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Molecular Epidemiological Study of Hepatitis B Virus Among Migrant Workers From Cambodia, Laos, and Myanmar to Thailand

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Although hepatitis B virus (HBV) infection is endemic in Southeast Asia, molecular epidemiological data on HBV circulating in some countries are limited. The aims of this study were to evaluate the seroprevalence of HBV and its genetic variability among migrant workers from Cambodia, Laos, and Myanmar in Thailand. Sera collected from 1,119 Cambodian, 787 Laotian, and 1,103 Myanmarese workers were tested for HBsAg. HBV DNA was amplified and the pre-S/S region was sequenced for genotyping and genetic mutation analysis. HBsAg was detected in 282 (9.4%). The prevalence of HBsAg among migrant workers from Cambodia, Laos, and Myanmar was 10.8%, 6.9%, and 9.7%, respectively. Of 224 subjects positive for HBV DNA, 86% were classified as genotype C (99% were subgenotype C1) and 11.6% were genotype B (30.8%, 34.6%, and 30.8% were sub-genotypes B2, B3, and B4, respectively). Various point mutations in the ''a'' determinant region were detected in approximately 18% of these samples, of which Ile126Ser/Asn was the most frequent variant. Sequencing analysis showed that 19.1% of samples had pre-S mutations, with pre-S2 deletion as the most common mutant (7.7%) followed by pre-S2 start codon mutation (3.8%) and both pre-S2 deletion and start codon mutation (3.3%). High prevalence of HBV infection (approximately 7–11%) was found among migrant workers from Cambodia, Laos, and Myanmar, which may reflect the current seroprevalence in their respective countries. The data also demonstrated that HBV sub-genotype C1 was the predominant strain and various mutations of HBV occurring naturally were not uncommon among these populations. ***J. Med. Virol. 82:1341–1349, 2010.*** © 2010 Wiley-Liss, Inc.

KEY WORDS: hepatitis B virus; seroprevalence; genotype; mutation; Southeast Asia

INTRODUCTION

Hepatitis B virus (HBV) infection is one of the major causes of chronic liver disease ranging from chronic hepatitis to cirrhosis and hepatocellular carcinoma (HCC) [Ganem and Prince, 2004]. HBV, a member of the family *Hepadnaviridae*, is a relaxed-circular double-stranded DNA virus of approximately 3,200 bp in length, with four overlapping open-reading frames encoding the polymerase (P), precore (PC)/core (C), envelope (pre-S1/pre-S2/S), and X proteins [Ganem and Prince, 2004]. HBV shows remarkable genetic variability and is

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classified currently into at least eight genotypes, designated A to H and four major serotypes, including *ayw*, *ayr*, *adw*, and *adr* [Norder et al., 1992; Kramvis et al., 2005]. Each genotype can be divided further into subgenotypes based on 4–8% divergence of the viral genome. HBV genotype and sub-genotype distribution appears to show varying geographic patterns [Allain, 2006; McMahon, 2009]. For example, genotypes A and D are predominant in Western countries and India, whereas genotypes B and C are common in Southeast Asia, China, and Japan. Genotype E is restricted to Africa, while genotypes F and H are found in indigenous populations in Alaska and Central and South America. In Asia, subgenotype B1 is predominant in Japan, while subgenotypes B2–5 prevail in other countries. Sub-genotype C1 is prevalent mainly in Southeast Asia, whereas subgenotype C2 is found commonly throughout the Far East as, for example, in Japan, China, and Korea [Allain, 2006; McMahon, 2009].

Chronic HBV infection and its related hepatic complications are important particularly in Southeast Asian countries where the prevalence of the infection is relatively high, varying from 3% to 6% in Singapore, Malaysia, and Brunei to approximately 6–12% in Indonesia, Philippines, Myanmar, Laos, Cambodia, and Vietnam [Lingao et al., 1989; Alexander et al., 1990; Sebastian et al., 1990; Amirudin et al., 1991; Budihusodo et al., 1991; Thüring et al., 1993; Lansang, 1996; Merican et al., 2000; James et al., 2001; Nakai et al., 2001; Caruana et al., 2005; Thuy et al., 2005; Srey et al., 2006; Jutavijittum et al., 2007; Duong et al., 2009; Utama et al., 2009]. In Thailand, the prevalence of HBV infection has declined upon implementation of the national HBV vaccination program, with present prevalence of approximately 4% [Theamboonlers et al., 1999; Luksamijarulkul et al., 2002; Chongsrisawat et al., 2006; Suwannakarn et al., 2008]. The predominant HBV genotypes in this region are genotypes C and B (Fig. 1). At present, a large number of migrant workers, originating from these countries, are employed in various sectors of Thai industries located in Bangkok and neighboring provinces. In 2006, registered and non-registered foreign workers in Thailand were approximately 1.8 million migrants [Martin, 2007]. Growing influx of migrant populations may influence the prevalence of HBV infection and the resulting disease burden in Thailand. The present study has been aimed at evaluating the HBV seroprevalence and its genetic variability, including genotypes, antigenic subtypes, and mutations present among these migrant workers. In addition, the phylogenetic relatedness of HBV strains isolated from these subjects was investigated.

MATERIALS AND METHODS

Study Populations

The serum samples of migrant workers collected for a routine health check-up were stored at −70°C until

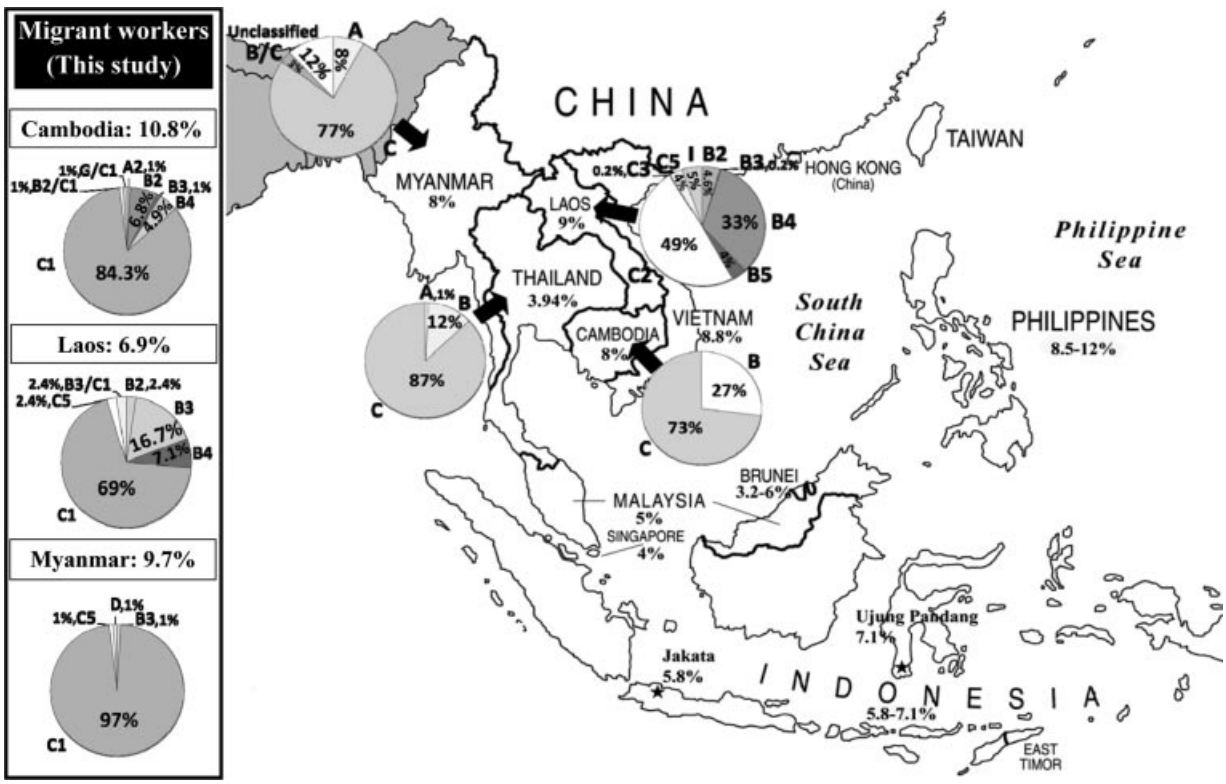

Fig. 1. The prevalence and genotypes of HBV infection in Southeast Asia countries derived from previous reports. Charts in the left corner demonstrate the prevalence and subgenotypes among migrant workers from Cambodia, Myanmar, and Laos in this study.

further analysis. In this study, 3,009 serum samples collected from 1,119 Cambodians (353 females, 763 males, and 3 unidentified), 787 Laotians (413 females, 364 males, and 10 unidentified), and 1,103 Myanmarese (582 females, 423 males, and 98 unidentified) were tested for hepatitis B surface antigen (HBsAg) by using a commercial automated ELISA (Murex Biotech Limited, Dartford, Kent, England). Samples positive for HBsAg were subjected to further analysis aimed at molecular characterization of HBV. The project had been approved by the ethical committee of the Faculty of Medicine, Chulalongkorn University.

HBV DNA Extraction, Amplification, and Sequencing

HBV DNA was extracted from 100 μm each of HBsAg-positive sera. The respective serum samples were incubated in lysis buffer (10 mM Tris–HCl, pH 8.0; 0.1 M EDTA, pH 8.0; 0.5% SDS; and 20 mg/ml proteinase K) at 50°C for 60 min followed by phenol/chloroform/isoamyl alcohol extraction and ethanol precipitation. DNA pellet was re-suspended in 30 μl of distilled water. The pre-S1/pre-S2/S region was amplified using primers Pre-S1F+ (5′-GGG TCA CCA TAT TCT TGG GAA C-3′: position 2814–2835) and R5 (5′-AGC CCA AAA GAC CCA CAA TTC-3′: position 1015–995) The total 25-μl reaction volume consisted of 10 μl of 2.5× 5 PRIME MasterMix solution (5 PRIME GmbH, Hamburg, Germany), 0.5 μl of 25 μM forward and reverse primers, 2 μl of DNA template and sterile distilled water. The thermocycler was programmed for HBV DNA amplification as follows: initial denaturation at 94°C for 3 min followed by 40 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 1.30 min and a final extension step at 72°C for 7 min. The HBV DNA amplicons were separated by 2% agarose gel electrophoresis at 100 V for 60 min and stained with ethidium bromide. PCR product size was estimated in comparison with a 100-bp DNA ladder under UV light. The expected products were excised from the gel and purified using the Perfectprep® Gel Cleanup Kit (Eppendorf, Hamburg, Germany). The purified samples were sent to a commercial DNA sequencing company (First BASE Laboratories Sdn Bhd, Selangor Darul Ehsan, Malaysia) for sequencing. Nucleotide sequences were edited by Chromas Lite program version 2.01 (Technelysium Pty Ltd, Queensland, Australia) and assembled by SeqMan (DNASTAR Lasergene Software, Madison, WT).

Genotyping, Subtyping, and Phylogenetic Analysis

Each sequence was aligned with each available human genotype stored at the GenBank database (National Center for Biotechnology Information, Bethesda, MD) by Clustal X program version 2.0.10 (European Bioinformatics Institute, Cambridge, UK). Based on these alignments phylogenetic trees were constructed for genotyping using Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0 (The Biodesign Institute, Tempe, AZ) for genotyping. The neighbor-joining method by Tamura-3 parameter was used for constructing phylogenetic trees. Uncompleted sequences were genotyped by the Viral Genotyping Tool (National Center for Biotechnology Information). Genetic recombinants were further determined by SimPlot program and bootscanning analysis (Simplot version 3.5.1, Baltimore, MD). HBV nucleotides were translated into amino acid sequences using the translation tool in ExPASy Proteomics Server (available on: http://www.expasy.ch/tools/dna.html). Subsequently, subtypes were identified based on the amino acids at positions 122 and 160 of the S protein.

HBV Mutation Analysis

HBV sequences were evaluated for mutations and deletions in the pre-S1/pre-S2 regions. The amino acids at positions 120 and 160 of the S protein were indicative for "a" determinant mutations.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD), and percentages as appropriate. Comparisons among groups were analyzed by the Pearson χ^2 or Fisher's exact test for categorical variables and by one-way ANOVA Bonferroni adjustment for quantitative variables. *P*-values below 0.05 were considered significant. All statistical analyses were performed using the SPSS software for Windows 17.0 (SPSS, Inc., Chicago, IL).

RESULTS

HBsAg Detection

HBsAg was detected in 282 of 3,009 (9.4%) samples. This group comprised 121 Cambodians (10.8%), 54 Laotians (6.9%), and 107 Myanmarese (9.7%). Among these subjects, HBV DNA was detected in 102 Cambodians (84.3%), 42 Laotians (77.8%), and 80 Myanmarese (74.8%) (Table I).

Distribution of HBV Genotypes and Serotypes

All sequences obtained from this study were submitted to the GenBank database (accession nos. GQ855313–GQ85570 and GQ856585). Phylogenetic analysis was performed based on the pre-S1/pre-S2/S genes. Of those positive for HBV DNA, 194 of 224 (86.6%) cases were determined as genotype C (99% and 1% were sub-genotypes C1 and C5, respectively), 25 (11.2%) cases were identified as genotype B (32%, 36%, and 32% were sub-genotypes B2, B3, and B4, respectively), 1 (0.44%) case as genotype A (sub-genotype A2), and 1 (0.44%) case as genotype D. As for antigenic subtype distribution, *adr* was the most common (68.3%), followed by *ayw* (8.9%), *adw* (6.7%), and *ayr* (0.9%). The prevalence of HBV genotype and subtype with respect to geographic location is shown in

TABLE I. Prevalence of HBV Genotypes and Subtypes in Migrant Workers

	Cambodia (n = 1,119)	Laos (n = 787)	Myanmar (n = 1,103)	Total (n = 3,009)	*P*-value
No. HBsAg pos.	121 (10.8)	54 (6.9)	107 (9.7)	282 (9.4)	0.013*
No. HBV DNA pos.	102 (84.3)	42 (77.8)	80 (74.8)	224 (79.4)	0.008*
Gender (M/F/ND[a])	81 (79.4):20 (19.6):1 (1)	31 (73.8):11 (26.2):0	46 (57.5):28 (35):6 (7.5)	158 (70.5):59 (26.3):7 (3.2)	0.030*
Age (years; mean ± SD)	29.2 ± 8.6	26.2 ± 7.4	28.3 ± 6.1	28.3 ± 7.6	NS
15–20 (M/F/ND/total)	14:0:0	8:3:0	7:3:0	29 (18.4):6 (10.2):0:35 (15.6)	
21–30	37:13:0	18:6:0	19:16:6	74 (46.8):35 (59.3):6 (85.7):115 (51.3)	
31–40	19:4:0	3:1:0	18:9:0	40 (25.3):14 (23.7):0:54 (24.1)	
41–50	10:3:1	2:1:0	2:0:0	14 (8.9):4 (6.8):1 (14.3):19 (8.5)	
ND	1:0:0	0:0:0	0:0:0	1 (0.6):0:0:1 (0.5)	
Genotype					
A2[b]	1 (1.0)	0 (0)	0 (0)	1 (0.44)	NS
B	13 (12.7)	11 (26.2)	1 (1.25)	25 (11.2)	0.000*
B2	7 (6.9)	1 (2.4)	0 (0)	8 (3.6)	
B3	1 (1.0)	7 (16.7)	1 (1.3)	9 (4.0)	
B4	5 (4.9)	3 (7.1)	0 (0)	8 (3.6)	
C	86 (84.3)	30 (71.4)	78 (97.5)	194 (86.6)	0.000*
C1	86 (84.3)	29 (69.0)	77 (96.3)	192 (85.7)	
C5	0 (0)	1 (2.4)	1 (1.25)	2 (0.9)	
D[b]	0 (0)	0 (0)	1 (1.25)	1 (0.44)	NS
Suspected recombination					NS
B2/C1	1 (1.0)	0 (0)	0 (0)	1 (0.44)	
B3/C1	0 (0)	1 (2.4)	0 (0)	1 (0.44)	
G/C1	1 (1.0)	0 (0)	0 (0)	1 (0.44)	
Subtype					
adr	76 (74.5)	20 (47.6)	57 (71.25)	153 (68.3)	0.000*
adw	9 (8.8)	5 (11.9)	1 (1.25)	15 (6.7)	NS
ayr	1 (1.0)	1 (2.4)	0 (0)	2 (0.9)	NS
ayw	6 (5.9)	12 (28.6)	2 (2.5)	20 (8.9)	0.000*
Could not be identified	10 (9.8)	4 (9.5)	20 (25.0)	34 (15.2)	

NS, no statistical significance.
Data were expressed as mean ± SD, n (%).
[a]Data not available.
[b]*Pre-C* gene could not be amplified.
*P-values <0.05.

Table I. There were significant differences in genotype and serotype distribution among groups. Briefly, Cambodians and Laotians had significantly higher prevalence of genotype B but had significantly lower prevalence of genotype C than those of Myanmarese ($P < 0.05$). In addition, Laotians had significantly higher prevalence of serotype *ayw* but had significantly lower prevalence of serotype *adr* than those of Cambodians and Myanmarese ($P < 0.05$).

Although the entire genome were not sequenced in this study, three isolates with suspected inter-genotype recombinants were identified (isolate 31 with genotype B2/C1, accession no. GQ855407; isolate 612 with genotype B3/C1, accession nos. GQ855454 and GQ855560; and isolate 3794 with genotype G/C1, accession no. GQ856585). Isolate 31 proved to be a recombinant of sub-genotypes B2 and C1, with its recombination breakpoint estimated at nucleotide 573 (Fig. 2A). Isolate 3794 represented a recombinant of genotypes G/C1 with its recombination breakpoints between nucleotides 2854 and 56 (Fig. 2B). Isolate 612 was classified as sub-genotype B3 in the pre-S/S gene but showed sub-genotype C1 between nucleotides 1554 and 1974 (figure not shown).

Prevalence and Characterization of the "a" Determinant Mutations

In this study, various point mutations in the "a" determinant region were detected in 35 out of 194 (18.0%) HBV isolates. Mutations were found in 19/94 (20.2%) of Cambodian samples, 6/38 (15.8%) of Laotian samples, and 10/62 (16.1%) of Myanmarese samples. The most frequent mutation in Cambodian, Laotian, and Myanmarese isolates was Ile126Ser/Asn. In addition, multiple point mutations in the "a" determinant region were detected in six isolates (Supplement 1). Amino acid sequence alignment of the partial S region of these 35 isolates is shown in Figure 3.

Prevalence and Characterization of Pre-S/S Mutations

Sequencing of the amplicons showed that pre-S mutations were detected in 40 of 209 cases (19.1%). In this study, the prevalence of pre-S mutations/deletions among Cambodian, Laotian, and Myanmarese migrant workers was 18.4%, 15.0%, and 22.5%, respectively. As for the prevalence of site-specific pre-S/S mutations, pre-S2 deletion was the most common (7.7%), followed

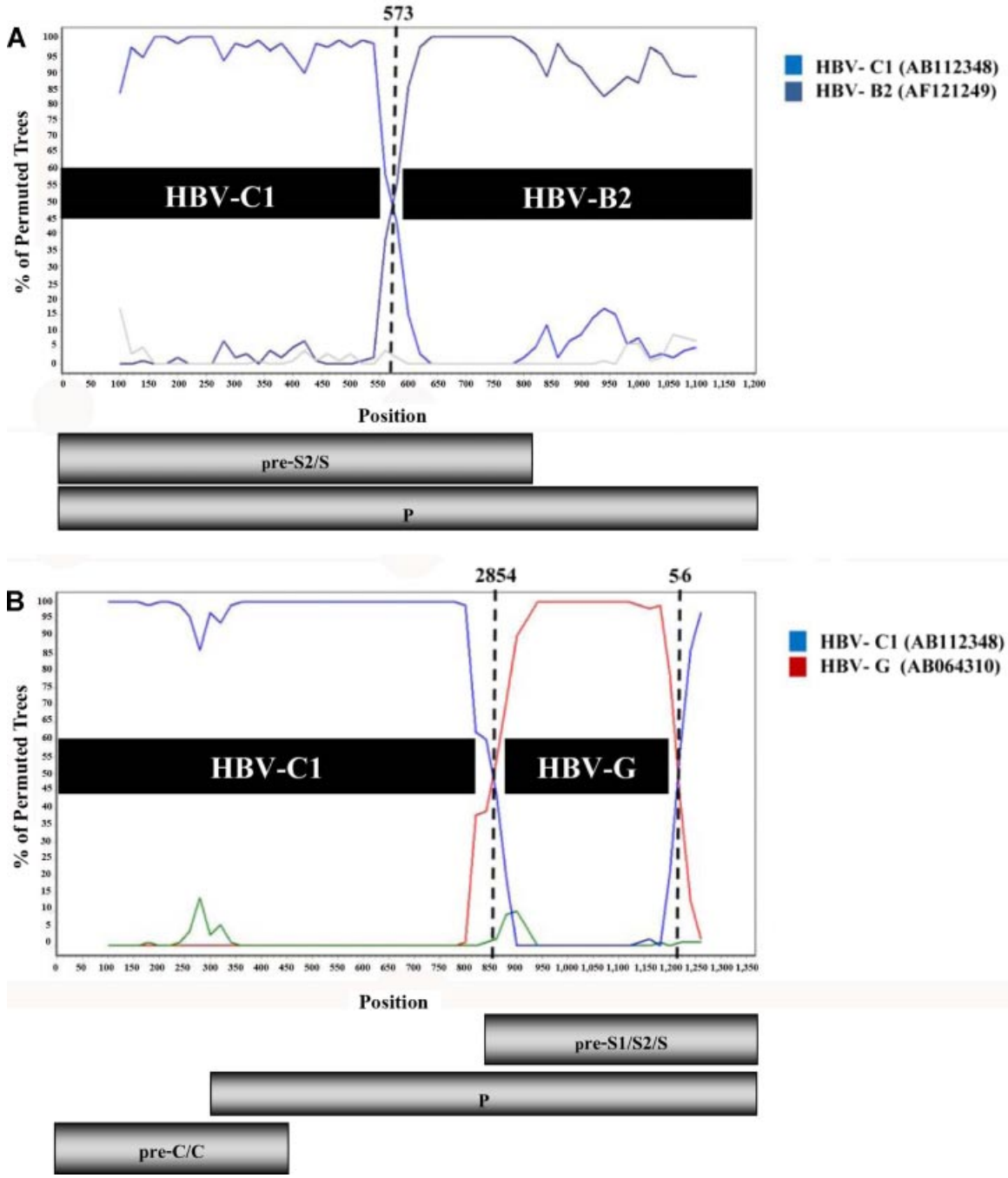

Fig. 2. Bootscanning analysis of suspected recombinant isolates. **A**: Complete S gene of isolate 31 was compared with HBV-B2 (AF121249) and HBV-C1 (AB112348); (**B**) isolate 3794, nucleotide positions 2006–157, was compared with HBV-C1 (AB112348) and HBV-G (AB064310). Dashed line(s) indicate(s) the breaking point(s) of recombination. The number above the dashed line indicates the nucleotide position of each isolate compared with the reference strain (NC_003977).

by pre-S2 start codon mutation (3.8%); both pre-S2 deletion and start codon mutation (3.3%); pre-S1 deletion (1.4%); pre-S2 start codon deletion and pre-S2 deletion (1.0%); pre-S1 start codon mutation and pre-S1 deletion (0.5%); pre-S1 start codon deletion and pre-S2 deletion (0.5%) and pre-S2 start codon mutation and pre-S2 deletion (0.5%). Amino acid sequence alignment of the entire pre-S1/pre-S2 region of the 40 samples is shown in Figure 4.

DISCUSSION

Although chronic HBV infection prevails in Southeast Asia, the data on its molecular epidemiology in some

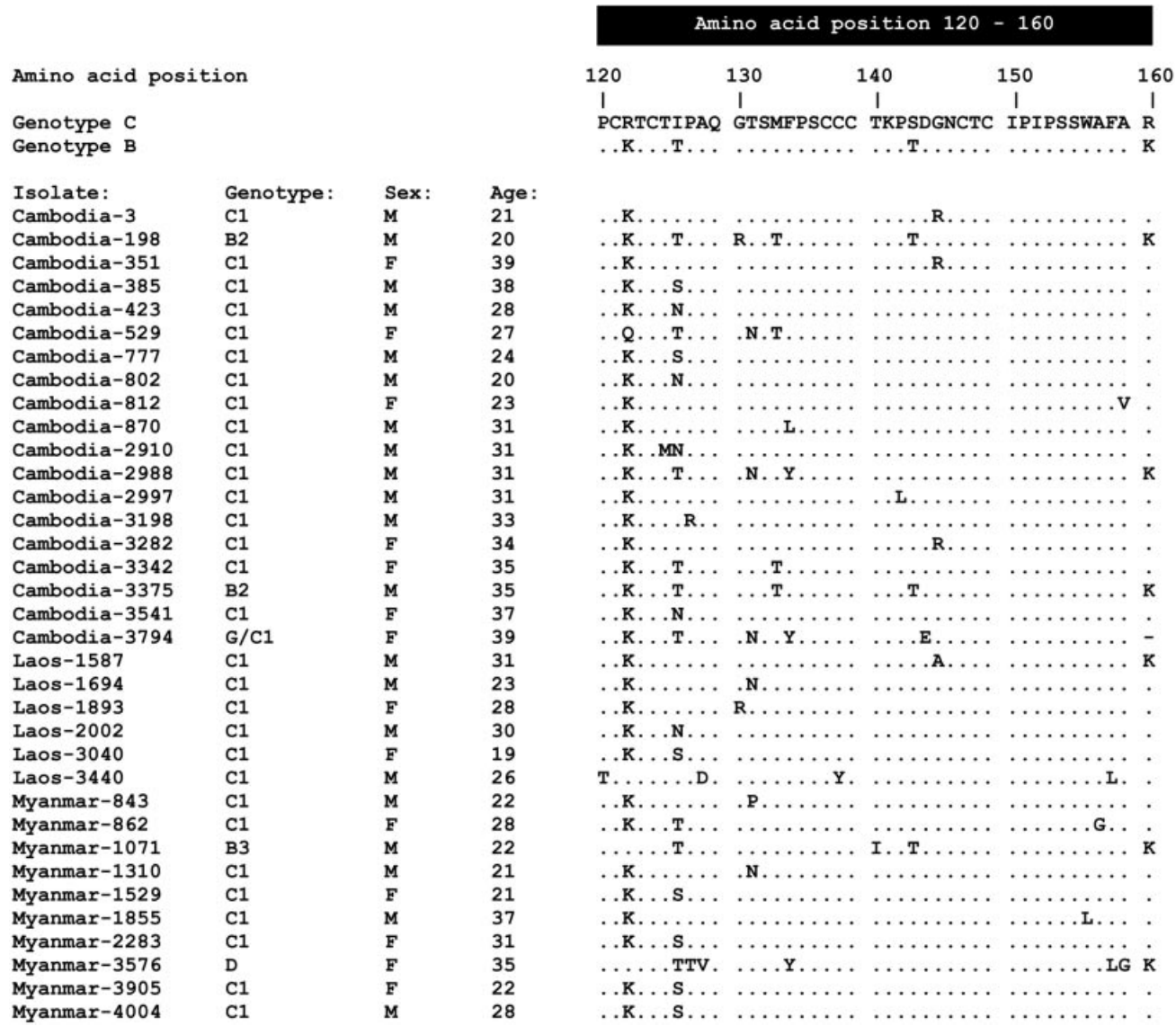

Fig. 3. Amino acid sequence alignment of the "a" determinant region of 35 samples.

countries in this part of the world are still limited. This has been the first comparative study on molecular characterization of HBV circulating in Cambodia, Laos, and Myanmar. This study, which included identification of both viral genotypes and subtypes in a significant number of HBV carriers from these countries, demonstrated that the predominant HBV strains belong to categories C1/*adr*, which accounted for more than 85% of cases. In this study, the most dominant strains of HBV found in migrant workers from the both countries (Myanmar and Laos) were HBV genotypes C1 and B3. In contrast to other previous studies, the most dominant strains of HBV in Laos were sub-genotype C2 and B4 [Olinger et al., 2008], and in Myanmar genotypes C and A [Nakai et al., 2001]. The difference may be the results of different population bring studied sampling. In this study, most of the migrant workers came from the boundaries between Thailand and Laos or Myanmar, whereas other reports studied in the population from the capital cities. These findings are not surprising but reflect the typical genotypes and subtypes circulating in Southeast Asia. The seroprevalence of HBsAg in these migrant workers was approximately 7–11%, similar to previous reports on seroprevalence in these countries but higher than a recent nationwide survey in Thailand (4%) [Theamboonlers et al., 1999; Luksamijarulkul et al., 2002]. This difference in seroprevalence among populations reflects a steady and remarkable decrease in chronic HBV carrier rate among Thai populations after the 1992 implementation of universal HBV vaccination.

HBV strains resulting from genomic recombination between different genotypes have been increasingly recognized in various parts of the world. In Asia, recombination of genotypes B/C has been reported in China, Hong Kong, Indonesia, Taiwan, Thailand, and

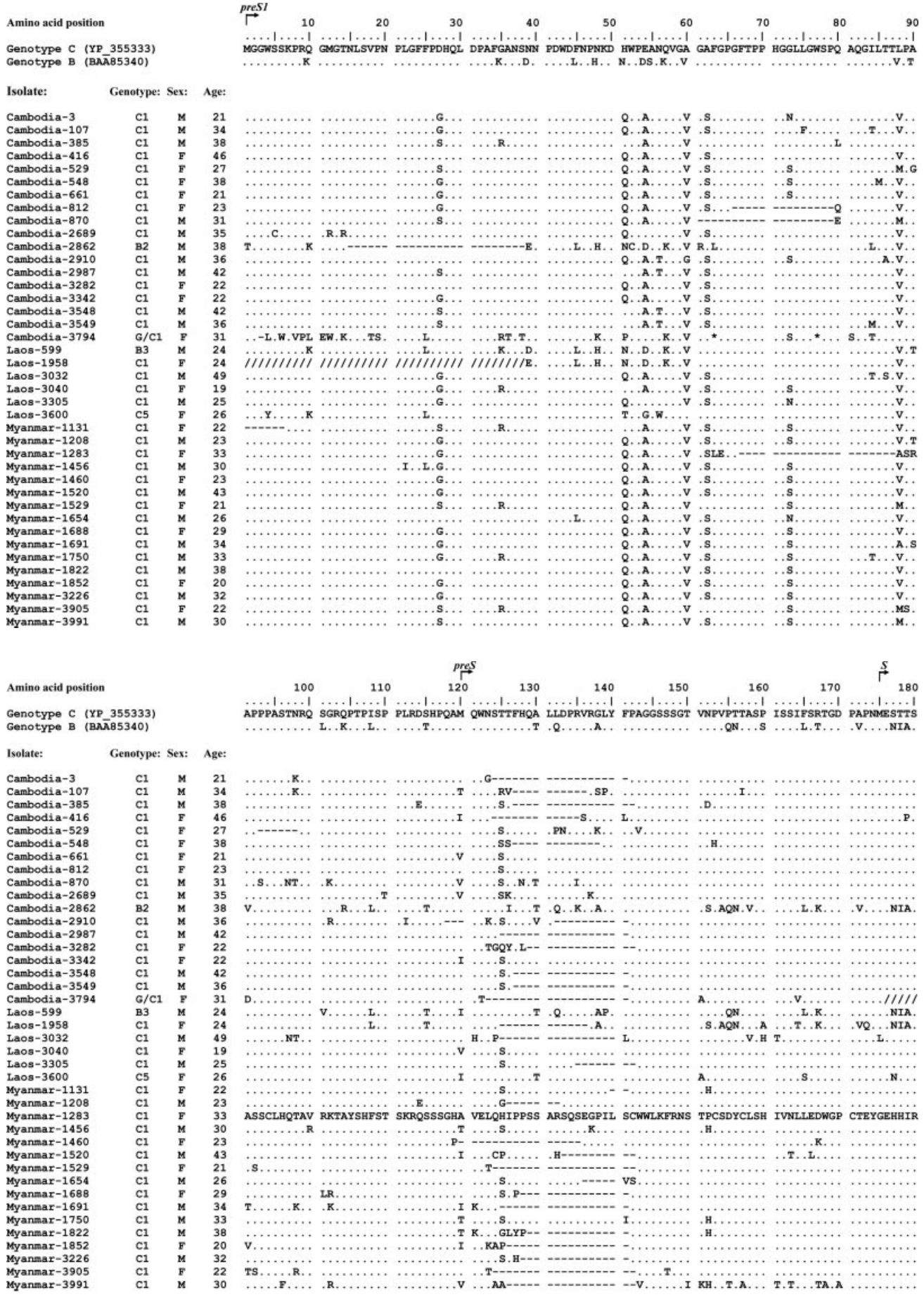

Fig. 4. Amino acid sequence alignment of the entire pre-S1/pre-S2 region of 40 samples.

Vietnam [Sugauchi et al., 2002], whereas recombination of genotypes C/D has been detected in Tibet and China [Cui et al., 2002; Wang et al., 2005]. In addition, recombinants between genotypes A/C and genotypes A/D have been documented in Vietnam [Hannoun et al., 2000] and India [Chauhan et al., 2008], respectively. Recently, a novel genotype I, with a complex recombination involving genotypes C, A, and G has been reported in Vietnam and Laos [Huy et al., 2008; Olinger et al., 2008]. Although the entire genome sequence was not determined in this study, three HBV isolates with suspected inter-genotype recombinants were identified. It is of note that a hybrid of genotypes B3/C1 in this study displayed recombination breakpoints in the vicinity of the pre-C/C region, which is the most common site of inter-genotype recombination as previously described [Sugauchi et al., 2002]. Another recombinant of genotypes G/C with its recombination breakpoints between nucleotides 2006 and 157 was also demonstrated in this study. Interestingly, the site of breakpoints in this recombinant was different from that found in a hybrid of genotypes G/C identified previously in a Thai patient with HCC [Suwannakarn et al., 2008].

Amino acid substitutions within the "a" determinant domain could lead to conformational changes which may interfere with active and passive immunization against HBV infection [Carman et al., 1990]. The most common vaccine escape mutant resulted from the mutation at position 145 (Gly145Arg), which is located in the second loop of the "a" determinant [Carman et al., 1990]. In this study, however, the most common amino acid substitution found in Cambodian, Laotian, and Myanmarese samples was located at position 126. In addition, the prevalence of "a" determinant mutants among chronic carriers from these countries was approximately 15–20%, which was slightly higher than the prevalence among random chronic carriers recently reported (6–12%) [Echevarria and Avellón, 2006]. It has been proposed that vaccination might have increased a selection pressure on the emergence of surface mutants in relation to wild-type HBV, as has been observed in several regions of the world [Carman et al., 1990; Cooreman et al., 2001; Coleman, 2006]. For example, a previous study in Taiwan demonstrated an increase in the prevalence of "a" determinant mutants in children from 7.8% before to 23.1% 15 years after the introduction of universal vaccination against HBV [Hsu et al., 2004]. High prevalence of the variants among migrant workers in this study, however, might not be associated with previous vaccination because the coverage rates of HBV vaccine administration in their countries are generally low [Caruana et al., 2005; Soeung et al., 2009]. Thus, it is speculated that these mutants within the "a" determinant region might have emerged in response to natural immunoselective pressure of the host. These infectious mutants have been circulating among individuals chronically infected with the virus.

HBV pre-S mutations/deletions occurring naturally have been reported frequently in chronic HBV carriers. It has been shown that pre-S deletion mutants tend to accumulate during a later stage of persistent HBV infection, including cirrhosis and HCC [Chen et al., 2006]. In fact, the prevalence of these mutations/deletions is rather variable and different, ranging from 0% to 36%, between diverse geographic areas [Huy et al., 2003]. In this study, the prevalence of pre-S mutations/deletions among Cambodian, Laotian, and Myanmarese migrant workers amounted to 18.4%, 15.0%, and 22.5%, respectively, which was higher than that determined by the previous study conducted on Thai populations (9.5%) [Suwannakarn et al., 2008]. As for the site of mutations, this study showed that pre-S2 deletion was the most common mutation type, followed by pre-S2 start codon mutation and the combined pre-S2 deletion and start codon mutation. These results were in agreement with those reported recently from Japan, Korea, and Thailand, according to which deletion in pre-S2 regions and pre-S2 start codon mutations was among the most prevailing [Huy et al., 2003; Choi et al., 2007; Suwannakarn et al., 2008].

In conclusion, high seroprevalence of HBsAg (approximately 7–11%) was found among migrant workers from Cambodia, Laos, and Myanmar, which may reflect the present prevalence of HBV infection in their respective countries. HBV sub-genotype/subtype C1/*adr* was the predominant strain circulating in these migrant workers. In addition, the "a" determinant variants were found frequently in these populations, and might not be attributed to vaccine-induced mutation. Finally, pre-S mutations, especially pre-S2 deletions and pre-S2 start codon mutations were not uncommon among these populations.

REFERENCES

Alexander MJ, Sinnatamby AS, Rohaimah MJ, Harun AH, Ng JS. 1990. Incidence of hepatitis B infection in Brunei Darussalam—Analysis of racial distribution. Ann Acad Med Singapore 19:344–346.

Allain JP. 2006. Epidemiology of hepatitis B virus and genotype. J Clin Virol 36:S12–S17.

Amirudin R, Akil H, Akahane Y, Suzuki H. 1991. Hepatitis B and C virus infection in Ujung Pandang, Indonesia. Gastroenterol Jpn 26:184–188.

Budihusodo U, Sulaiman HA, Akbar HN, Lesmana LA, Waspodo AS, Noer HM, Akahane Y, Suzuki H. 1991. Seroepidemiology of HBV and HCV infection in Jakarta, Indonesia. Gastroenterol Jpn 26: 196–201.

Carman WF, Zanetti AR, Karayiannis P, Waters J, Manzillo G, Tanzi E, Zuckerman AJ, Thomas HC. 1990. Vaccine-induced escape mutant of hepatitis B virus. Lancet 336:325–329.

Caruana SR, Kelly HA, De Silva SL, Chea L, Nuon S, Saykao P, Bak N, Biggs BA. 2005. Knowledge about hepatitis and previous exposure to hepatitis viruses in immigrants and refugees from the Mekong Region. Aust N Z J Public Health 29:64–68.

Chauhan R, Kazim SN, Kumar M, Bhattacharjee J, Krishnamoorthy N, Sarin SK. 2008. Identification and characterization of genotype A and D recombinant hepatitis B virus from Indian chronic HBV isolates. World J Gastroenterol 14:6228–6236.

Chen BF, Liu CJ, Jow GM, Chen PJ, Kao JH, Chen DS. 2006. High prevalence and mapping of pre-S deletion in hepatitis B virus carriers with progressive liver diseases. Gastroenterology 130: 1153–1168.

Choi MS, Kim DY, Lee DH, Lee JH, Koh KC, Paik SW, Rhee JC, Yoo BC. 2007. Clinical significance of pre-S mutations in patients with genotype C hepatitis B virus infection. J Viral Hepat 14:161–168.

Chongsrisawat V, Yoocharoen P, Theamboonlers A, Tharmaphornpilas P, Sinlaparatsamee S, Chaiear K, Khwanjaipanich S, Poovorawan Y. 2006. Hepatitis B seroprevalence in Thailand: 12 years after hepatitis B vaccine integration into the National Expanded Programme on Immunization. Trop Med Int Health 11:1496–1502.

Coleman PF. 2006. Detecting hepatitis B surface antigen mutants. Emerg Infect Dis 12:198–203.

Cooreman MP, Leroux-Roels G, Paulij WP. 2001. Vaccine- and hepatitis B immune globulin-induced escape mutations of hepatitis B virus surface antigen. J Biomed Sci 8:237–247.

Cui C, Shi J, Hui L, Xi H, Zhuoma, Quni, Tsedan, Hu G. 2002. The dominant hepatitis B virus genotype identified in Tibet is a C/D hybrid. J Gen Virol 83:2773–2777.

Duong TH, Nguyen PH, Henley K, Peters M. 2009. Risk factors for hepatitis B infection in rural Vietnam. Asian Pac J Cancer Prev 10:97–102.

Echevarria JM, Avellón A. 2006. Hepatitis B virus genetic diversity. J Med Virol 78:S36–S42.

Ganem D, Prince AM. 2004. Hepatitis B virus infection—Natural history and clinical consequences. N Engl J Med 350:1118–1129.

Hannoun C, Norder H, Lindh M. 2000. An aberrant genotype revealed in recombinant hepatitis B virus strains from Vietnam. J Gen Virol 81:2267–2272.

Hsu HY, Chang MH, Ni YH, Chen HL. 2004. Survey of hepatitis B surface variant infection in children 15 years after a Nationwide Vaccination Programme in Taiwan. Gut 53:1499–1503.

Huy TT, Ushijima H, Win KM, Luengrojanakul P, Shrestha PK, Zhong ZH, Smirnov AV, Taltavull TC, Sata T, Abe K. 2003. High prevalence of hepatitis B virus pre-s mutant in countries where it is endemic and its relationship with genotype and chronicity. J Clin Microbiol 41:5449–5455.

Huy TT, Sall AA, Reynes JM, Abe K. 2008. Complete genomic sequence and phylogenetic relatedness of hepatitis B virus isolates in Cambodia. Virus Genes 36:299–305.

James L, Fong CW, Foong BH, Wee MK, Chow A, Shum E, Chew SK. 2001. Hepatitis B seroprevalence study 1999. Singapore Med J 42: 420–424.

Jutavijittum P, Yousukh A, Samountry B, Samountry K, Ounavong A, Thammavong T, Keokhamphue J, Toriyama K. 2007. Seroprevalence of hepatitis B and C virus infections among Lao blood donors. Southeast Asian J Trop Med Public Health 38:647–649.

Kramvis A, Kew M, François G. 2005. Hepatitis B virus genotypes. Vaccine 23:2409–2423.

Lansang MA. 1996. Epidemiology and control of hepatitis B infection: A perspective from the Philippines, Asia. Gut 38:S43–S47.

Lingao AL, Torres NT, Muñoz N, Lansang MA, West SK, Bosch FX, Domingo EO. 1989. Mother to child transmission of hepatitis B virus in the Philippines. Infection 17:275–279.

Luksamijarulkul P, Thammata N, Tiloklurs M. 2002. Seroprevalence of hepatitis B, hepatitis C and human immunodeficiency virus among blood donors, Phitsanulok Regional Blood Center, Thailand. Southeast Asian J Trop Med Public Health 33:272–279.

Martin P. 2007. Introduction: Foreign workers in Thailand. In: Martin P, editor. The economic contribution of migrant workers to Thailand: Towards policy development. Bangkok: International Labour office. pp 1–6.

McMahon BJ. 2009. The influence of hepatitis B virus genotype and subgenotype on the natural history of chronic hepatitis. Hepatol Int 3:334–342.

Merican I, Guan R, Amarapuka D, Alexander MJ, Chutaputti A, Chien RN, Hasnian SS, Leung N, Lesmana L, Phiet PH, Sjalfoellah Noer HM, Sollano J, Sun HS, Xu DZ. 2000. Chronic hepatitis B virus infection in Asian countries. J Gastroenterol Hepatol 15:1356–1361.

Nakai K, Win KM, Oo SS, Arakawa Y, Abe K. 2001. Molecular characteristic-based epidemiology of hepatitis B, C, and E viruses and GB virus C/hepatitis G virus in Myanmar. J Clin Microbiol 39: 1536–1539.

Norder H, Hammas B, Löfdahl S, Couroucé AM, Magnius LO. 1992. Comparison of the amino acid sequences of nine different serotypes of hepatitis B surface antigen and genomic classification of the corresponding hepatitis B virus strains. J Gen Virol 73:1201–1208.

Olinger CM, Jutavijittum P, Hübschen JM, Yousukh A, Samountry B, Thammavong T, Toriyama K, Muller CP. 2008. Possible new hepatitis B virus genotype, Southeast Asia. Emerg Infect Dis 14: 1777–1780.

Sebastian VJ, Bhattacharya S, Ray S, Daud JH. 1990. Prevalence of hepatitis B-surface antigen in the pregnant women of Brunei Darussalam. Southeast Asian J Trop Med Public Health 21:123–127.

Soeung SC, Rani M, Huong V, Sarath S, Kimly C, Kohei T. 2009. Results from Nationwide Hepatitis B Serosurvey in Cambodia using simple and rapid laboratory test: Implications for National Immunization Program. Am J Trop Med Hyg 81:252–257.

Srey CT, Ijaz S, Tedder RS, Monchy D. 2006. Characterization of hepatitis B surface antigen strains circulating in the Kingdom of Cambodia. J Viral Hepat 13:62–66.

Sugauchi F, Orito E, Ichida T, Kato H, Sakugawa H, Kakumu S, Ishida T, Chutaputti A, Lai CL, Ueda R, Miyakawa Y, Mizokami M. 2002. Hepatitis B virus of genotype B with or without recombination with genotype C over the precore region plus the core gene. J Virol 76: 5985–5992.

Suwannakarn K, Tangkijvanich P, Thawornsuk N, Theamboonlers A, Tharmaphornpilas P, Yoocharoen P, Chongsrisawat V, Poovorawan Y. 2008. Molecular epidemiological study of hepatitis B virus in Thailand based on the analysis of *pre-S* and *S* genes. Hepatol Res 38:244–251.

Theamboonlers A, Jantaradsamee P, Kaew-In N, Tangkijvanich P, Hirsch P, Poovorawan Y. 1999. The predominant genotypes of hepatitis B virus in Thailand. Ann Trop Med Parasitol 93:737–743.

Thüring EG, Joller-Jemelka HI, Sareth H, Sokhan U, Reth C, Grob P. 1993. Prevalence of markers of hepatitis viruses A, B, C and of HIV in healthy individuals and patients of a Cambodian province. Southeast Asian J Trop Med Public Health 24:239–249.

Thuy le TT, Ryo H, Van Phung L, Furitsu K, Nomura T, 2005. Distribution of genotype/subtype and mutational spectra of the surface gene of hepatitis B virus circulating in Hanoi, Vietnam. J Med Virol 76:161–169.

Utama A, Octavia TI, Dhenni R, Miskad UA, Yusuf I, Tai S. 2009. Hepatitis B virus genotypes/subgenotypes in voluntary blood donors in Makassar, South Sulawesi, Indonesia. Virol J 6:128.

Wang Z, Liu Z, Zeng G, Wen S, Qi Y, Ma S, Naoumov NV, Hou J. 2005. A new intertype recombinant between genotypes C and D of hepatitis B virus identified in China. J Gen Virol 86:985–990.