Genotype and Serotype Identification of Hepatitis B Virus in Chronic Hepatitis B Patients Treated with Telbivudine

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Abstract. Hepatitis B is an inflammatory liver disease caused by hepatitis B virus (HBV). In Indonesia, the prevalence of Hepatitis B Surface Antigen (HBsAg) is 9.4%, categorizing the country as endemic hepatitis B. HBV has been classified into at least ten genotypes and four serotypes. Each genotype has different clinical significance and virologic characteristics, which can be an independent risk factor of Hepatocellular Carcinoma in addition to the male sex, older age, and positive Hepatitis B Envelope Antigen status. Telbivudine antiviral therapy suppressed the virus, but in case of resistance mutations, the mutant might grow continuously due to drugs inefficiency. This study aimed to detect the genotypes and serotypes of HBV in hepatitis B chronic patients after 12 weeks of telbivudine treatment. The subject of this study involved 26 patients with chronic hepatitis B, receiving 12 weeks treatment of telbivudine in the gastrohepatology division of the RSUP Wahidin Sudirohusodo hospital. The HBV genotype was identified by analyzing the HBV P gene, while the serotype was detected by deducing the nucleotide of the HBV S gene that overlapped with the P gene. Nine samples (75%) were genotype b, and three samples (25%) were genotype c. For serotype, six (50.0%), three (25.0%), and three (25.0%) samples were ayw, adw, and adr, respectively. Genotype b is generally associated with less progressive liver disease than genotype c. Genotypes b and c are prevalent in highly endemic areas in which the perinatal or vertical transmission play an important role in spreading the virus.

Keywords: Genotype, Hepatitis B Virus, Serotype, Telbivudine Treatment

1. Introduction

An estimation of 4.0 to 20.3% of hepatitis B cases in a healthy population of Indonesia has ranked this country with moderate to high endemicity of hepatitis B infection [1]. In 2007, the prevalence of Hepatitis B surface antigen (HBsAg) was 9.7% in men and 9.3% in women [2]. Meanwhile, the prevalence of people infected with hepatitis B virus-hepatitis B core antibody (HBc) was 34%,
indicating that a third of Indonesia's people have been infected with HBV. The highest hepatitis B virus infection in Indonesia found in various Provincials, including East Nusa Tenggara, Papua, South Sulawesi, Central Sulawesi, Maluku, Southeast Sulawesi, North Sulawesi, Aceh, West Nusa Tenggara, Central Kalimantan, North Sumatra, and South Kalimantan [2].

At least ten hepatitis B virus (HBV) genotypes (A to J) with distinct geographic distributions and several HBV mutants, including precore/core promoter mutations and pre-S/S deletion mutations, have been recognized to be not only predictive of liver disease progression but also associated with response to antiviral therapy. HBV genotypes and variants may serve as viral genetic markers in predicting disease progression as well as helping physicians for optimizing individualized antiviral therapy in clinical practice [3]. Genotypes B and C are found mostly in the Asia Pacific countries, including Indonesia [4,5].

One of chronic hepatitis B (CHB) treatments is the lifelong oral administration of nucleos(t)ide analogs such as Telbivudine. The antiviral nucleos(t)ide analogs targeted the HBV polymerase protein-translated from the HBV P gene, which is responsible for the reverse transcription process during HBV replication. However, HBV drug-resistant mutants often arise from such treatment. Currently, three Telbivudine-resistant mutants have been recognized, namely M204I, L80I/V and/or L180M [3]. The antiviral will suppress HBV titer to an undetectable level, unless in the presence of drug-resistant mutants, which can still replicate even with the antiviral pressure [6,7]. According to various international guidelines, the primary therapy for CHB is considered failed if it could not decrease the HBV DNA titer more than 1 log10 IU/mL after 12 weeks of compliant administration. Therefore, monitoring of antiviral therapy in CHB patients should be performed at week 12 [8-10].

The pathogenic differences among various HBV genotypes have been partially clarified and influenced the clinical outcomes of HBV infection [3]. Hence, the identification of HBV genotype is crucial for many reasons. Furthermore, monitor developments are necessary for those who received antiviral therapy based on infecting vhb genotype. This study aimed to detect the genotypes and serotypes of HBV in hepatitis b chronic patients after 12 weeks of Telbivudine treatment.

2. Materials and Methods
As many as 26 CHB patients received 600 mg daily dose of Telbivudine for 12 weeks were recruited from the RSUP Wahidin Sudirohusodo hospital. Venous blood samples (5 mL) were taken at week 12 of treatment. The HBV DNA was extracted, and P gene region amplified with nested polymerase chain reaction (PCR) method. The PCR method began with a pre-denaturation step at 94°C for 5 minutes, followed by denaturation (94°C for 30 s), annealing (55°C for 30 s), and extension steps (72°C for 1 m) for the first 35 cycles and second 25 cycles of PCR phase, and ended with a final extension step at 72°C for 7 min. Sequences of the oligonucleotide primers are listed in Table 1. Amplification products were visualized using ethidium bromide-stained 1.5% agarose gel under ultraviolet light. All experiments were carried out with the Kwok and Higuchi’s rules, to avoid any cross-contamination [11].

Positive PCR products were purified using a Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech Ltd., Taipei, Taiwan) and sequenced using a Bigdye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA) on an automatic sequencer (Applied Biosystems 337 DNA, Perkin Elmer). The resulting nucleotide sequences were aligned and compared with reference sequences from the GenBank (HBV genotype B, Acc no. M54923 and HBV genotype C, Acc. no. GQ358153) using the BioEdit v.7.0.5 software. Phylogenetic analysis with 1.000 bootstrapping and neighbor-joining method using MEGA v 5.1 software was used to classify the HBV isolates into genotypes based on 4% nucleotide heterogeneity of the overlapping HBV S gene region (12). HBV serotypes were determined using the HBsAg analysis (which is deduced from nucleotide S gene), based on amino acid variations in positions 122 and 160 [12-14].
3. Results and Discussion

The P gene amplification results showed 12 positive samples (46.2%) and 14 negative samples (53.8%). In the positive sample, VHB DNA was detected. The test continued with the direct sequencing method to determine nucleotide sequences, followed by the genotype analysis. The 12 positive samples consisted of 9 (75%) HBV genotype B and 3 (25%) HBV genotype C (Figure 1). This result was in agreement with a previous study by Thedja and co-workers. The study reported the HBV genotype distribution in Makassar, Indonesia, which is dominated by HBV genotypes B and C [15]. Both genotypes are commonly found in regions of East Asia and Southeast Asia, the Pacific islands, and Pakistan. Meanwhile, genotypes A and D are found in India and the Philippines, and the genotype D is common in the Pacific islands. HBV genotypes B and C are often found in areas with high vertical transmission, while genotypes A, D, E, F, G, and H occurred in areas with the horizontal transmission [16]. In addition to genotypes, VHB serotypes can also be determined by conducting HBsAg analysis (which is deduced from nucleotide S gene), based on amino acid variations in positions 122 and 160 [12-14]. Six samples (50.0%) obtained were the final subtypes, three samples (25.0%) were adw subtypes, and three samples (25.0%) were adr subtypes. Identification of HBV genotype is crucial for many reasons. The pathogenic differences among various HBV genotypes have been partially clarified and influenced the clinical outcomes of HBV infection. HBV genotypes in viral factors are not only predictive of clinical progression but also related to interferon (IFN)-α treatment response [17].

![Figure 1. Bioedit analysis of HBsAg protein amino acid VHB genotype.](attachment:image.png)

Clear HBV genotype-related associations exist between clinical outcomes and treatment efficacy in patients with CHB [18]. A study conducted in China has investigated the reasons for the longer immune clearance period in HBV patients infected with genotype C as compared to genotype B. These including a higher level of viral replication, a high hepatic histological activity, recurrent or persistently high ALT levels and IFN, nucleos(t)ide analogs, and low response to treatment. The possible relationship among genotypes B and C and peripheral blood follicular helper T (Tfh) cells in CHB patients under treatment has been investigated. Tfh cells play a major role in spreading signals that affect cellular division, helping the activation of B cells and the regulation of humoral response. In
addition, Tfh cells secreted specific cytotoxic T lymphocyte (CTL) interleukin (IL)-21, to sustain long-acting, effective, and antiviral immunity in chronic infection. High serum HBV DNA and ALT ratios in patients with genotype C might be related to lower peripheral blood Tfh cell levels. The lowering levels also decreased the levels of IL-21 as compared to the genotype B. The study also reported low levels of HBV-specific CTL [19].

Chronic hepatitis B patients can be successfully treated using nucleos(t)ide analogs (NAs), which was the choice of therapy for patients in this study. The NAs work mainly by inhibiting the hepatitis B virus (HBV) DNA polymerase activity and thus suppress the HBV replication [20]. However, among 26 samples studied, 12 samples showed a positive HBV DNA. These cases emerged a necessity for continuous observation with a long period of treatment. Persistent HBV replication may trigger strong and continued immune responses against the virus, resulting in even more severe liver damages [21].

4. Conclusion
Genotypes B and C were dominant genotypes found in patients of this study, while the dominant serotypes were ayw, adw, and adr. Further prospective research is needed to evaluate the treatment outcomes of patients with more numbers, to provide more evidence of the relationship between genotype and treatment development.

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