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Prevalence of HBV infection among different HIV-risk groups in Hai Phong, Vietnam

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Running head: HBV in Vietnamese **HIV**-risk **groups**

Abstract

Hepatitis B virus (HBV) infection in Hai Phong, northern Vietnam, was characterized by analyzing the prevalence and genotype distribution of HBV as well as co-infection with human immunodeficiency virus type 1 (HIV-1) among five different groups at risk for HIV infection. Plasma samples were collected from intravenous drug users (n=760, anti-HIV-1 antibody positive rate: 35.9%), female sex workers (n=91, 23.1%), seafarers (n=94, 0%), pregnant women (n=200, 0.5%), and blood donors (n=210, 2.9%) in 2007 [Ishizaki et al, 2009]. Samples were screened for the hepatitis B surface antigen (HBsAg) and anti-HBs antibody and analyzed genetically. The cumulative HBV incidence rate (HBsAg+anti-HBs) was 53.2% (10.7%+42.5%) in intravenous drug users, 51.6% (11.0%+40.6%) in female sex workers, 54.3% (9.6%+44.7%) in seafarers, 50.5% (12.5%+38.0%) in pregnant women, and 51.0% (18.1%+32.9%) in blood donors; there was no significant difference among these groups. Of 163 HBsAg-positive samples, 113 could be analyzed genetically. Phylogenetic analysis, based on the preS1 region, revealed genotype B4 was most prevalent (90/113; 79.6%), followed by C1 (17.7%), I1 (1.8%), and B2 (0.9%). There was no significant difference in HBV genotype distribution among different HIV infection-risk groups. The prevalence of HBsAg was 10.3% (31/301) in HIV-1-infected individuals and 12.5% (132/1054) in non-HIV-1 infected individuals, which was not significant. In addition, no significant difference in HBV genotype distribution was observed between HBV/HIV co-infected and HBV mono-infected groups. These results suggest that, although HBV and HIV share modes of transmission, major transmission routes of HBV have been different from those of HIV in Hai Phong, Vietnam.

Keywords:

Introduction

In 2008, universal infant immunization for hepatitis B virus (HBV) was implemented in more than 177 countries, according to WHO's recommendation [WHO, 2009a]; yet, HBV infection is still a serious worldwide public health concern. More than 350 million individuals in the world are estimated to have chronic HBV infection, which is a leading cause of cirrhosis and hepatocellular carcinoma worldwide [Lavanchy, 2004; WHO, 2009a]. The prevalence of chronic HBV infection varies geographically: high (more than 8%) in areas such as South East Asia, China, and Sub-Sahara Africa; intermediate (2-7%) in areas such as South Central and South West Asia, and Eastern and Southern Europe; and low (less than 2%) in Australia, New Zealand, Northern and Western Europe, and North America [Alter, 2003; Lavanchy, 2004]. The predominant mode of transmission varies according to the endemicity of HBV infection. In high endemic areas, perinatal transmission is the main mode of transmission; whereas, sexual contact amongst high risk individuals such as homosexual males, heterosexuals with many sexual partners, or injection drug users (IDUs) is the predominant mode in low endemic areas [Alter, 2003; Lavanchy, 2004].

Eight HBV genotypes, A-H, have been identified based on molecular analyses [Schaefer, 2007]. The distribution of genotypes and their sub-genotypes differs geographically [Norder et al., 2004; Schaefer, 2007]. In Asia, genotypes B and C are predominant [Tran et al., 2004a]. It has been reported that HBV genotypes and sub-genotypes differ in virological characteristics [Sugiyama et al., 2006; Liaw et al., 2009], natural clinical courses, and responses to antiviral therapy [Suzuki et al., 2005; Dienstag et al., 2008, Osiowy et al., 2010]. Recently, a new HBV genotype, I, was identified in Vietnam [Hannoun et al., 2000; Tran et al., 2008]; Laos [Olinger et al., 2008], France, in a child who was adopted from Vietnam [Colson et al., 2009]; China [Yu et al., 2010]; eastern India [Arankalle et al.,

2010]; and Canada, from an adult who immigrated from Vietnam in 1998 [Osiowy et al., 2010].

Vietnam is one of the HBV endemic countries. The prevalence of chronic HBV infection varies from 3.0% to 23.0% in each area and ethnic group [Tran et al., 1993; Song et al., 1994; Katelaris et al., 1995; Kakumu et al., 1998; Hipgrave et al., 2003; Nguyen et al., 2007; Duong et al., 2009]. Prevalent HBV genotypes are B (sub-genotype B4) and C (sub-genotypes C1 and C5) [Tran et al., 2003; Tran et al., 2004b; Le et al., 2005; Truong et al., 2007; Nguyen et al., 2009]. Hai Phong is the largest port city in northern Vietnam, with a high prevalence of human immune deficiency virus type 1 (HIV-1). Recently, the characteristics of HIV-1 among different HIV infection-risk groups [Ishizaki et al., 2009] and the existence of multiple infection routes of hepatitis C virus (HCV) among intravenous drug users [Tanimoto et al., 2010] in Hai Phong were reported. However, there are few reports on the molecular epidemiology of HBV in Hai Phong.

To investigate the characteristics of HBV infection in Hai Phong, northern Vietnam, the prevalence and genotype distribution of HBV as well as co-infection with HIV-1 were analyzed among five different HIV infection-risk groups in the city.

Subjects and Methods

Subjects

Residents of Hai Phong in northern Vietnam were invited to join this study, as reported previously [Ishizaki et al, 2009]. After thorough ethical clearance and informed consent, blood samples were collected from 1,355 participants from April to October 2007. The participants had different risks for HIV infection and were categorized into five groups: (1) IDUs, who were concentrated in rehabilitation centers in Hai Phong (n=760; all male; mean age: 34.1 years old, age range: 19–65; anti-HIV-1 antibody positive rate: 35.9%); (2) female sex workers (FSWs), who had been commercial sex workers previously and were concentrated in a rehabilitation center (n=91; mean age: 24.8 years old, age range: 17–42; anti-HIV-1 antibody positive rate: 23.1%); (3) seafarers, who worked for marine companies (n=94; all male; mean age: 32.5 years old, age range: 20–56; anti-HIV-1 antibody positive rate: 0%); (4) pregnant women, who attended antenatal clinics (n=200; mean age: 30.8 years old, age range: 15–50; anti-HIV-1 antibody positive rate: 0.5%); and (5) blood donors (n=210; female/male: 69/140, one person whose gender was not known; mean age: 31.2 years old, age range: 16–58; anti-HIV-1 antibody positive rate: 2.9%). The plasma samples were tested with immunochromatography assay kits for HBsAg (DINA SCREEN HBsAg II; Abbott Japan, Tokyo, Japan) and for anti-HBs antibody (DINA SCREEN AUSAB; Abbott Japan).

DNA extraction and amplification

HBV DNA was extracted from 100 µl of HBsAg-positive plasma using the SMITEST EX-R&D nucleotide extraction kit (Genome Science Laboratories, Fukushima, Japan) according to the manufacturer's instructions. The preS1 region of the HBV gene was amplified by nested polymerase chain reaction (PCR) using AmpliTaq Gold (Applied Biosystems, Japan) with primers HBPr1 (position: 2850-2868, 5'-GGGTCACCATATTCTTGGG-3') and

HBr135 (position: 803-822, 5'-CAAAGACAAAAGAAAATTGG-3') for the first round, and HBPr2 (position: 2867-2888, 5'-GAACAAGAGCTACAGCATGGG-3') and HBPr3 (position: 3226-3246, 5'-CCACTGCATGGCCTGAGGATG-3') for the second round [Stuyver et al., 2000]. The first and second round PCR were performed with one cycle at 94°C for 10 min, followed by 40 cycles at 94°C for 30 sec, 50°C for 30 sec, and 72°C for 1 min, with a final extension of 72°C for 10 min. PCR products were visualized by ethidium bromide staining of samples electrophoresed on an agarose gel.

The full-length HBV genome was amplified as two overlapping fragments with primers HB8F (position: 1824-1843, 5'-TTCACCTCTGCCTAATCATC-3') and HB6R (position: 1784-1803, 5'-TAGGCATAAATTGGTCTGTT-3') for the large fragment, and HB7F (position: 1611-1630, 5'-GAGACCACCGTGAACGCCCA-3') and HB7R (position: 2048-2072, 5'-CCTCACCATACAGCACTCAGG-3') for the small fragment. The large fragment was further amplified, using nested PCR, as 11 overlapping fragments, as descried previously [Sugauchi et al., 2001]. PCR for the large fragment was performed using KOD FX (Toyobo, Osaka, Japan) with one cycle at 92°C for 2 min, followed by 30 cycles at 98°C for 10 sec, 50°C for 30 sec, and 68°C for 3.5 min with a final extension at 68°C for 7 min. PCR of the small fragment and the second round of PCR for the large fragment were performed using AmpliTaq Gold DNA polymerase (Applied Biosystems, Japan) with one cycle at 94°C for 10 min, followed by 30 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 10 min.

Sequence analysis and HBV genotyping

The amplified products were sequenced directly and analyzed with an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) with BigDye Terminator v1.1 (Applied Biosystems). Multiple alignments were performed using ClustalW with minor manual

adjustments [Thompson et al., 1997] and the phylogenetic tree was constructed by the Neighbor-Joining method with 1,000 bootstrap replicates and visualized by the NJplot Win program. References of complete HBV genome sequences were retrieved from GenBank (GenBank/EMBL/DDBJ); their accession numbers are indicated in Figs. 1 and 2. The *Chi*-square test was used for the calculation and *p* values less than 0.05 were considered statistically significant. The GenBank accession numbers of the sequences derived from this study are AB562351-AB562461 for the preS1 region and AB562462-AB562463 for the complete genomes.

Results

HBV prevalence

From a total of 1,355 individuals from five different HIV infection-risk groups in Hai Phong, 163 (12.0%) were positive for HBsAg and 547 (40.4%) were positive for anti-HBs antibody. The HBV cumulative incidence rate (HBsAg + anti-HBs) was 53.2% (10.7% + 42.5%) in IDUs (n=760), 51.6% (11.0% + 40.6%) in FSWs (n=91), 54.3% (9.6% + 44.7%) in seafarers (n=94), 50.5% (12.5% + 38.0%) in pregnant women (n=200), and 51.0% (18.1% + 32.9%) in blood donors (n=210). The higher prevalence of HBsAg in blood donors (18.1%) was significant compared to the other groups (10.9%, $p<0.05$); however, there was no significant difference in HBV cumulative incidence rates among these groups.

Genotype distribution

Genetic analysis for the HBV preS1 region was successful in 113 of the 163 HBsAg-positive samples. The phylogenetic analysis revealed the most prevalent HBV genotype was B4 (n=90, 79.6%), followed by C1 (n=20, 17.7%), I1 (n=2, 1.8%), and B2 (n=1, 0.9%) (Fig. 1 and Table 1). No HBV strains isolated from a group formed a clear cluster, except for the seven genotype B4 strains isolated from pregnant women; although, the bootstrap value of the cluster was not significant (Fig. 1) and those strains did not form a cluster in a phylogenetic tree based on the precore region (data not shown). Two genotype I1 strains isolated from an IDU and FSW, respectively, were confirmed by a phylogenetic analysis based on full-genome sequences (Fig. 2). There was no significant difference in HBV genotype distribution among these different HIV infection-risk groups; though, there was some tendency that genotype B4 was more prevalent and genotype C1 less prevalent in pregnant women than IDUs (Table 1).

Comparison of HBV genotypes between HBV-positive groups with and without HIV-1 co-infection

HIV-1 prevalence among the same groups in Hai Phong was reported to be 35.9% (273/760) in IDUs, 23.1% (21/91) in FSWs, 0% (0/94) in seafarers, 0.5% (1/200) in pregnant women, and 2.9% (6/210) in blood donors [Ishizaki et al, 2009]. The prevalence of HBsAg was 10.3% (31/301) in HIV-1-infected individuals and 12.5% (132/1054) in non-HIV-1 infected individuals, not a significant difference between these two groups (p=0.29). In addition, there was no significant difference in HBV genotype distribution between HBV/HIV-1 co-infected and HBV mono-infected groups (Table 2). Those who were infected with HBV genotype I1 (n=2, IDU and FSW) and B2 (n=1, pregnant woman) were not co-infected with HIV-1 (Table 2).

Discussion

The prevalence of HBsAg in Hai Phong was 12.0%, which is consistent with the results of previous studies in Vietnam [Tran et al., 1993; Song et al., 1994; Kakumu et al., 1998; Hipgrave et al., 2003; Nguyen et al., 2007; Duong et al., 2009]. No significant difference in HBsAg prevalence was observed among IDUs, FSWs, seafarers, and pregnant women; while, the higher prevalence of HBsAg in blood donors (18.1%) was significant compared to the other groups (10.9%, $p<0.05$). These findings may be because the majority of blood donors, recruited for this study in 2007, were paid blood donors, who have high risks of infectious diseases [van der Poel et al., 2002]. However, the Vietnamese government has been involved in the implementation of a voluntary non-remunerated blood donation system [WHO, 2009b], and HBsAg prevalence among blood donors in Hai Phong decreased to 11.5% (n=200) in 2009 (unpublished data).

In the current study, the cumulative HBV-infection incidence rate, calculated by adding the HBsAg-positive and anti-HBs anitibody-positive rates, was 52.4% (range: 50.5% - 54.3%), which is within the range of previous reports from Vietnam (47.7 % - 79.2%) [Song et al., 1994; Hipgrave et al. 2003]. There was no significant difference in the cumulative incidence rate among the five different HIV infection-risk groups, suggesting that major transmission routes of HBV are different from those of HIV-1 in Hai Phong, Vietnam.

As mentioned above, the predominant mode of HBV transmission varies according to the endemicity of HBV infection. In high-prevalent areas (more than 8% of chronic HBV infection rate), perinatal transmission is the main mode of transmission [Alter, 2003; Lavanchy, 2004]. Therefore, vertical transmission is considered the main mode of HBV transmission in Hai Phong, northern Vietnam. However, the cumulative prevalence of HBV infection increases as children grow up in Vietnam [Katelaris et al., 1995; Hipgrave et al.,

2003; Duong et al., 2009]. In addition, several risk factors of HBV infection have been reported in Vietnam, such as poor infection control activities in health-care settings, the male gender, and a low level of education [Nguyen et al., 2007; Duong et al., 2009]. Therefore, horizontal HBV transmission also plays a role in Vietnam.

The most common HBV genotype in Hai Phong was genotype B4 (80.5%), followed by C1 (17.7%), which is consistent with previous reports [Tran et al, 2003; Tran et al., 2004b; Le et al., 2005; Truong et al., 2007; Nguyen et al.; 2009]. It is interesting that two HBV genotype I1 strains (HPA184_07 and HPC017_07) were also identified in this study, and they clustered with Vietnamese and Laos strains, respectively (Fig. 2). Genotype I, consisting of sub-genotypes I1 and I2, was first reported in an individual from Vietnam in 2000 [Hannoun et al., 2000] and then in Laos, China, eastern India, and other countries in which people originated from Vietnam [Tran et al., 2008; Olinger et al., 2008; Colson et al., 2009; Yu et al., 2010; Arankalle et al, 2010; Osiowy et al., 2010]. The genotype I is reported to be a recombinant of genotypes A, C, and G [Yu et al., 2010], but the origin of the parental strains and the time of occurrence are not clear yet. Further epidemiological investigation is needed to understand the origin of this new genotype.

In the current study, no significant difference was observed in HBV genotype distribution among the different HIV infection-risk groups. In addition, only 10.8% of the HIV-1-infected individuals were co-infected with HBV, and no significant difference was observed in HBV genotype distribution between HBV/HIV-1 co-infected and HBV mono-infected groups. These results suggest the major transmission routes of HBV are different from those of HIV-1 in Hai Phong, Vietnam.

It was reported that HBV genotype distribution changed in Japan, especially among individuals who acquired HBV through sexual contact, after the successful control of vertical HBV transmission by the introduction of HBV immunization [Kobayashi et al., 2008]. In Vietnam, universal infantile immunization for HBV was introduced in 2002 [WHO 2009c]; therefore, a reduction of HBV prevalence and a shift of HBV genotype distribution is expected in the near future.

HIV-1 infection is still limited to IDU and FSW groups; however, the endemic of HIV-1 infection seems to have started in the general population in Hai Phong [Ishizaki et al, 2009]. As HBV and HIV-1 share modes of transmission and those who are co-infected with HBV and HIV need specific treatment options [Koziel et al., 2007], longitudinal monitoring of the trend of HBV prevalence and genotypes distribution together with the trend of HIV-1 prevalence is necessary to assess the proper strategies for HBV and HIV-1 infection control in Vietnam.

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Figure legends

Fig. 1. Phylogenetic tree of 113 HBV strains from IDUs, FSWs, seafarers, pregnant women, and blood donors based on the preS1 region. Filled squares, HBV strains from IDUs; open circles, HBV strains from FSWs; open double circles, HBV strains from seafarers; triangle, HBV strains from pregnant women; closed diamond, HBV strains from blood donors; +, co-infection with HIV.

Fig. 2. Phylogenetic tree of genotype I based on the full-length genome. HBV genotype I1 strains (HPA 184-07 and HPC 017-07), identified in the current study, are shown in bold. Boot strap values greater than 700 are shown.

Fig. 1

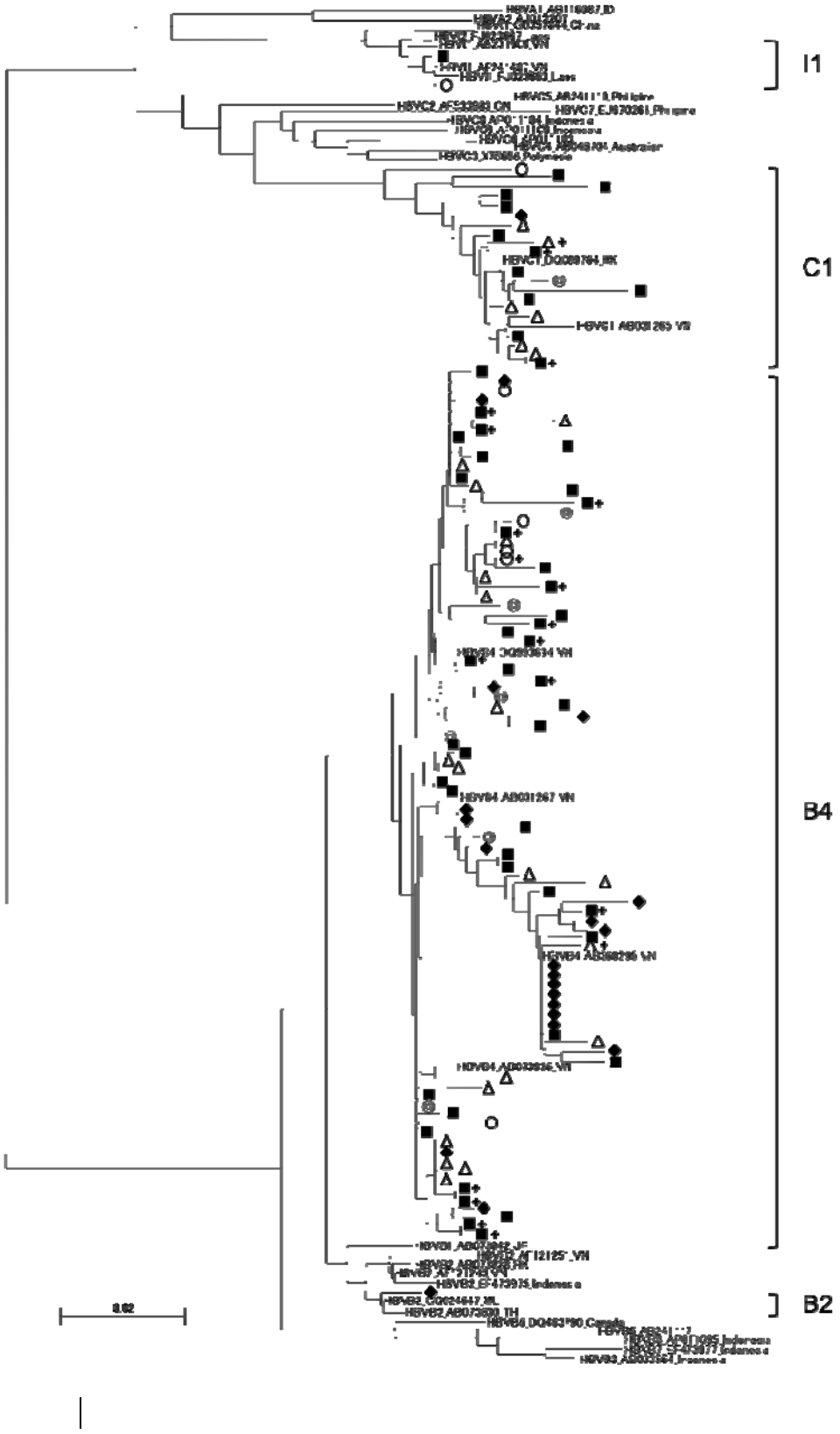

Fig. 2

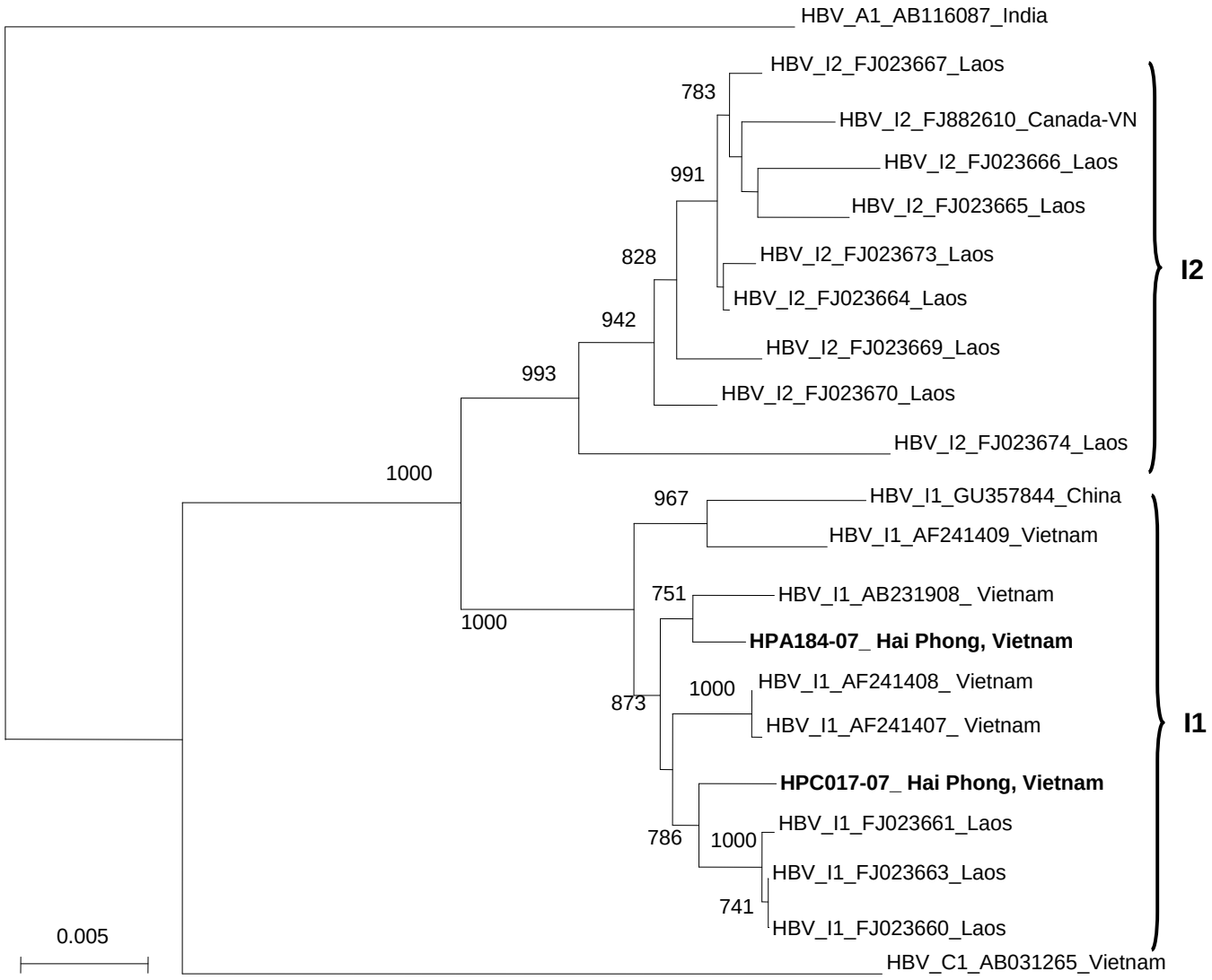
HBV_A1_AB116087_India
HBV_I2_FJ023667_Laos
783
HBV_I2_FJ882610_Canada-VN
HBV_I2_FJ023666_Laos
991
HBV_I2_FJ023665_Laos
828
HBV_I2_FJ023673_Laos
HBV_I2_FJ023664_Laos
942
HBV_I2_FJ023669_Laos
993
HBV_I2_FJ023670_Laos
HBV_I2_FJ023674_Laos
I2
1000
967
HBV_I1_GU357844_China
HBV_I1_AF241409_Vietnam
751
HBV_I1_AB231908_ Vietnam
1000
HPA184-07_ Hai Phong, Vietnam
1000
HBV_I1_AF241408_ Vietnam
873
HBV_I1_AF241407_ Vietnam
HPC017-07_ Hai Phong, Vietnam
786
1000
HBV_I1_FJ023661_Laos
HBV_I1_FJ023663_Laos
741
HBV_I1_FJ023660_Laos
I1
0.005
HBV_C1_AB031265_Vietnam

Table1: Distribution of HBV genotypes of the preS1 region among different groups

Groups	PCR positive	Genotype B4		Genotype C1		Genotype I1		Genotype B2	
	n	n	%	n	%	n	%	n	%
IDUs	52	40	76.9	11	21.2	1	1.9	0	0
FSWs	7	5	71.4	1	14.3	1	14.3	0	0
Seafarers	7	6	85.7	1	14.3	0	0	0	0
Pregnant women	22	20	90.9	1	4.5	0	0	1	4.5
Blood donors	25	19	76.0	6	24.0	0	0	0	0
Total	113	90	79.6	20	17.7	2	1.8	1	0.9

Table 2: Comparison of HBV genotypes between HBV-positive group with and without HIV co-infection

Groups	n	Genotype B4		Genotype C1		Genotype I1		Genotype B2	
		n	%	n	%	n	%	n	%
HBV + HIV	19	16	84.2	3	15.8	0	0	0	0
HBV single	94	74	78.7	17	18.1	2	2.1	1	1.1
Total	113	90	79.6	20	17.7	2	1.8	1	0.9