-925. doi:10.1136/pgmj.66.781.923.
- Kott. 2010. Uptake Mechanism of Hepatitis B Virus into Susceptible Primary Hepatocyte Cultures. Doctor Rerumnaturalium. University of Giessen.
- Lindh M, Anderson, AS, Gusdal A. 1997. Genotypes, nt 1858 variants, and geographic origin of hepatitis B virus large-scale analysis using a new genotyping method. *J Infect Dis.* 175(6):1285-93. doi: 10.1086/516458.
- Lusida MI, Surayah SH. 2003. Genotype and subtype analyses of hepatitis B virus (HBV) and possible coinfection 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.* 47(12): 969-975. doi:10.1111/j.1348-0421.2003.tb03457.x.

- Lusida I, Victor EN, Handajani RS, Fujii MN, Sasayama M, Utsumi T, Hotta H. 2008. Novel subgenotypes of hepatitis B virus genotypes C and D in Papua, Indonesia. *J Clin Microbiol.* 46(7):2160-2166. doi:10.1128/JCM.01681-07.
- Mans et al. 2004. HBV: Where are we going? in: Hepatitis B and D protocols Vol II: Immunology, Model Systems, and Clinical Studies. Edited by Hamatake RK and Lau JYN, New Jersey: Humana Press.
- Norder H, Courcoue AM, Magnius LO. 1994. Complete genomes, relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. *Virology* 198(2):489-503. doi:10.1006/viro.1994.1060.
- Nurtjahyani SD, Handajani R. 2013. Detection of hepatitis B virus DNA among abdominal typhus patients with hepatitis B virus co-infection in Tuban district based on nested PCR technique. *J Biol Agri Healthcare.* 3(6):101-105.
- Okamoto H, Tsuda F, Sakugawa H, Sastrosoewignjo RI, Imai M, Miyakawa Y, Mayumi M. 1988. Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. *J Gen Virol.* 69:2575-2583. doi:10.1099/0022-1317-69-10-2575.
- Riset Kesehatan Dasar. 2007. Laporan Nasional 2007. Badan Penelitian dan Pengembangan Kesehatan Departemen Kesehatan, Republik Indonesia.
- Quarleri J. 2014. Core promoter: a critical region where the hepatitis B virus makes decisions. *World J Gastroenterol.* 20(2):425-35.
- Stuyver L, Gendt SD, Geyt CV, Zoulim F, Fried M, Schinazi RF, Rossau R. 2000. A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. *J Gen Virol.* 81:67-74. doi:10.1099/0022-1317-81-1-67.
- Tatematsu K, Tanaka Y, Kurbanov F, Sughauci F, Mano S, Maeshiro T, Nakayoshi T, Wakuta M, Miyakawa Y, Mizokami, M. 2009. 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.* 83(20):10538-10547. doi:10.1128/JVI.00462-09.
- Telenta PFS, Poggio GP, Lopez JL, Gonzales J, Lemberg A, Campos RH. 1997. Increased prevalence of genotype F hepatitis B virus isolates in Buenos Aires, Argentina. *J Clin Microbiol.* 35(7):1873-1875.
- Tulara NK. 2013. Concurrent infection with malaria, dengue, and hepatitis A virus together. *J Trop Dis.* 3(1):1-2.
- Utsumi T, Yano Y, Hotta H. 2014. Molecular epidemiology of hepatitis B virus in Asia. *World J Med Genet.* 4(2): 19-26. doi:10.5496/wjmg.v4.i2.19.
- World Health Organization. 2015. Hepatitis B. [Internet]. cited 2014 Nov 25. Available from: <http://www.who.int/mediacentre/factsheets/fs204/en/>.
- Yano Y, Utsumi T, Lusida MI, Hayashi Y. 2015. Hepatitis B virus infection in Indonesia. *World J Gastroenterol.* 21(38):10714-10720. doi: 10.3748/wjg.v21.i38.10714,
- Zainal A, Andriani F, Endriani R 2009. Distribusi genotipe dan subtipe virus hepatitis B pada penderita hepatitis B kronik di Pekanbaru. *Majalah Kedokteran Berkala* 43(3):105-111.
- Zhang Q, Teh JGX, Chan WPW. 2015. A patient with typhoid fever, *Giardia lamblia* gastroenteritis and hepatitis E. *Proc Singapore Healthcare.* 1-3.