RINGKASAN

GENOTIPE DAN SUBTIPE VIRUS HEPATITIS B PADA PENDONOR DARAH DENGAN *HEPATITIS B SURFACE ANTIGEN* (HBsAg) POSITIF DI JAYAPURA, PROVINSI PAPUA, INDONESIA

Pada tahun 2000, *Core Working Party for Asia-Pasific Consensus on Hepatitis B and C* menyatakan bahwa Indonesia memiliki tingkat endemisitas hepatitis B sedang sampai tinggi. Angka pengidap virus hepatitis B di antara pendonor darah sukarela di sebelas kota besar di Indonesia berkisar antara 2,1%-9,5%, sementara itu di Jayapura, Provinsi Papua, angka tersebut pernah mencapai 17,5%. Pada saat ini, delapan genotipe dan sembilan subtipe virus hepatitis B telah teridentifikasi di seluruh dunia. Baik genotipe maupun subtipe virus hepatitis B memperlihatkan perbedaan dalam distribusi geografis, karakteristik klinik dan virologik serta dapat memberikan informasi historik tentang pola migrasi nenek moyang penduduk setempat. Data epidemiologi molekuler yang tersedia saat ini tentang pola genotipe dan subtipe virus hepatitis B di Jayapura bersumber dari beberapa penelitian yang dilakukan dengan menggunakan spesimen serum yang diambil pada waktu lebih dari satu dekade yang lalu dengan jumlah sampel terbatas. Hal ini berarti bahwa gambaran terkini tentang pola genotipe dan subtipe virus hepatitis B di Jayapura, khususnya pada kelompok pendonor darah, belum diketahui.

Penelitian ini bertujuan untuk menentukan pola genotipe dan subtipe virus hepatitis B pada pendonor darah dengan *Hepatitis B surface Antigen* (HBsAg) positif di Jayapura.

Sampel serum diperoleh dari pendonor darah di Unit Transfusi Darah Palang Merah Indonesia Cabang Jayapura dari bulan September 2004 sampai Januari 2005. Sampel serum diuji saring untuk deteksi HBsAg dengan metode *immunochromatography* dan selanjutnya dilakukan penentuan HBsAg dengan metode *enzyme-linked immunosorbent assay*. Pada sampel serum dengan HBsAg positif ditentukan genotipe dan subtipe virus hepatitis B. DNA virus hepatitis B diekstraksi dari 60 µl sampel serum menggunakan DNAzol Reagent (Invitrogen). Amplifikasi bagian dari gen S dilakukan dengan *polymerase chain reaction* (PCR) menggunakan *primer sense* P7 (5'-GTG GTG GAC TTC TCT CAA TTT TC-3; posisi 256-278) dan *primer antisense* P8 (5'-CGG TAW AAA GGG ACT CAM GAT-3'; posisi 796-776). Jika amplifikasi PCR *first-round* ini negatif, PCR *second-round* dilakukan menggunakan *primer sense* HBS1 (5'-CAA GGT ATG TTG CCC GTT TG-3'; posisi 455-474) dan *primer antisense* HBS2 (5'-AAA GCC CTG CGA ACC ACT GA-3'; posisi 713-694). Kondisi siklus untuk kedua *round* PCR adalah 40 siklus pada 94°C selama 1 menit, 55°C selama 1 menit dan 72°C selama 2 menit. Produk PCR divisualisasikan dengan elektroforesis menggunakan gel agarose 2% yang telah diwarnai dengan ethidium bromide. Produk PCR dipurifikasi menggunakan QIAquick PCR Purification Kit (Qiagen), selanjutnya di-*label* menggunakan Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) dan disekuens

menggunakan ABI Prism 310 Genetic Analyzer (Perkin Elmer). Sekuens nukleotida virus hepatitis B dari pendonor darah di Jayapura dibandingkan dengan sekuens nukleotida virus hepatitis B dari bank data DNA internasional (DDBJ/EMBL/GenBank). Genotipe virus hepatitis B ditentukan berdasarkan persentase homologi lebih dari 96% pada level gen S menggunakan perangkat lunak komputer Genetyx-Mac version 10.1.2 (Software Development Co., Ltd., Tokyo, Jepang). Pohon filogenetik direkonstruksi menggunakan *Unweighted Pair Group Method using Arithmetic averages* (UPGMA) *clustering*. Sekuens nukleotida virus hepatitis B dikonversi menjadi sekuens asam amino dan dilakukan *multiple alignment*. Subtipe virus hepatitis B ditentukan dengan analisis substitusi asam amino pada posisi 122, 127, 134, 159, 160 dan 177 pada gen S.

Empat puluh tiga (4,6%) dari 925 pendonor darah memperlihatkan HBsAg positif, dengan rentang usia 18,0-47,7 tahun, dan rata-rata usia 29,7 tahun (laki-laki:perempuan = 42:1). Tiga puluh enam (83,7%) dari 43 sampel berasal dari pendonor darah asli Papua, sedangkan sisanya berasal dari non Papua. DNA virus hepatitis B terdeteksi pada 40 (93,0%) dari 43 sampel; 17 (42,5%) dari 40 sampel pada PCR *first-round* dan 23 (57,5%) sampel lainnya pada PCR *second-round*. Pada 27 (62,5%) dari 40 sekuens nukleotida sampel dilakukan penentuan genotipe dan subtipe virus hepatitis B. Dua puluh tiga (85,2%) dari 27 isolat termasuk genotipe C, 2 (7,4%) isolat termasuk genotipe B dan 2 (7,4%) isolat termasuk genotipe D. Subtipe *adr* (85,2% dari 27 isolat) merupakan subtipe virus hepatitis B predominan, diikuti subtipe *adw2* (7,4%) dan subtipe *ayw2* (7,4%). Semua subtipe *adr* termasuk dalam genotipe C, demikian pula semua subtipe *adw2* dalam genotipe B dan semua subtipe *ayw2* dalam genotipe D. Tiga belas (56,5%) dari 23 isolat subtipe *adr* tidak memiliki determinan *q*, sedangkan 10 (43,5%) isolat lainnya memiliki determinan *q*. Berdasarkan analisis filogenetik sebagian gen S, 20 (87,0%) dari 23 isolat virus hepatitis B C/*adr* pada penelitian ini membentuk satu *cluster* terpisah, 1 (4,3%) isolat dalam *cluster* Melanesia dan Polinesia serta 2 (8,7%) isolat dalam *cluster* Jepang, Korea dan Cina.

Hasil penelitian ini memperlihatkan bahwa virus hepatitis B genotipe C predominan pada pendonor darah dengan HBsAg positif di Jayapura. Hasil ini mirip dengan hasil penelitian sebelumnya dan menguatkan fakta bahwa virus hepatitis B genotipe C predominan di bagian paling timur Indonesia. Penelitian ini juga memperlihatkan bahwa virus hepatitis B subtipe *adr* predominan. Tiga observasi sebelumnya pada murid Sekolah Dasar etnis Papua, pendonor darah dan penduduk dewasa asli Papua di Jayapura juga memperlihatkan pola yang sama. Hasil penelitian ini menegaskan kembali fakta bahwa Jayapura berada dalam zona *adr*. Pada tahun 1997, Mulyanto *et al.* menyatakan bahwa nenek moyang penduduk bagian paling timur Indonesia yang terinfeksi virus hepatitis B subtipe *adr* tampaknya datang dari Melanesia di mana subtipe *adr* banyak ditemukan. Isolat virus hepatitis B dari *New Caledonia* (Melanesia) dan *French Polynesia* (Polinesia) termasuk dalam subtipe *adrq-*. Menariknya, hasil penelitian ini justru memperlihatkan bahwa 43,5% isolat virus hepatitis B subtipe *adr* di Jayapura memiliki determinan *q* seperti isolat dari Jepang, Korea, Cina, Vietnam, Myanmar dan Thailand. Temuan ini membuka pemikiran baru yang masih perlu dikaji lebih jauh

tentang adanya pola migrasi tambahan dari nenek moyang penduduk asli Papua. Hasil analisis filogenetik pada penelitian ini dapat menjadi pendorong untuk dilakukannya penelitian lebih lanjut untuk mengetahui kekerabatan isolat virus hepatitis B C/*adr* dari Jayapura dengan isolat subgrup C1 dari Asia Tenggara serta dengan isolat dari populasi Aborigin di Australia.

Sebagai kesimpulan, hasil penelitian ini menguatkan temuan sebelumnya bahwa Jayapura termasuk dalam zona C/*adr*. Namun, dengan teridentifikasinya subtipe *adrq+* terungkap fakta bahwa virus hepatitis B C/*adr* di Jayapura tidak hanya dikaitkan dengan virus hepatitis B C/*adr* dari Melanesia dan Polinesia, seperti diasumsikan selama beberapa tahun terakhir. Penelitian lebih jauh diperlukan untuk mengklarifikasi karakteristik *cluster* virus hepatitis B C/*adr* dari Jayapura yang terpisah, baik dari *cluster* Melanesia dan Polinesia maupun dari *cluster* Jepang, Korea dan Cina.

SUMMARY

HEPATITIS B VIRUS GENOTYPES AND SUBTYPES AMONG *HEPATITIS B SURFACE ANTIGEN* (HBsAg)-POSITIVE BLOOD DONORS IN JAYAPURA, PAPUA PROVINCE, INDONESIA

In the year 2000, Core Working Party for Asia-Pasific Consensus on Hepatitis B and C stated that Indonesia had a moderate to high endemicity of hepatitis B. Hepatitis B virus (HBV) carrier rate among voluntary blood donors in eleven large cities in Indonesia ranges from 2.1% to 9.5%, while in Jayapura, Papua Province, it may reach 17.5%. To date, eight genotypes and nine subtypes of HBV have been identified worldwide. Both HBV genotypes and subtypes differ with each other in geographical distribution, clinical and virological characteristics, and can also provide historical information on the migration pattern of the ancestor of local population. The available molecular epidemiological data on HBV genotypes and subtypes pattern in Jayapura were obtained from several studies with limited samples conducted more than a decade ago. This indicates that a most current picture on HBV genotypes and subtypes pattern in Jayapura, particularly those in blood donors, is still unknown.

The objective of this study was to identify HBV genotypes and subtypes pattern among Hepatitis B surface Antigen (HBsAg)-positive blood donors in Jayapura.

Serum samples were taken from blood donors visited Blood Transfusion Unit, Indonesian Red Cross, Jayapura, from September 2004 to January 2005. Serum samples were screened for HBsAg detection using immunochromatography method and then were subjected to HBsAg determination using enzyme-linked immunosorbent assay method. Those with HBsAg-positive were subjected to HBV genotype and subtype determination. HBV DNA was extracted from 60 µl serum samples using DNAzol Reagent (Invitrogen). The amplification of part of the S gene was performed using polymerase chain reaction (PCR) with sense primer P7 (5'-GTG GTG GAC TTC TCT CAA TTT TC-3; positions 256-278) and antisense primer P8 (5'-CGG TAW AAA GGG ACT CAM GAT-3'; positions 796-776). If this first-round PCR amplification was negative, second-round PCR was carried out using sense primer HBS1 (5'-CAA GGT ATG TTG CCC GTT TG-3'; positions 455-474) and antisense primer HBS2 (5'-AAA GCC CTG CGA ACC ACT GA-3'; positions 713-694). The cycle condition for both PCR rounds were 40 cycles in 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes. Amplification products were visualized on 2% agarose gel electrophoresis stained with ethidium bromide. PCR products were purified using QIAquick PCR Purification Kit (Qiagen) and then labelled using Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) and sequenced by means of ABI Prism 310 Genetic Analyzer (Perkin Elmer). HBV nucleotide sequences of blood donors in Jayapura were compared to those from international DNA data bank (DDBJ/EMBL/GenBank). HBV genotype was determined based on the homologous percentage more than 96% in the S gene level using computer software Genetyx-Mac version 10.1.2 (Software Development Co., Ltd., Tokyo, Japan). Phylogenetic tree was reconstructed by Unweighted Pair

Group Method using Arithmetic averages (UPGMA) clustering. HBV nucleotide sequences were converted into amino acid ones and multiple aligned. HBV subtype was determined using analysis of amino acid substitutions at positions 122, 127, 134, 159, 160, and 177 in the S gene.

Forty-three (4.6%) of 925 blood donors were HBsAg-positive, with age ranging from 18.0-47.7 years, and average age of 29.7 years (male:female = 42:1). Thirty-six (83.7%) of 43 samples were obtained from native Papuan and the rest were from non-native Papuan. HBV DNA was detected in 40 (93.0%) of 43 samples; 17 (42.5%) of 40 samples at first-round PCR and 23 (57.5%) others at second-round PCR. Twenty-seven (62.5%) of 40 samples' nucleotide sequences were subjected to HBV genotype and subtype determination. This study showed that 23 (85.2%) of 27 isolates tested belonged to genotype C, 2 (7.4%) to genotype B, and 2 (7.4%) to genotype D. Subtype *adr* (85.2% of 27 isolates) was found to be the predominant HBV subtype, followed by *adw2* (7.4%) and *ayw2* (7.4%). All of subtype *adr* were included in genotype C, as all of *adw2* in genotype B and *ayw2* in genotype D. Thirteen (56.5%) of 23 isolates of subtype *adr* had no *q* determinant, while 10 (43.5%) others had *q* determinant. Based on phylogenetic analysis of part of the S gene, 20 (87.0%) of 23 isolates of HBV C/*adr* in this study formed a distinct cluster, 1 (4.3%) belonged to the Melanesian and Polynesian cluster and 2 (8.7%) to the Japanese, Korean and Chinese cluster.

This study showed that HBV genotype C was predominant among HBsAg-positive blood donors in Jayapura. This result was similar with previous study and confirmed the fact that HBV genotype C was predominant in the easternmost part of Indonesia. This study also found that HBV subtype *adr* is predominant. The three previous observations on Papuan elementary school children, blood donors and adult population of native Papuan in Jayapura also showed the same pattern. This study confirmed the fact that Jayapura was within the *adr*-zone. In the year 1997, Mulyanto *et al.* stated that the ancestor of the inhabitants of the easternmost part of Indonesia who were infected with HBV subtype *adr* most likely came from Melanesia, where subtype *adr* is largely found. HBV isolates from New Caledonia (Melanesia) and French Polynesia (Polynesia) were classified into subtype *adrq-*. Interestingly, this study showed that 43.5% of HBV isolates subtype *adr* in Jayapura did have *q* determinant as those found from Japan, Korea, China, Vietnam, Myanmar, and Thailand. This finding disclosed a new insight that deserved further investigation on the presence of additional migration pattern of the ancestor of native Papuan. Phylogenetic analysis results in this study should be a drive for conducting further studies on the genetic relatedness of HBV C/*adr* isolates from Jayapura with subgroup C1 isolates from southeast Asia and with Australian Aborigin isolates.

In conclusion, this study confirmed previous findings that Jayapura belonged to C/*adr*-zone. However, the identification of subtype *adrq+* disclosed the fact that HBV C/*adr* in Jayapura related not only to HBV C/*adr* from Melanesia and Polynesia as assumed during the last several years. Further studies were needed to clarify the characteristics of cluster of HBV C/*adr* from Jayapura, which was separated from the Melanesian and Polynesian cluster, and from the Japanese, Korean and Chinese cluster.

ABSTRACT

HEPATITIS B VIRUS GENOTYPES AND SUBTYPES AMONG *HEPATITIS B SURFACE ANTIGEN* (HBsAg)-POSITIVE BLOOD DONORS IN JAYAPURA, PAPUA PROVINCE, INDONESIA

Eight genotypes and nine subtypes of hepatitis B virus (HBV) have been identified worldwide. Both HBV genotypes and subtypes differ with each other in geographical distribution, clinical as well as virological characteristics, and can also provide historical information on the migration pattern of the ancestor of local population. We carried out DNA extraction, amplification with polymerase chain reaction and sequencing of serum samples of Hepatitis B surface Antigen-positive blood donors in Jayapura, Papua Province, Indonesia. The 27 obtained nucleotide sequences were compared with HBV nucleotide sequences from international DNA data bank for genotype determination. HBV subtype were determined using analysis of amino acid substitutions at positions 122, 127, 134, 159, 160, and 177 in the S gene. Twenty-three (85.2%) of 27 isolates tested belonged to genotype C, 2 (7.4%) to genotype B, and 2 (7.4%) to genotype D. Subtype *adr* (85.2% of 27 isolates) was found to be the predominant HBV subtype, followed by *adw2* (7.4%) and *ayw2* (7.4%). All of subtype *adr* were included in genotype C, as all of *adw2* in genotype B and *ayw2* in genotype D. Thirteen (56.5%) of 23 isolates of subtype *adr* had no *q* determinant as those found from Melanesia and Polynesia. Interestingly, 10 (43.5%) others had *q* determinant as those found from Japan, Korea, and China. This study confirmed previous findings that Jayapura belonged to C/*adr*-zone. However, the identification of subtype *adrq+* disclosed the fact that HBV C/*adr* in Jayapura related not only to HBV C/*adr* from Melanesia and Polynesia as assumed during the last several years. Based on phylogenetic analysis of part of the S gene, 20 (87.0%) of 23 isolates of HBV C/*adr* in this study belonged to a distinct cluster, which was separated from the Melanesian and Polynesian cluster, and from the Japanese, Korean and Chinese cluster.

Keywords : genotypes, subtypes, hepatitis B virus, HBsAg-positive blood donors, Papuan, Indonesia.